Pak. J. Bot., 55(SI): 41-51, 2023. DOI: http://dx.doi.org/10.30848/PJB2023-SI(5)

INNATE STRATEGIC POTENTIAL OF OLIVE TREES TO MITIGATE DROUGHT IN WATER DEFICIT REGIMES

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Abstract

Olive is becoming most interesting for cultivation in arid and semiarid areas owing to its economic significance, drought tolerance, and capacity to withstand shallow poor-quality soils beyond the Mediterranean Region. The study area was not included among the suitable areas for olive cultivation during the first phase in Pakistan. The evaluation of olive germplasm under water deficit at new climatic zones has become a dire need of the moment under the climate change scenario. The present study was designed to evaluate the natural adaptability potential of olive cultivars of diverse geographical origins to water scarcity in the study area. Four selected cultivars (BARI-1, Ottobratica, Leccino, and BARI-2) were grown under hotter and dryer climates and subjected to four water deficit levels (25%, 50%, 75%, and 100% field capacity). The effects of olive cultivars, water deficit levels, and their interactions on the following parameters were estimated using standard protocols. The extent of all considered parameters was diversely affected and significantly different among the cultivars except chlorophyll contents. The highest amounts of TFA, TSS, POD, and APX were found in BARI-1, followed by Leccino, Ottobratica, and BARI-2. Leccino was better than BARI-1 in proline, SOD, and CAT, while BARI-2 occupied the lowest rank. The highest protein contents and chlorophyll were found in BARI-2 and Ottobratica, respectively, compared to others. The pattern of variation among all the above parameters was highly significant and directly correlated with the deficit levels except protein contents. TSP decreased with increasing water deficit, while all other parameters increased with increasing water stress. There was no significant difference between 75% and 50% FC levels in chlorophyll contents. The highest value of proline and the augmented activities of POD and APX were found in Leccino at 25% FC, while CAT activity in the same cultivar was higher at 75% FC. 25% FC announced the utmost parts of TFA, TSS, and SOD activity in Bari-1. 75% FC showed the highest rate of TSP in Bari-2 and chlorophyll contents by 75% FC in Ottobratica. Generally, based on observations, all studied cultivars seem to be drought tolerant, while Leccino, Bari-1, Bari-2, and Ottobratica were more tolerant from one another, respectively, and may survive under water deficit conditions (25% FC) successfully at the climate of the study area.

Key words: Drought stress, Antioxidant, Climate change, Olive culture, Ottobratica, Leccino.

Introduction

Unfavorable environmental factors commonly impact plant performance in agricultural and wild contexts (Bandurska, 2022). According to Cramer *et al*., (2011), just 3.5% of the world's land area is unaffected by ecological restrictions. As a result, plants are often subjected to abiotic stressors that hinder agricultural output and interfere with healthy growth (Raza *et al*., 2019). Drought is typically associated with high evapotranspiration, which causes continual loss of water through transpiration. In many parts of the globe, it is regarded as the most prevalent climate-related limitation (Raza *et al*., 2019; Rojas, 2020). Drought causes a decrease in water content (water deficit), which causes stress (strain) in plant tissues, which has a detrimental influence on plant physiology, proliferation, reproductive, and agricultural productivity (Claeys & Inzé, 2013).

The degree of dehydration is influenced by the length and intensity of the stress as well as adaptive characteristics that prevent water loss (such as smaller sized leaves, leaves are cuticular or tomentose, as well as folding leaves) and promote water absorption from deeper soil layers (extensive, vertically oriented root system). Stomatal behavior in crassulacean acid (CAM) plants, which have an alternate pathway of carbon absorption that happens throughout the night (Lambers *et al*., 2008; Taiz & Zeiger, 2010), is another illustration of an adaptive characteristic preventing water loss.

