



Drought induced molecular and physiological adaptations in *Zizyphus mauritiana* and *Salvadora oleoides* under climate change scenario

Muhammad Asif^{1*}, Junaid Naseer², Tanveer Hussain Turabi², Rashid Iqbal Khan³, Ayesha Maryam⁴

¹Department of Botany, The Islamia University of Bahawalpur, Pakistan.

²Institute of Forest Sciences, The Islamia University of Bahawalpur, Pakistan.

³Horticultural Research Institute, National Agricultural Research Center, Islamabad, Pakistan.

⁴Department of Agricultural Sciences, Allama Iqbal Open University, Islamabad, Pakistan.

Article Info

Keywords:

Drought stress, Field capacity, Leaf functioning, Photosynthetic pigments.

Date:

Received: 12 January 2023

Accepted: 17 May 2023

Published: 30 December 2023

Volume: 02 (01), 2023

Abstract

Water scarcity is one of the most impactful emerging challenges around the globe, enhancing the competition between domestic and agricultural usage. This scenario is even worse in arid and semi-arid areas owing to the low availability of water resources. The current research trial aimed to check the adaptability potential and leaf functioning of *Zizyphus mauritiana* and *Salvadora oleoides* under water stress. A pot experiment was conducted in the Forest Nursery and Experimental Area, Institute of Forest Sciences, The Islamia University of Bahawalpur. The four levels of water scarcity i.e., 60% field capacity (FC), 50% FC, 40% FC and 30% FC along with control were evaluated following a complete randomized design (CRD). The outcomes exhibited that water stress has a profound impact on the growth of both tree species. Water stress has reduced the plant height (41% & 43%), stem diameter (6.47% & 4.17%), shoot fresh weight (10.6% & 10.2%) and root fresh weight (40.21% & 44.07%) w.r.t control in *Z. mauritiana* and *S. oleoides* respectively. A similar trend was observed in biochemical parameters where the stress has induced higher enzymatic activities such as SOD (1.8 & 1.18 mg-l protein), POD (5.81 & 2.79 mg-l protein), CAT (1.93 & 0.68 mg-l protein) and MDA levels (0.039 & 0.034 mol g-l FW) respectively. Moreover, the photosynthetic pigments such as chlorophyll a, chlorophyll b, total chlorophyll and carotenoids also exhibited antagonistic responses towards drought stress. The overall results showed that *Z. mauritiana* performed better as compared to *S. oleoides* under drought stress. The outcomes of the study provide meaningful insights regarding the plantation on marginal lands in arid to semi-arid regions.

1. Introduction

Drought is a natural complex environmental phenomenon that is triggered for an extended period because of inadequate water availability for the ecosystem and humans (Sun *et al.*, 2018). It is a multidimensional abiotic stress and prompts because of dry spells and rainfall depreciation (Gogoi and Tripathi, 2019). Due to climate change, variations in global patterns of high temperature, relatively low humidity, and high wind characteristics along with the timing of rains, duration, and intensity of rain (Raghuwanshi *et al.*, 2019). Drought is categorized into four recognizable classes, one is meteorological drought (precipitation loss), hydrological drought, (scarce and low water supply), agricultural drought (soil moisture loss), and socio-economic drought (failure of demand as well as supply of different commodities because of drought) (Wilhite, 2005; Sung and Stagge, 2022).

*Email: www.muhammadasif786@gmail.com

Drought stress has direct impacts on plant growth, water relations with water-soluble nutrients, yield, membrane integrity, photosynthesis mechanism, and pigment contents (Muhammad *et al.*, 2012). Stress decreased cell division along with expansion rate, stem elongation, declined stomatal oscillations, leaf size, root proliferation, capability of water use, and plant nutrients in addition to water relations with reduced crop productivity (Li *et al.*, 2009; Farooq *et al.*, 2009). Growth of plants involves the meristematic cell division which occurs because of cell elongation. The meristematic activity of cells due to water deficiency leads to a decline elongation of cells resulting in a decline in cell division as well as the growth of plants (Kumar *et al.*, 2022). Physiological parameters include several leaves, root area, leaf surface area, plant height, tillers, shoot and root fresh along with dry weight, and root length are negatively affected by drought, but some parameters show hydrotropism phenomena like shoot and root length (Anjum *et al.*, 2011). Under severe drought stress leaf area as well as the number of leaves decreased because of low turgor pressure (Sahoo *et al.*, 2022). It also greatly impacts the biomass and height of plants as water scarcity conditions decline the cell expansion at the early vegetative phase by decreasing leaf area along with elongation of the internode (Waraich and Ahmad, 2010; Hammad and Ali, 2014).

Water deficiency increases abscisic acid (ABA) biosynthesis which causes the reduction in stomatal conductance to reduce the transpiration losses (Shinozaki *et al.*, 2006). In the influence of severe water scarcity conditions cell elongation in higher vascular plants can be prohibited because of the disturbance of the stream of xylem water towards elongating surrounding cells (Mutluay *et al.*, 2022). The decline in elongation of cells, expansion as well and mitosis results in a decline in leaf surface, plant growth, and plant height under water stress (Hussain *et al.*, 2008).

Photosynthesis is greatly sensitive to water deficiency conditions, as its stress directly influences the crop's photosynthetic capacity. Under drought stress key factors that decrease carbon fixation are decreased leaf size along with number, stomatal closure, imperfect ATP synthesis as well as carboxylation enzymes activities, and ruin of photosynthetic mechanism (Costa *et al.*, 2022). Mostly drought stress impacts carbon fixation classified into non-stomatal along with stomatal limitations. Crop plants show primary responses by closing the stomata under drought stress. By closing stomata removal of water by transpiration is reduced simultaneously stomatal CO₂ intake is also reduced (Sperlich *et al.*, 2016). Transpiration along with photosynthesis rates are reduced because of decreased stomatal conductance in kidney-shaped beans (*Phaseolus vulgaris*) (Miyashita *et al.*, 2005). Similarly, drought stress increasingly reduced the CO₂ assimilation rates due to which stomatal conductance is decreased as in coconut plants (Gomes *et al.*, 2010). Stomatal limitations are not enough to reduce the negative effects of decreased stomatal conductance under water deficiency conditions on photosynthesis indicating the non-stomatal constraints involvement (Gad *et al.*, 2023). Non-stomatal limitations for carbon fixation are changed activities of essential enzymes and reduced synthesis, imperfect ATP synthesis, heavy oxidative load, photosynthetic pigments, and photorespiration (Farooq *et al.*, 2009). Photosynthetic pigments include carotenoid contents, xanthophylls, and chlorophyll contents play an important role in carbon fixation by capturing the solar radiation to drive photosynthetic apparatus. Chlorophyll a along with chlorophyll b contents are greatly reduced under drought stress in marigolds (Asrar and Elhindi, 2011).