Plants have been compelled to evolve special adaptation features and the capacity to adapt (acclimate) to unfavorable environments since they are stationary creatures. Morphological, architectural, and physiological features that promote growth under challenging circumstances are examples of adaptation. The process of acclimatization, also known as hardening, involves biochemical, physiological, and structural changes that enable adaptation to new ecological circumstances. It should be differentiated from adaptability, which often refers to qualities shaped by evolution and genetically determined. Plant plasticity, which controls various intricate molecular and cellular responses, such as changes in hormonal balance and transcriptional regulation, determines a plant's capacity to adapt (Lambers *et al*., 2008). The plant's reaction is influenced by the length and intensity of the stress element and by genetic characteristics that govern how effectively a plant can withstand stress. Plants may be in a condition of eustress or distress dependent on the intensity and length of the pressure (Bandurska, 2022). A small amount of stress induces a mild strain (eustress), which sets off reactions that aid in coping with hazardous situations. According to (Hideg *et al*., 2012), stress brought on by a large dosage of stressors rapidly induces a stressful state in plants, resulting in physiological instability, death, or activation of defense mechanisms against stress damage. The condition of distress in plant tissues is impacted by harmful environmental variables, which generates a signal that sets off metabolic processes that lead to resistance, such as avoidance and/or endurance mechanism.

A complicated feature that results from modifications at the molecular, physiological, and metabolic levels is plants' resilience to stress stimuli. It allows plants to thrive, recuperate, and reproduce even in challenging circumstances (Bandurska, 2022). These pathways are crucial in natural biological resistance (stress survival). Although these mechanisms are frequently activated, which is crucial and essential to plant users, they often do, nor prevent drought's damaging effects on growth and yields (agricultural resistance). Therefore, there is a need for ongoing, in-depth study that will increase the knowledge needed to develop agricultural genotypes with high yields and resistance to drought (Bandurska, 2022).

Olive (*Olea europaea* L.) is an evergreen water-tolerant plant. The increased understanding of the nutritional and medicinal benefits of olive fruit and oil-bioactive ingredients has contributed to the recent surge in olive culture (Valente *et al*., 2020). The olive tree is suitable for the Mediterranean-type climate, where plants are frequently subjected to protracted dry periods throughout the growth season (Khalil & El-Ansary, 2020). Olives' metabolic and nutritive value are affected by strategies of olive trees responding to climate change-related occurrences, which are becoming more common in the Mediterranean (Valente *et al*., 2020). Because water stress can have a damaging impact on plant development and agronomic yield, it is recognized as one of the significant challenges impacting olive tree growth (Ahmadipour *et al*., 2018). Water is critical, most common limiting factor for plant dispersion, survival, and agronomic production, with drought and restoration responses; essential to their viability and productivity. Olive is a well-adapted plant to recurring drought occurrences, but at a high cost to carbon stocks and CO_2 supply (Brito *et al*., 2020). Screening of drought-tolerant olive cultivars has become essential for developing sustainable dry-land agriculture. Nonetheless, despite the critical need for these instruments in the current climate of global change, physiological indicators connecting drought resistance with mechanistic consequences functioning at the cellular level remain absent, particularly under severe stress (Baccari *et al*., 2020).

The olive is becoming most interesting for cultivation in arid and semiarid areas owing to its economic significance, drought tolerance, and capacity to withstand shallow, poor-quality soils beyond the Mediterranean Region. The International Olive Oil Council (IOOC) recognized the recent introduction of olives in Pakistan. Still, the study area was not included among the suitable areas for olive cultivation during the first phase in Pakistan (Cima & Urbano, 2008). The study area, including the vast desert of Cholistan at Bahawalpur, may be a potential area for the cultivation of olives and may play an essential role in reducing the import bill of edible oil if explored and provided with suitable irrigation facilities. The evaluation of olive germplasm under water deficit at new climatic zones has become a dire need of the moment under the climate change scenario. The present study was designed to evaluate morphological and physio-biochemical mechanisms responsible for the adaptability of four olive cultivars, particularly to a harsh set of environmental conditions and the suitability of Olive Plant Genetic Resources (OPGR) under hotter and dryer climate of Bahawalpur under severe water deficit conditions.

Materials and Methods

Study site: The study was performed at the experimental site of the Department of Botany, Faculty of Chemical and Biological Sciences, Baghdad-ul-Jadeed campus, The Islamia University of Bahawalpur at Bahawalpur, Punjab, Pakistan. The site was located at 29°22'44.5"N 71°45'45.2"E (Google Maps 2022), 401-421 feet above sea level (Pakistan topographic map, elevation, relief 2022).

Climatic conditions of the study area: The study region is one of the warmest and driest in Pakistan, with average yearly temperatures of 27.16ºC, summer monthly average temperatures of 35.42ºC, and winter average monthly temperatures of 18ºC. The hottest months are May and June, with an average maximum temperature of 48.5ºC, sometimes exceeding 50ºC, and average night temperatures of 27ºC. Similarly, the most incredible months are December and January, when temperatures can drop below 7ºC, with occasional ground frost and minimum night temperatures up to 03ºC. Mean annual rainfall varies between 150 to 200 mm with 2-3 years of prolonged drought interruption; nevertheless, an overall favorable environmental condition is expected for vegetation growth, characteristic of the arid zone. The wettest months are July and August, with a maximum mean precipitation of 55 mm; the driest months are November and December, with an average minimum rainfall of 1.5 mm. The wind movement is South-Western in the summer and North-Eastern in the winter (Fig. 1).

The mean monthly wind speed ranges from 7 km/h in October through December to 13 km/h in June-July, with a minimum rate of 3 km/h in Oct-Dec. to a maximum speed of 21 km/h in June (Meteoblue, 2022b). The maximum monthly average humidity was noted to be 38 percent during December. On the other hand, the lowest humidity was worth 16 percent in May, with the highest rainy days (8.5) in July and the lowest rainy days (0.4) in November. The longest days were found in June (avg. 14 h, 0 min) and shortest in December (Avg. 14 h, 18 min), with the most average sunshine (12 h, 06 min) in May to the least (7h, 30 min) in January. The month with the most sunshine days was December (30.7 days) to the least sunshine days (20.1 days) in July. The highest UV index (UVI) was experienced in April-July (UVI 9) and lowest in December and January (UVI 5), with maximum cloud cover (19%) in August to minimum cloud cover (03%) in October and 10 km visibility through the year. The average atmospheric pressure in Bahawalpur ranges from 1018 mbar in January to 996.2 mbar in July (Atlas, 2021).