In plants, oxidative stress is imposed because of drought stress which results in the synthesis of different ROS species like hydroxyl radicals (OH•), singlet oxygen (1O₂), hydrogen peroxide (H₂O₂), and superoxide (O₂⁻) (Indradewa *et al.*, 2020). Underwater deficiency conditions reactive oxygen species (ROS) are synthesized because of a greater amount of O₂ inside the leaf which causes stress and sensitivity problems in plants (Waraich *et al.*, 2011). The CO₂ intake is prohibited because of closed stomata as well a higher amount of oxygen in stomata produces ROS species under water deficiency stress which ruptures the membranes and causes leaking which mainly affects the rate of photosynthesis, plant growth, and respiration. ROS species also greatly disturb the synthesis of various cellular constituents including nucleic acid, lipids, carbohydrates, and proteins (Waraich *et al.*, 2011). At the cellular level because of H₂O₂ and O₂⁻ various chemical reactions occur like the Fenton reaction which involves an iron-catalyzed reaction, and various enzyme reactions like peroxidases, oxidase, and xanthine which decrease the NADPH (nicotinamide adenine dinucleotide phosphate hydrogen), lipoxygenases as well as oxidase (Mattos and Moretti, 2015).

Drought stress significantly affects the uptake of nutrients including potassium (K), phosphorous (P) along nitrogen (N) in plants. Nutrient uptake decreases in roots because of a decrease in soil moisture which reduced the nutrient diffusion rate from the matrix of soil to the absorbing root surface as well as translocation to leaves (Wang *et al.*, 2022). The absorption ability of crop plants decreased under drought stress due to reduced transpiration rates, membrane permeability, and declined active transport (Knipfer *et al.*, 2023). Therefore, transportation of nutrients from root to shoot is reduced because of the curtailed absorbing ability of crop plants under water deficiency stress. Water deficiency decreases the function of nitrate reductase by affecting the nitrogen uptake and nitrogen metabolism, as water scarcity causes the reduction in water level in tissues of plants which causes the reduction in water potential, it leads to a reduction in leaf elongation, pigment contents, photosynthetic rate of leaf, photosystem II damages, changes the protein synthesis, cell membrane properties and nitrogen

metabolism due to which leaf area and plant height is reduced (Ahmad and Anjum, 2023). Drought stress also decreases the phosphorus uptake in plants which reduces the area and water content in fresh leaves (Singh *et al.*, 2022).

Zizyphus mauritiana is an evergreen tree and shrub which is widely distributed in sub-tropical and tropical areas. *Z. mauritiana* which is a tropical fruit tree commonly called “Desert apple” “Indian jujube” “Indian plum” “Ber” “Chinese apple” and “Malay apple” belongs to the family Rhamnaceae and is cultivated in regions of mild temperate (Jbawi *et al.*, 2023). *Z. mauritiana* is a medicinal plant. Its fruits are used in ulcers and cuts. They are also used against various diseases like fevers, pulmonary ailments, dysentery, and fresh wound healing (Mohankumar *et al.*, 2022). *Salvadora oleoides* is also a medicinal plant with multipurpose activities that belong to the Salvadoraceae family and is commonly called “Mithi Jal” and “Peelu”. Its plant has fruits also known as desert grapes. Desert grapes are sweet with pulpy material containing rich amounts of calcium (Tandon *et al.*, 2018). This plant is a natural source of lipoxygenase, and bioactive antioxidants (Saleem *et al.*, 2020). Bahawalpur region has a true Cholistan desert along with shifting dunes and is characterized by strong summer winds, extreme temperature, low relative humidity, extreme evaporation rate, and sporadic precipitation. Therefore, there is a dire need to identify the best xerophytic tree species for sandy desert regions. So, a pot experiment was conducted to evaluate the growth potential and eco-physiological response of *Z. mauritiana* and *S. oleoides* in desert environments.

Medical plants are the main source of modern medicines which have been used in the treatment of the various health disorders of mankind from ancient times (Dar *et al.*, 2017). About 80% population of developing countries uses these medicinal plants against different kinds of diseases. Most of the rural people rely on these plants due to their easy availability (Hussain *et al.*, 2018). *Moringa oleifera* belongs to monogeneric family, the Moringaceae, and is a plant with high medicinal and nutritional importance. It is distributed throughout the tropical and subtropical parts of the World representing the Asia, Europe, Oceania and America. Plants parts like leaves, pods, flowers and seeds are considered food sources of high nutritional value. This plant is also a good source of vitamins, minerals, proteins and has different biotechnological and pharmacological potential (Brilhante *et al.*, 2017). *Moringa oleifera* requires a temperature and precipitation range of 25-30°C and 250-1500 mm (annual rainfall) respectively; while it can survive up to a temperature of 48 °C. It can be found between 600 m to 2200 m elevations. Well-drained clay or sandy loamy soil are suitable for their growth. During water stress condition, plant shed their leaves to overcome the water loss. In dry conditions, it requires water during their initial growth stages for at least two months (Abdoun *et al.*, 2022).

Azadirachta indica is widely spread in southern region of Asia and Africa. Its various parts including fruit, flowers, leaves, bark, oil and gum are being used as medicine in the treatment of contagious diseases such as heart disease, diabetes, cancer and hypertension (Islas *et al.*, 2020). It can grow best in a temperature range of 21 to 32°C and a precipitation range of about 400 to 1200 mm (annual rainfall). Well-drained sandy and deep soils are best for their growth (Jibo *et al.*, 2018). Organic materials and plant nutrients are found in sewage water. The cheap way for the disposal of sewage water is agriculture. Sewage water in agriculture may add substantial to lighten the burden of using fresh water resources. Organic matter with plant nutrients (N, K, P, S, Ca, Mn, Cu and Zn) in considerable quantity are present in waste water which is obtained from different sources and hence increases the crop yield (Nagajyothi *et al.*, 2009). Sharma, (2011) said that the content of nitrogen is highest in urea for all fertilizers with solid nitrogenous. Urea can be subjected in irrigated crops in dry form to the soil or dissolve form and useful through the irrigation water (Epstein and Bloom, 2005). Due to the large availability of volumes, sugarcane husk can be utilized as a medium for plant growth. Plants of sugarcane have high efficiency of bioconversion sunlight after catching in photosynthesis. They can fix the dry matter annually of about 55 tons per hectare of land on large and small scales in industries all over the world for its utilization as raw material as it produces tons of bagasse. Waste material from agriculture is used for high production and is also cherished for the disposal of waste and costs (Sidana, 2014). Hussain *et al.* (2013) also described the importance of advanced planting techniques in maximizing the yield of food crops.