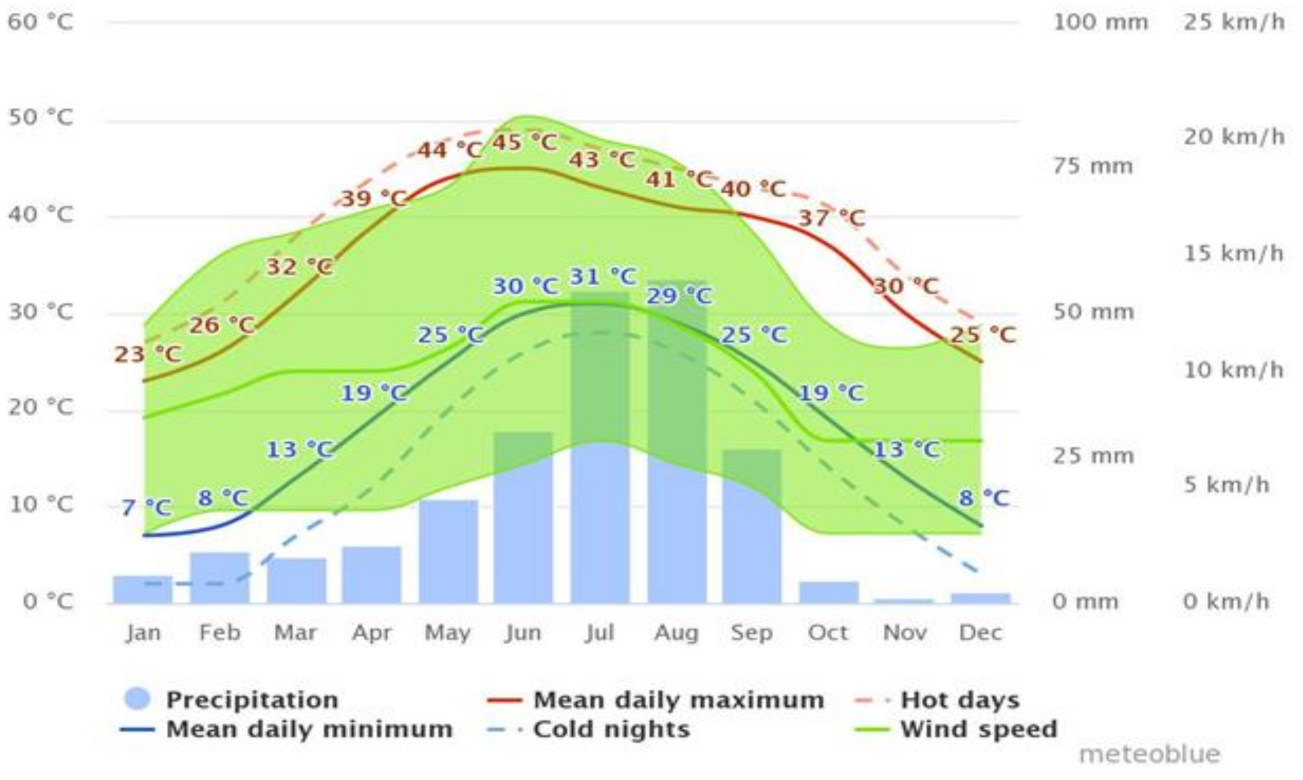

Fig. 1. The maximum daily average (solid red line) and mean daily minimum temperature (solid blue line) at Bahawalpur each month. The standard of the hottest day and coldest night (dashed red and blue lines) of each month during the previous 30 years. (Source: Meteoblue (Meteoblue 2022b).

Five distinct seasons retail in the study area, characterized by varied durations. Monsoon (July and August), Autumn (November), Summer (May to October), Spring (March & April), and Winter (December to February), as reported by (Sukhera & Pasha 1987). The annual Global horizontal irradiation (GHI) strikes with an average intensity of 1923.1 kWh/m^2 and direct normal irradiation (DNI) of 1504.5 kWh/m^2 per year. The monthly direct normal irradiation ranges from 160.518 kWh/m2 in October to 92.101 kWh/m2 in July, and the daily mean ranges from 2.971 kWh/m2 in July to 5.178 kWh/m2 in October (Atlas, 2021).

The soil in the studied region is of the transporting type and was formed from two kinds of parental material: aeolian sandy and clayey alluvium type. Its texture ranges from loamy-sand to sandy-loam to sandy. The soils are saline-sodic with calcareous mass, minimal organic content, and high pH and electrical conductivity (Akram *et al.*, 1986).

The data on temperature and precipitation trends, along with their anomalies for a long average of 43 years (1979-2021) over the larger region of Bahawalpur (30.00 N, 73.25 E), showing the increasing mean annual temperatures with linear climate change trend (-0.1°C anomaly) and declining mean annual precipitation with decreasing trend of 4.8 mm over 43 years (Meteoblue, 2022a). Bahawalpur Division falls under a vast area of Cholistan, keeping in view the Global warming due to industrial shift and climate change; it may be a potential crop area for Olive cultivation under prevailing environmental conditions with adequate water supply (Cima & Urbano, 2008).

Plant material: One-year-old, self-rooted plants of *Olea europaea* L. (Oleaceae) were collected from a standardized nursery farm at the Center of Excellence for Olive Research and Training (CEFORT), Barani Agricultural Research Institute (BARI), Chakwal, Punjab, Pakistan, and included four cultivars from different geographical origins: BARI-1, BARI-2 from Pakistan, Ottobratica and Leccino from Italy. For further research, taxonomically authenticated (Dr. Azhar Hussain Naqvi, senior scientist at BARI) plant material was brought to the Botany Department, Faculty of Chemical and Biological Sciences, the Islamia University of Bahawalpur. Voucher specimens were deposited at the department's herbarium as reference material. Germplasm was kept under natural conditions for 90 days to acclimate to the study area's climate. Some selected morphological and biochemical parameters were investigated to assess the adaptability potential of a typical Mediterranean tree species under water deficit conditions applied at the study area.

Experiment design and treatments application: The experiment was designed as fully factorial with two factors, i.e., 04 most responsive cultivars already screened in another experiment (data not given here), comprising of BARI-1, Ottobratica, Leccino, and BARI-2 under 04 water regimes (100%, 75%, 50%, and 25% field capacity) during summer with 03 replications. Almost one and half years old, self-rooted, Pot-grown (08-inch flexible plastic containing a mixture of sand: soil: peat moss in a ratio 1:1:1) olive trees of selected cultivars with similar canopy, leaf number, and leaf area were subjected to water deficit. The field capacity of the soil mixture was calculated before the stress application initiation and maintained at 100%, 75%, 50%, and 25% throughout the experiment. Standard soil weight was multiplied by the value of field capacity (%) to calculate the amount of water to be added to each treatment. The weight of each plant before transplantation was also estimated after determining the amount of water, such that the total weight of the containers comprised soil, plant, pot, and water. Four plants for each treatment were kept separate for destructive calculation of live plant weight (as plants were gaining weight due to continuous growth) fortnightly to standardize the amount of water to be added to each treatment for maintaining the required field for each treatment (Imakumbili, 2019). All plants were weighed daily each evening to estimate the amount of water that transpired throughout the study period, according to Sofo *et al*., 2005; Petridis *et al*., 2012. Accordingly, soil water contents were maintained by adding the measured quantity of water that transpired during the daytime. The deficit treatments were carried out for eight weeks, at the end of 2nd month, leaves were gathered from robust, current-year, sun-exposed foliage that was completely grown for mentioned biochemical parameters, and data for other morphological parameters were recorded. The current experiment was limited to the summer period (June-July), as drought and temperature stresses are mostly confined to this part of the year. The effects of Olive cultivars, water deficit levels, and their interactions on the following parameters were estimated using standard protocols.

Measured traits: At the conclusion of the trial, leaves were sampled from various treatments, collected, prepared, and transported to the laboratory for the evaluation of selected features. Different biochemical parameters such as total free amino acids (TFA), total Proline, total soluble proteins (TSP), total soluble sugars (TSS), and four antioxidant enzymes enumerating, Catalase (CAT), Peroxidase (POD), ascorbate peroxidase (APX), Superoxide Dismutase (SOD) activities and Chlorophyll contents (SPAD values), were estimated according to standard protocols as given in the following paragraphs.

Collection, storage, and processing of leaf samples for extraction: Fully expanded, sun-exposed, healthy, and mature current-year fresh leaves were randomly collected from each cultivar, packed in zip polybags, and labeled for each treatment on the same day. The collected leaves of the same age (to avoid the error) were cleaned with distilled water and stored at -80ºC for further extraction and analyses.

Determination of biochemical parameters: The ninhydrin test was used to determine the total free amino acid concentration, according to šircelj *et al*., (2005). To create the standard curve, ethanol (80%) was used as blank, and leucine as a common normal amino acid. The Bates *et al*., (1973) protocol was opted to estimate the proline contents. D-Proline as a standard curve, and toluene was employed as a blank. The amount of total soluble proteins (TSP) was calculated by Bradford (1976), and bovine serum-albumin was used as standard. The procedure for extracting and quantifying total soluble sugars (TSS) was followed as described by Buysse & Merckx (1993). The results were expressed as milligram or microgram per gram fresh weight. A compact portable chlorophyll meter (SPAD-502), manufactured by Konica Minolta Sensing Korea Co., Ltd., was used to measure chlorophyll from different deficit treatments. Ten mature, sun-exposed, fully developed leaves from the canopy of each replicate were randomly measured for chlorophyll contents.

Antioxidant enzymes assays: APX activity was estimated using spectrophotometer (Nakano & Asada, 1981). The SOD activity was determined as described by Giannopolitis & Ries (1977). Catalase activity was evaluated by the Aebi’s method (Aebi, 1984). The peroxidase activity (absorbance) was estimated as by Herzog (1973). The extraction of enzymes was carried out at 4°C (Yucel *et al*., 2014) and results were expressed in unit per milligram fresh weight.