Due to high demand and severe collection practices, a large number of medicinal plant species are considered threatened (Riaz *et al.*, 2021). Thus, efforts are being made to conserve medicinal plants by increasing the wild population otherwise the medicinal species may be vanished. Genetic stocks of these medicinal plants are conserved by propagation in the nursery. The nursery provides planting materials for these species for cultivation. On a large-scale, cultivation brings more species which helps to reduce the pressure on the wild stocks. *Moringa oleifera* and *Azadirachta indica* are native to Bahawalpur and very important medicinal plants. Climatic factors, topography, elevations and edaphic factors are favorable for growth. That's why there is a dire need to experiment to check the effect of different nursery techniques on the growth of these medicinal plants on experimental sites.

2. Materials and Methods

2.1: Study site and climatic conditions

The experiment was conducted at Forest Nursery and Experimental Area (29.3981° N, 71.6908° E) Institute of Forest Science, The Islamia University of Bahawalpur. The climate of Bahawalpur is characterized by strong summer winds, extreme

temperatures, low relative humidity, extreme evaporation rate and sporadic precipitation. The annual temperature ranges between 6°C to 50°C and rainfall between 90-200 mm.

2.2: Application and treatment of drought stress

The diseased free, uniform size, six months old seedlings of *Z. mauritiana* and *S. oleoides* were collected from Forest Nursery and Experimental Area. The seedlings were transplanted into earthen pots (13 x 17 cm). The drought stress in various levels i.e., T₁ (control), T₂ (60% field capacity (FC)), T₃ (50% FC), T₄ (40% FC) and T₅ (30% FC) were applied after 30 days of transplanting. The plants were kept under natural growing conditions and data was recorded for six months.

2.3: Measurement of growth parameters

2.3.1 Vegetative growth parameters

The stem diameter (mm) of *Z. mauritiana* and *S. oleoides* was measured at the collar point with the help of a digital caliper, which was marked beforehand. Plant height (cm) of *Z. mauritiana* and *S. oleoides* was measured with the help of measuring tape of the entire individual from collar point to apex. After harvesting the plant shoot as well as root sections of *Z. mauritiana* and *S. oleoides* by using weighting balance (Electronic Scale JJ3000B) were immediately weighted by placing them into paper bags. By doing this all samples of *Z. mauritiana* and *S. oleoides* were placed at 74°C in a heating oven (Thermal Electric Thermostat Drying Oven DGH - 9202 series) for 10 days. The dry weight of all these samples was recorded after removing them from the heating oven. Biomass partitioning in two types was divided one was root biomass other was shoot biomass. Shoot biomass was measured by using the following formula:

$$\text{Shoot biomass (\%)} = \text{Shoot dry biomass} / \text{total biomass} \times 100$$

Root biomass was measured by using the following formula:

$$\text{Root biomass (\%)} = \text{Root dry biomass} / \text{total biomass} \times 100$$

The total biomass was measured by using the given formula:

$$\text{Total biomass} = \text{Shoot dry biomass} + \text{Root dry biomass}$$

2.4: Determination of biochemical parameters

The sample extraction for analysis of various enzymes was done following the protocol of Naqvi *et al.* (2011). The 0.5g of plant tissue was ground in mortar and pestle and mixed with 2 ml of 50 mM phosphate buffer and extract was prepared. Extracts were filtered and then at 8000 rpm centrifuged for 10 minutes and supernatant was collected and stored at -80°C.

2.4.1: Determination of Superoxide Dismutase (SOD)

The SOD activity was determined by using the Giannopolitis and Ries, (1977) method in which the ability of SOD to inhibit the photoreduction of Nitroblue tetrazolium (NBT) was determined. The SOD determination reaction solution contains 0.22 g methionine in 15 ml of distilled water and 0.015g of NBT in 17.5 ml of distilled water. 0.013 g of riboflavin in 17.5 ml of distilled water, 0.2 molar buffer, and 0.0375g of Triton-X in 17.5 ml of distilled water. The reaction solution containing the tube before adding riboflavin was placed under a UV lamp for 15 minutes. With the help of a spectrophotometer at 560 nm absorbance of the solution was measured.

2.4.2: Determination of Peroxidase (POD)

According to Liu and Huang (2000) method, POD activity was measured. In this method POD solution was prepared which contains 20 mM guaiacol, 50 mM phosphate buffer (pH 5), 40 mM H₂O₂, and 0.1 ml enzyme extract. By using a spectrophotometer at 470nm frequency changes in the absorbance of the reaction solution were measured.

2.4.3: Determination of Catalase (CAT)

The CAT activity was determined with the help of Liu *et al.* (2010) described method. For determination of CAT activity reaction solution contains 1 ml 5.9 Mm and 0.1 ml enzyme extract, 1.9 ml phosphate buffer (pH 7). A reaction solution of approximately 200 µl was kept in 96 well plates and at 240 nm absorbance was taken every 20 seconds.

2.4.4: Determination of Malondialdehyde (MDA)

The MDA contents were measured by using the method of Heath and Packer (1968). In this method, 200 µl enzyme extract was homogenized with 200 µl of 0.5% thiobarbituric acid (TBA). Then in homogenate 200 µl of 20% trichloroacetic acid (TCA) was added. The mixture was heated at 95°C for 30 minutes and then quickly cooled in an ice bath for 5 minutes. After centrifugation at 4000 rpm for 10 min, the absorbance of the supernatant was recorded at 532 nm and 600 nm. The MDA content was calculated based on the extinction coefficient equal to 155mM⁻¹cm⁻¹.

$$\text{MDA} = (532-600)/1.56 \times 10^5$$

2.4.5: Determination of total phenolic content

The Folin-Ciocalteu method was used to measure the total phenolic contents (Jagadish *et al.*, 2009; Slinkard and Singleton, 1992). Leaf sample 1g added with 5ml extraction mixture (methanol, acetone, HCl (90:8:2) and homogenized with Pestel and mortar then centrifuged at 9000 rpm for 3 to 5 minutes. The extraction solution of 200 µl was mixed with 400 µl

Folin-ciocalteu reagent and then add 1400 µl of 5% Na₂CO₃. The mixture was incubated for 90 minutes at room temperature. Absorbance is measured at 760 nm using a UV-Vis spectrophotometer (Lambda EZ 201, Perkin Elmer, USA).

2.5: Determination of photosynthetic pigments

The photosynthetic pigments such as chlorophyll and carotenoid contents were measured by using Amon (1949) and Davis (1986) method. The leaf discs 0.5g were mixed with 10 ml 80% acetone at 10°C overnight and then at 14000 rpm centrifuged for 5 minutes. On the spectrophotometer (PG, T60U) absorbance of supernatant was measured at 645, 652, 663 and 480 nm. Chlorophyll contents were quantified by using the given formula:

$$\text{Chl}_a \text{ (mg g}^{-1} \text{ FW)} = [12.7(\text{OD}_{663}) - 2.69(\text{OD}_{645})] \times v/1000 \times w$$

$$\text{Chl}_b \text{ (mg g}^{-1} \text{ FW)} = [22.9(\text{OD}_{645}) - 4.68(\text{OD}_{663})] \times v/1000 \times w$$

$$\text{Chl}_{a+b} \text{ (mg g}^{-1} \text{ FW)} = [20.2(\text{OD}_{645}) - 8.02(\text{OD}_{663})] \times v/1000 \times w$$

Carotenoid contents were quantified by using given formula:

$$\text{Carotenoids (mg g}^{-1} \text{ FW)} = A^{\text{car}}/E_{\text{mx}}^{100}$$

Here

V= Volume of sample extract and W= Weight of the sample

$$A^{\text{car}} = (\text{OD}_{480}) + 0.114(\text{OD}_{645}); E_{\text{mx}}^{100} \text{ cm} = 2500$$

Total soluble protein is measured by Bradford (1976) method. In this method, a microcentrifuge tube contains a 50 µl sample and Bradford reagent 2 ml is added to the same tube. Whereas blank contained only Bradford reagent. At 595 nm frequency absorbance is taken. With the help of various concentrations of BSA (bovine serum albumin), a standard curve is prepared which is used to determine the protein content.

2.6: Statistical analysis:

The subjected data were analyzed through analysis of variance (ANOVA) under complete randomized design (CRD) by using statistics 8.1 software.

3. Results

3.1: Growth behavior under drought conditions

The impact of drought stress on the growth parameters of *Z. mauritiana* and *S. oleoides* was analyzed by measuring the plant height, stem diameter, shoot, and root fresh weight along with dry weight and total biomass. It was observed that the *Z. mauritiana* and *S. oleoides* showed significant variations in plant height and stem diameter under drought stress as indicated in Fig. 3.1 respectively. The observed results showed that the plant height and stem diameter of *Z. mauritiana* was maximum in T₁ (control), and it gradually reduced with the increased water deficit conditions. The minimum plant height and stem diameter were recorded in T₅ (30% FC) in *S. oleoides*. Likewise, under drought stress, the *Z. mauritiana* and *S. oleoides* depicted significant variations regarding the shoot and root fresh weight along with dry weight as indicated in Fig. 3.1 respectively. The minimum shoot and root fresh weight along with dry weight was recorded in T₅ (30% FC) in *S. oleoides*. The overall shoot and root fresh weight along with the dry weight of *Z. mauritiana* and *S. oleoides* at different stress levels ranged from 49.66 -43.66 g, 28.33 -11.66 g, 37.33 -20.66 g, and 25.33 -8.66 g respectively. In all treatments, *Z. mauritiana* performs better as compared to *S. oleoides*. The order of species response for the treatments was T₁ (control) > T₂ (60% FC) > T₃ (50% FC) > T₄ (40% FC) > T₅ (30% FC).

3.2: Biochemical responses under drought conditions

The impacts of drought stress conditions on the biochemical responses i.e., SOD, POD, CAT, MDA, and total phenolics of *Z. mauritiana* and *S. oleoides* showed significant differences (Fig. 3.2). The observed results showed that the SOD, POD, and MDA values of *Z. mauritiana* were maximum in T₅ (30% FC), and it gradually reduced with the decreased water deficit condition. The minimum SOD, POD, MDA, and antioxidants values were recorded in T₁ (control) in *S. oleoides*. Likewise, it was observed that *Z. mauritiana* and *S. oleoides* depicted significant variations regarding the CAT values under water deficit conditions as indicated in Fig. 3.2. The observed results showed that the CAT value of *S. oleoides* was maximum in T₅ (30% FC) and the minimum CAT value was recorded in T₁ (control) in *Z. mauritiana*. The overall CAT values of *S. oleoides* and *Z. mauritiana* at different stress levels ranged from 1.93-0.72 mg⁻¹. Similarly, the maximum phenolic values were recorded in T₅ (30% FC) in *Z. mauritiana* and minimum phenolics in T₁ (control) in *S. oleoides*. The overall phenolic values of *Z. mauritiana* and *S. oleoides* at different stress levels ranged from 358.41-129.32 mg g⁻¹ FW. The order of species response for the treatments was T₅ (30% FC) > T₄ (40% FC) > T₃ (50% FC) > T₂ (60% FC) > T₁ (control).

3.3: Photosynthetic activity under drought conditions

The impacts of water deficit conditions on photosynthetic activity in *Z. mauritiana* and *S. oleoides* were analyzed by quantifying chlorophyll a, b, a+b as well as carotenoids and total soluble protein. Under drought stress, it was observed that *Z. mauritiana* and *S. oleoides* depicted significant variations regarding the chlorophyll a as indicated in Fig. 3.3. The observed results showed that the chlorophyll a of *S. oleoides* was maximum in T₁ (control), and it gradually reduced with the increased water deficit condition. The minimum chlorophyll a was recorded in T₅ (30% FC) in *Z. mauritiana*. The results conclude that

the chlorophyll b of *Z. mauritiana* was maximum in T₁ (control) and minimum total chlorophyll b was recorded in T₅ (30% FC) in *S. oleoides*. The observed results showed that the carotenoid content of *S. oleoides* was maximum in T₁ (control), and it gradually reduced with the increased drought conditions. The minimum carotenoid levels were recorded in T₅ (30% FC) in *Z. mauritiana*. The overall carotenoid of *S. oleoides* and *Z. mauritiana* at different stress levels ranged from 356.30 - 81.00 mg g⁻¹ FW. Similarly, the results also depicted a significant variation in total soluble protein contents. The minimum total soluble protein was recorded in T₅ (30% FC) in *S. oleoides*. Among all treatments, *Z. mauritiana* performs better as compared to *S. oleoides*. The order of species response for the treatments was T₁ (control) > T₂ (60% FC) > T₃ (50% FC) > T₄ (40% FC) > T₅ (30% FC).

3.4 Physiological Parameters

The impacts of drought stress conditions on the physiological parameters i.e., instantaneous an intrinsic WUE, transpiration rate and stomatal conductance exhibited significant variations (Fig. 3.4). The outcomes expressed that the highest stomatal conductance and transpiration rate was recorded in T₁ (control) in *Z. mauritiana* and minimum in T₅ (30% FC) in *S. oleoides*. On the contrary, intrinsic WUE depicted a non-significant impact of drought stress among all treatments.