Statistical analysis

To summarize the primary data, MS Excel 2017 was used. Two-way complete factorial analysis of variance (ANOVA) was performed using Statistix 8.1(Statistix8.1, 2003), and multiple comparisons of means by applying the least significant difference (LSD) test were done at alpha 0.05. A multivariate test for principal component analysis (PCA) was performed using the software Minitab-19 (Tombesi *et al*., 2019) to reveal observations' score plot and the variables' loading plot.

Results

Biochemical compounds and antioxidant enzymes expressed among various olive cultivars: Biochemical parameters of various Olive cultivars expressed under water deficit managements are given in Fig. 2. Least significant test (LSD) is a post-hoc test after ANOVA (Table 1) to sort out significantly affecting treatment (factor); it uses pairwise comparison among treatments. Value for multiple comparisons using a t-test was calculated to identify the significant difference. The letters (A, B, C, etc.) represent different groups, among which the means differ significantly. Selected olive cultivars diversely expressed various biochemical parameters discussed under water deficit levels. All parameters' extent was significantly different among the cultivars except APX and chlorophyll. The highest amounts of TFA, TSS, and POD were found in BARI-1, followed by Leccino, Ottobratica, and BARI-2, while no significant difference between Leccino and BARI-1 in APX activity. Leccino was better than BARI-1 in terms of Proline, SOD, APX, and CAT, while the other two cultivars also occupied the same ranks as above for these parameters. The highest soluble protein and chlorophyll contents were found in BARI-2 and Ottobratica, respectively, whereas the Leccino showed the lowest values of both parameters. A significant difference in chlorophyll contents was present between BARI-1 and BARI-2 (Fig. 2A-I).

Table 1. Mean squares of biochemical parameters for four varieties of *Olea europaea* L. under four water deficit treatments.

Source	df	APX	TFA	Catalase	Peroxidase	Proline	TSP	SOD	TSS	Chlorophyll
Variety	3	41.1131***	139.357***	4672.69***	6388.73***	19945.7***	17.0048***	3768.81***	110.710***	983.46***
Treatment	3	8.1373***	24.532***	772.7***	544.33***	3185.69***	2.9836***	601.62***	20.075***	106.026ns
Variety*Treatment	9	1.4893***	8.279***	235.86***	135.64***	1225.19***	1.2238***	150.90***	3.209***	197.391*
Error	32	0.0100	0.010	0.01	0.01	0.01000	0.0100	0.01	0.010	65.332
CV		1.41	1.41	0.49	0.08	0.07	0.07	1.26	0.08	0.73

Legend: APX = Ascorbate Peroxidase, SOD = Superoxide Dismutase, TFA = Total Free Amino acids, TSP = Total Soluble Proteins, TSP = Total Soluble Sugars *, **, *** data is significant at p<0.05, <0.01, <0.001 respectively, ns = non significant (p>0.05)

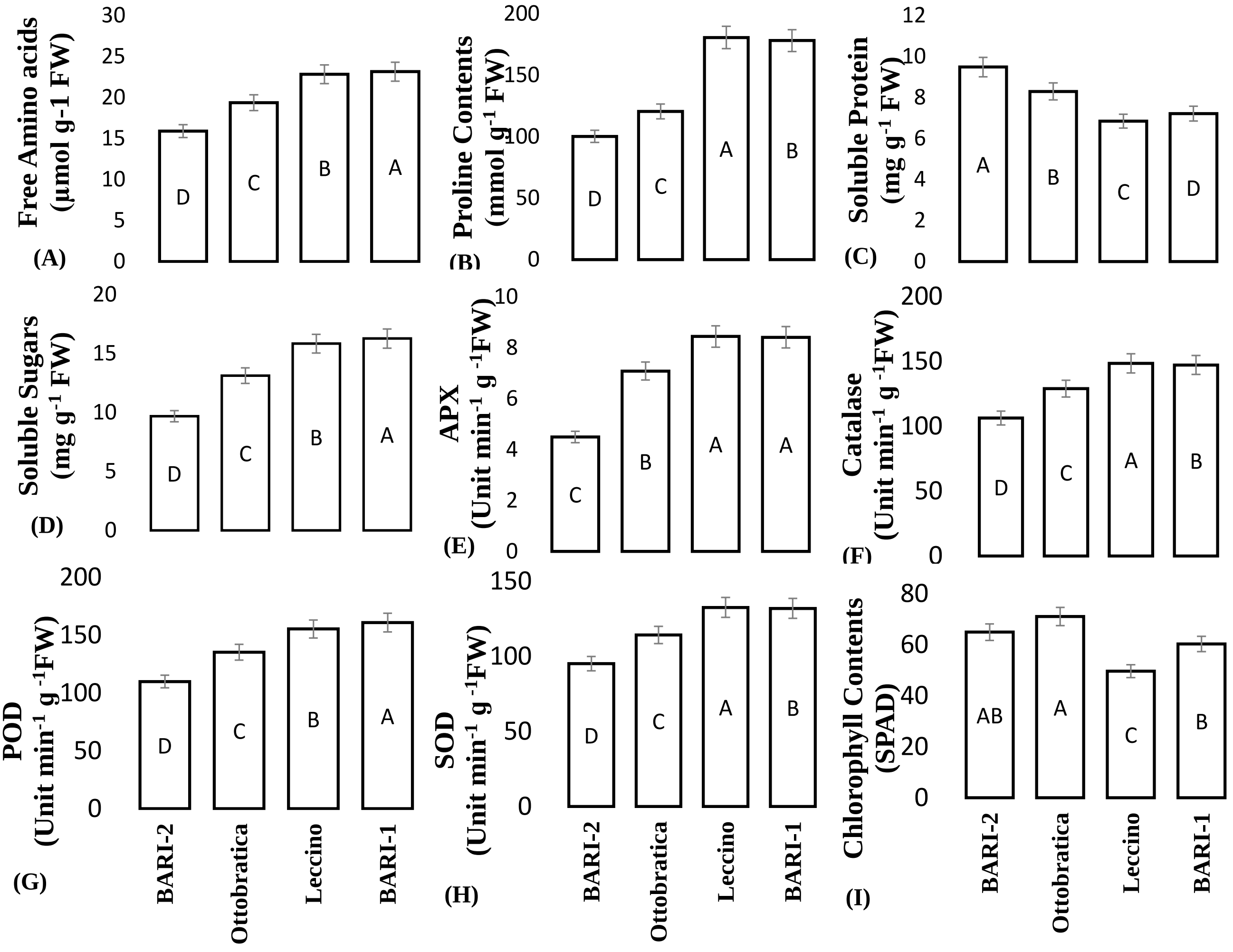

Fig. 2. Bar plots showing mean values (n=3) along with letters obtained after the least significant difference test ($LSD_{0.05}$) of nine studied variables concerning four olive cultivars.

Biochemical parameters in various olive cultivars expressed under water deficit regimes: Water deficit affected various biochemical parameters significantly; as depicted in multiple comparisons, four influential groups are visible with different letters in each parameter except chlorophyll contents. The least significant difference (LSD) test is a post-hoc test after ANOVA to sort out significantly affecting treatment (factor); it uses pairwise comparison among treatments. Value for multiple comparisons using a t-test is calculated to identify the significant difference. Various biochemical parameters discussed were affected diversely by water deficit levels applied in different olive cultivars. The difference between TFA, TSS, APX, free proline contents, Catalase, POD, and SOD activity, was highly significant among various water deficit treatments. The pattern of variation among all these parameters was directly correlated with the deficit levels; with increasing drought stress levels, the values of all above-mentioned parameters were also increased regularly (Fig. 3A-H).

Various water deficit treatments significantly affected all studied parameters among all selected cultivars except chlorophyll. The pattern of variation among all these parameters was directly correlated with the deficit levels except protein contents. Total soluble protein contents were decreased with increasing water deficit, while all other contents were increased with increasing water stress. A significant difference was not seen between 75% and 50% FC levels in Chlorophyll contents. The highest values of all mentioned traits were expressed under the lowest field capacity level (25%) and decreased along the increasing moisture gradient, while protein articulated the opposite trend (Fig. 3I).