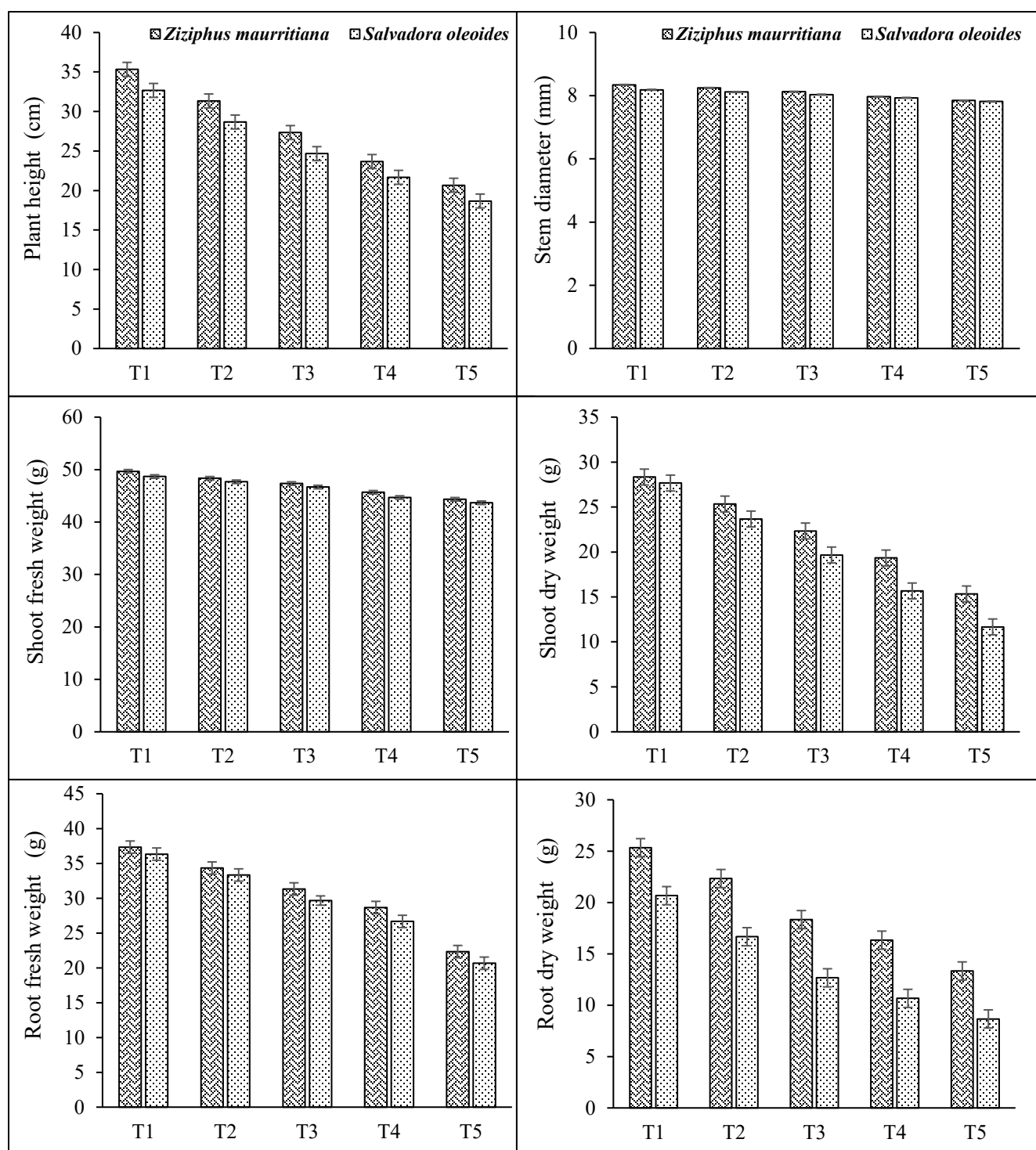


Figure 3.1: Growth potential of *Z. mauritiana* and *S. oleoides* under water deficit environment

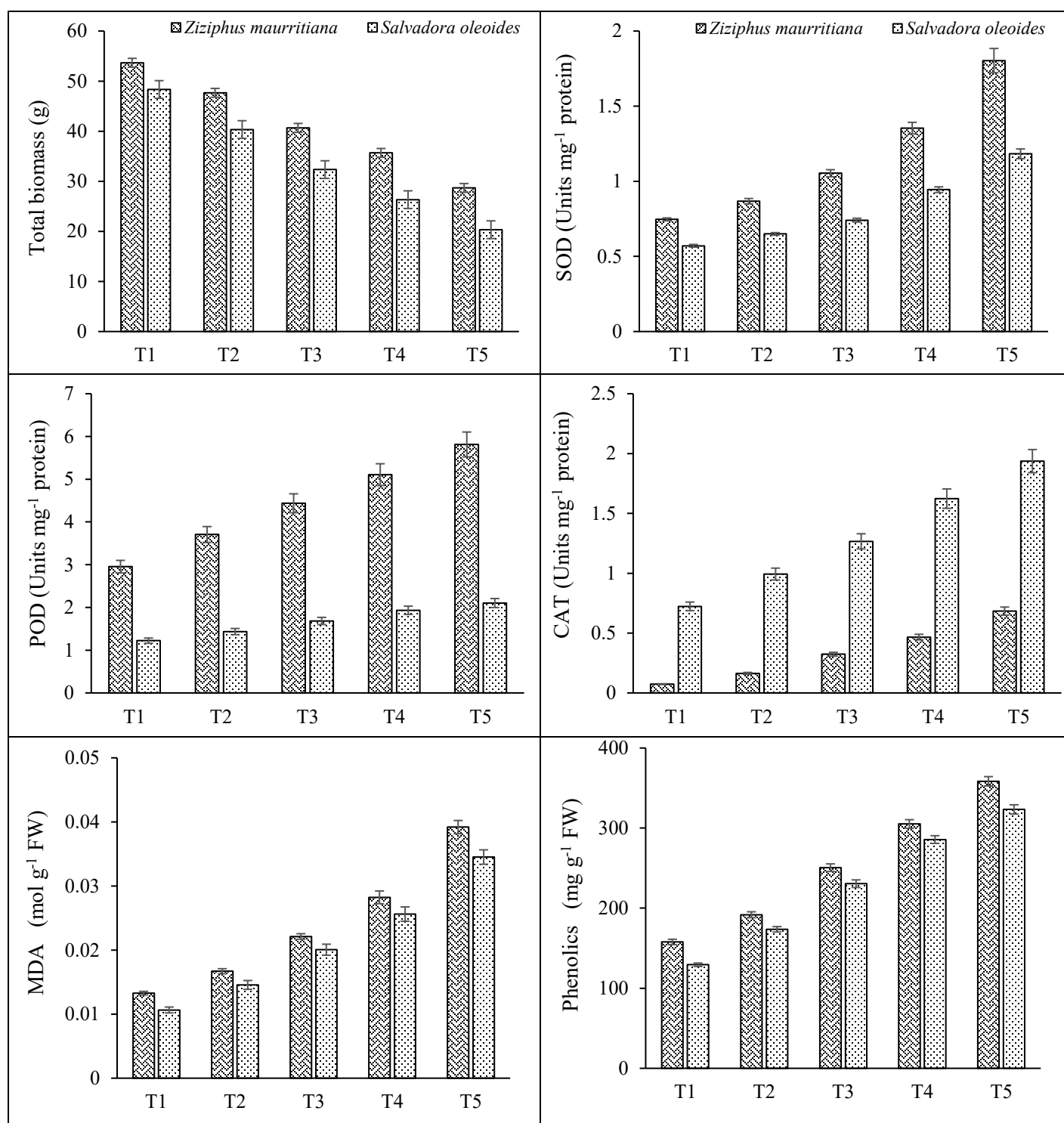
Drought induced adaptations in *Zizyphus mauritiana* and *Salvadora oleoides*

Figure 3.2: Biochemical response of *Z. mauritiana* and *S. oleoides* under water deficit environment

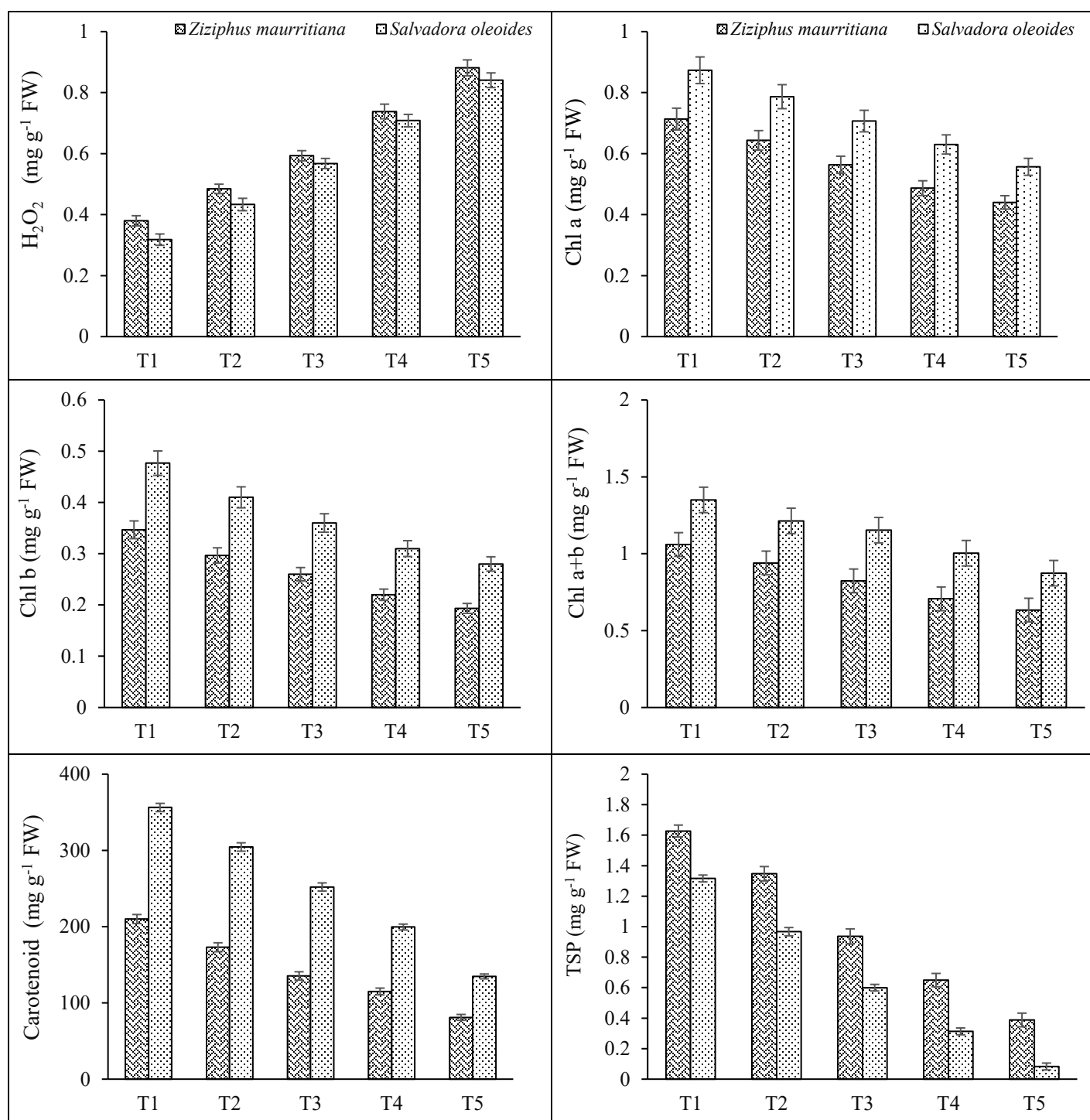
Drought induced adaptations in *Ziziphus mauritiana* and *Salvadora oleoides*

Figure 3.3: Photosynthetic activity in *Z. mauritiana* and *S. oleoides* under water deficit environment

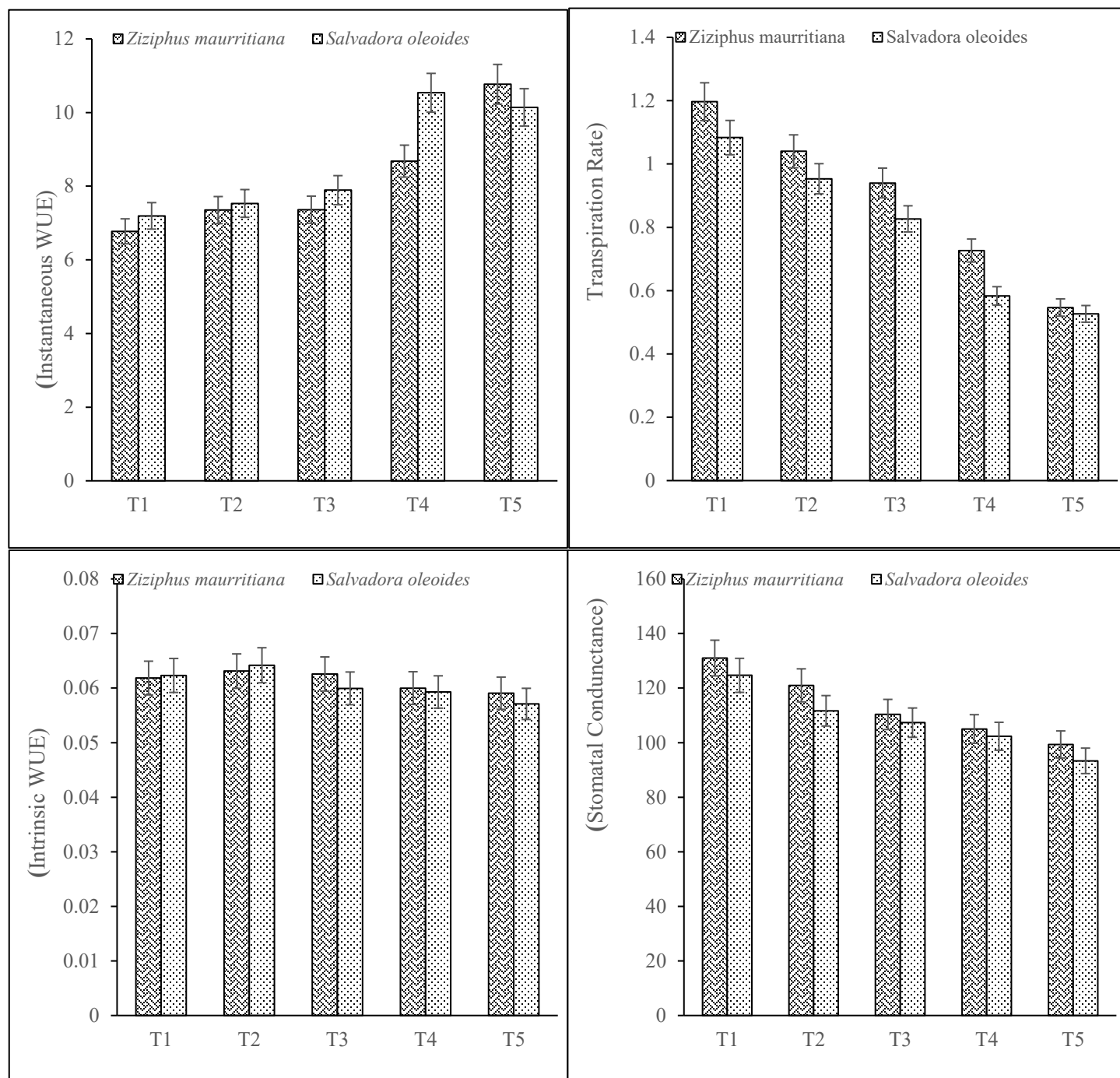
Drought induced adaptations in *Ziziphus mauritiana* and *Salvadora oleoides*

Figure 3.4: Physiological activity in *Z. mauritiana* and *S. oleoides* under water deficit environment

Discussion

4.1: Effect of drought stress on growth behavior

Drought is considered a major environmental factor that directly affects the physiological and morphological characteristics of plants (Naghmouchi *et al.*, 2021). These physiological, morphological, and biochemical responses to abiotic stress affect the distribution of *Z. mauritiana* and *S. oleoides* across arid and semi-arid ecosystems (Dávila *et al.*, 2022, Brown *et al.*, 2002). The results of the current study demonstrated that the overall plant height and stem diameter of *Z. mauritiana* and *S. oleoides* at different stress levels ranged from 18.66 -35.33 cm and 8.34 -7.81 mm respectively. Reduction in growth was the first behavior of plants toward water deficit stress to maintain the balance between metabolite synthesis and energy utilization for biomass synthesis and osmotic adjustment (Todaka *et al.*, 2015). Our study results across water deficit conditions depicted that reduction in growth in both species was an energy conservation strategy against water deficit conditions. With increasing water deficit conditions plant height along with stem diameter in both plant species significantly decreased. As, water deficit conditions cause impaired cell elongation, mitosis, and cell expansion which results in the decline of plant growth (Hussain *et al.*, 2008). In aerial parts of plants such biomass synthesis reduction was related to a decline in meristematic activity, and it was governed by lower leaf turgor pressure that resulted in decreased cell elongation (Muller *et al.*, 2014). Similarly, shoot and root fresh weight along with the dry weight of both species significantly reduced with increasing water deficit conditions as the moisture content of shoot and root was significantly decreased across treatments of drought stress. Such negative changes in growth traits caused the loss of turgor pressure because of a reduction in plant water potential along with a reduction in cell expansion and division (Mguis *et al.*, 2012). Reduction in shoot and root weight, plant height, and leaf were characteristics which mostly used as key traits to assess the water scarcity tolerance in plants (Farooq *et al.*, 2009). Our findings indicated that overall shoot and root fresh weight along with dry weight for *Z. mauritiana* at different stress levels ranged from 49.66 -43.66 g and 28.33 -11.66 g and for *S. oleoides* at different stress levels ranged from 37.33 -20.66 g and 25.33 -8.66 g respectively. Likewise, the total biomass of the plant was reduced significantly with increasing water deficit conditions which was a direct result of stomatal constraint (Flexas and Medrano, 2002; Drake *et al.*, 2018). Our findings revealed that the overall total biomass of *Z. mauritiana* and *S. oleoides* at different stress levels ranged from 53.66 to 20.33 g. The mechanism of stomatal closure inhibits water stress and at the same time through CO₂ intake limitation decreases biomass accumulation (Chaves and Oliveira, 2004).

4.2: Effect of drought stress on enzymatic activity

Plants require well-developed enzymatic along with non-enzymatic scavenging systems which function together to control the uncontrolled oxidation flow under different abiotic stress. By scavenging ROS protects the plant cells from oxidative impairment. Commonly, water deficit stress results in the overproduction of ROS which destroys the cell metabolism by oxidative damage of membrane protein, lipids as well as DNA (Kaushal and Wani 2016). Its synthesis takes place in various cell organelles like mitochondria, peroxisome, chloroplast, vacuoles, and various types of plant membranes (Kohli *et al.*, 2019). Various enzymatic oxidants include SOD, MDA, CAT, and POD which efficiently decrease the stress-induced reactive oxygen species accumulation (Zhao *et al.*, 2020; Hasaman *et al.*, 2020). The SOD and POD are major components of antioxidant defense or scavenging systems. SOD catalyzes the dismutation of O₂⁻ into H₂O₂ and oxygen (Saker and Oba, 2018). POD plays a prominent role in catalyzing hydrogen peroxide into water and oxygen (Liu *et al.*, 2019). The results across water deficit conditions demonstrated that SOD and POD values in *Z. mauritiana* and *S. oleoides* increased with increasing water deficient conditions and their values overall ranged between 1.80 - 0.57 mg⁻¹ protein and 5.81 - 1.22 mg⁻¹ protein respectively. The enrichments of these enzymes protect the chloroplast from eliminated superoxide and ROS (Sarkar *et al.*, 2018). Our results about drought stress showed that: CAT and MDA values in both species increased with increasing water deficient conditions which overall ranged between 1.93-0.073 mg⁻¹ protein and 0.039 - 0.010 mol g⁻¹ FW respectively. The CAT are heme-containing tetrameric enzymes that in coordination with SOD and POD use hydrogen peroxide as substrate and convert it into oxygen and water (Szalai *et al.*, 2021). Our findings across water deficit conditions demonstrated that levels of phenolic contents in both species increased with increasing water deficit conditions and ranged from 358.41-129.32 mg g⁻¹ FW. Among whole of the antioxidant enzymes catalase has one of the giant turnover rates: it's one molecule that can transform six million molecules of hydrogen peroxide (H₂O₂) into oxygen along with water per minute. Therefore, CAT is prominent in eliminating hydrogen peroxide which is synthesized by oxidase in peroxisome involved in purine metabolism, photorespiration, and β -oxidation of fatty acids (Gill and Tuteja 2010). According to Blokhina *et al.*, (2003) under water deficit conditions, lipid peroxidation is an indicator of oxidative impairment. MDA plays a prominent role in membrane lipid peroxidation. In lipid peroxidation evaluation pattern for *Zizyphus* species agrees with various species including *Salvadora* species treated with water scarcity (Bacelar *et al.*, 2006; Turkan *et al.*, 2005; Hassine and Lutts 2010). Several researchers have demonstrated the role of MDA in the inhibition of lipid peroxidation which is dependent on its ability to react with lipid alkoxyl radicals and lipid peroxy as well as interrupt the peroxidation chain (Li *et al.* 2012; Zhang *et al.* 2013; Sarropoulou *et al.*, 2012; Kabiri *et al.*,

2018). Phenolic compounds also act as antioxidant scavengers because of their high reactivity as electron or hydrogen donors for free radicals and therefore, perform a chain-breaking function to stabilize the unpaired electron (Huang *et al.*, 2005).

4.3: Effect of drought stress on photosynthetic activity

Photosynthetic pigments are major components that inhibit the rate of photosynthesis in specific cases. Our findings across water deficit conditions demonstrated that chlorophyll a, b, a+b, and carotenoid pigments decreased with increasing water deficit conditions in *Z. mauritiana* and *S. oleoides* and overall values at different stress levels ranged from 18.36-3.45 mg g⁻¹ FW, 12.76-0.60 mg g⁻¹ FW, 29.04 -6.78 mg g⁻¹ FW and 2.49 - 2.02 mg g⁻¹ FW respectively. Mafakheri *et al.* (2010) suggested that a decrease in chlorophyll content reduces the ability to capture light, ultimately leading to increased ROS production. The decline in chlorophyll content under water deficit treatment may be due to the decrease in pigment biosynthesis due to the inhibition of the activity of some enzymes, such as protochlorophyllide reductase and δ -aminolevulinic acid dehydratase (Bankaji *et al.*, 2014). Our results demonstrated that chlorophyll a and chlorophyll b levels significantly decreased with increasing water deficit conditions. The reduction in chlorophyll b is higher than chlorophyll a in *S. oleoides* and the decline in chlorophyll a is higher than chlorophyll b in *Z. mauritiana*. Carotenoids are accessory pigments that function as light-harvesting compounds and they also function as antioxidant defense systems by having the ability to scavenge singlet oxygen, and lipid peroxy-radicals as well as suppress superoxide synthesis and lipid peroxidation under water deficit conditions (Basma *et al.*, 2009). They act as lipophilic antioxidants which can purify various types of reactive oxygen species (Arora *et al.*, 2013). Reduction in photosynthetic pigments under water stress was also reported by Luffa Singh *et al.* (2013), who performed hydroponic experiments to investigate whether the exogenous addition of nitric oxide (NO) in the form of sodium nitroprusside (SNP) reduces arsenic (As) toxicity in *Luffa acutangula*.

Water deficit conditions induce significant impacts on the accumulation of total soluble protein. The proteins are involved in various processes, including altering plant metabolism and triggering plant defense network signaling under stress conditions (Zandalinas *et al.* 2017; Sarkar *et al.*, 2016). Our results across water deficit conditions demonstrated that total soluble protein decreased with increasing water deficit conditions and its overall values ranged between 1.62 -0.082 mg g⁻¹ FW. The decrease in soluble proteins during drought stress is likely due to a rapid decline in photosynthesis. Under water deficit conditions protein synthesis as well as photosynthesis significantly reduced or even stopped as no material is provided for protein synthesis (Singh *et al.*, 2020). The progressive decrease in total soluble protein due to water deficiency in plants is caused by proteolysis using amino acids released during the osmotic adaptation of plants. This fact suggests that recovery of this parameter is slow, probably because protein synthesis is dependent on other nitrogenous compounds (Costa, 2009). Our results are consistent with those of (Iqbal and Bano, 2009) who researched changes in membrane stability indices of antioxidant enzymes and seed proteins due to water stress. Some researchers stated that under water stress decreasing CO₂ assimilation may related to the restriction of CO₂ diffusion into the leaf, it also causes the inhibition of biochemical processes including Rubisco activity and ATP synthase (Singh *et al.*, 2020).

Conclusions

Drought causes the alternations in physiological, biochemical, and morphological responses of plants mainly by limiting the water content which leads to the closure of stomata and causes reduction in photosynthesis along with carbon assimilation. It declines stem extension, leaf size, meristematic activity, root proliferation, and reduction in water use efficiency. It also reduces the photosynthetic pigments, nutrition uptake as well and gas exchange which results in to decrease in plant productivity and growth. Furthermore, drought stress induces the overproduction of free radicals in the form of ROS species which result in oxidative stress leading to cell damage and death. Oxidative stress causes lipid peroxidation of the cell membrane which makes the cell membrane more permeable. However xerophytic plants against drought stress have developed different mechanisms that help them to grow very well under drought stress. Our results suggested that *Z. mauritiana* performs better growth as compared to *S. oleoides* against water deficit conditions.

Acknowledgment

The authors would like to thank the Institute of Forest Sciences and the Cholistan Institute of Desert Studies, The Islamia University of Bahawalpur, Pakistan for providing technical support and research facilities.

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