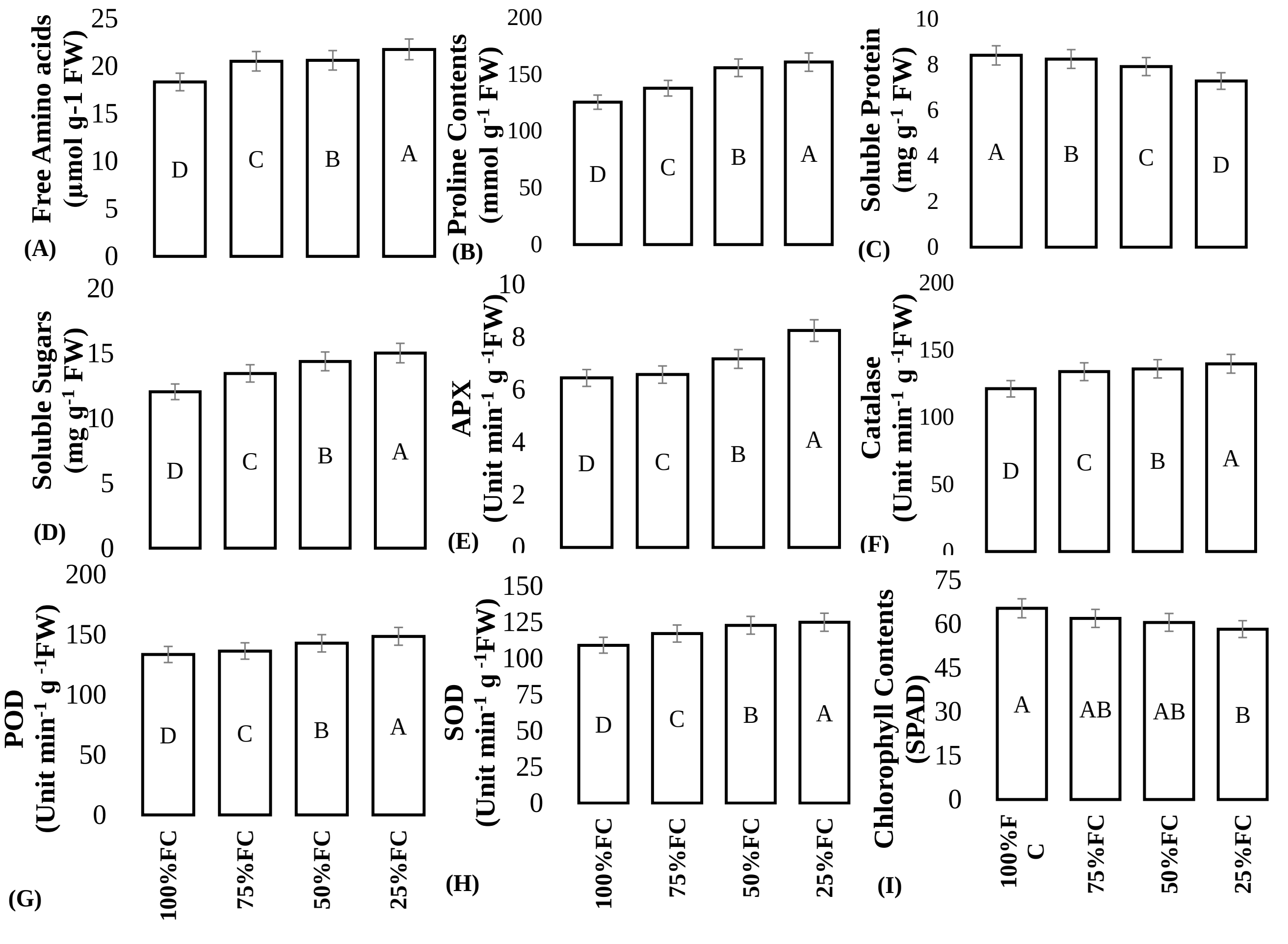

Fig. 3. Bar plots showing mean values (n=3) along with letters obtained after the least significant difference test ($LSD_{0.05}$) of nine studied variables concerning four deficit levels.

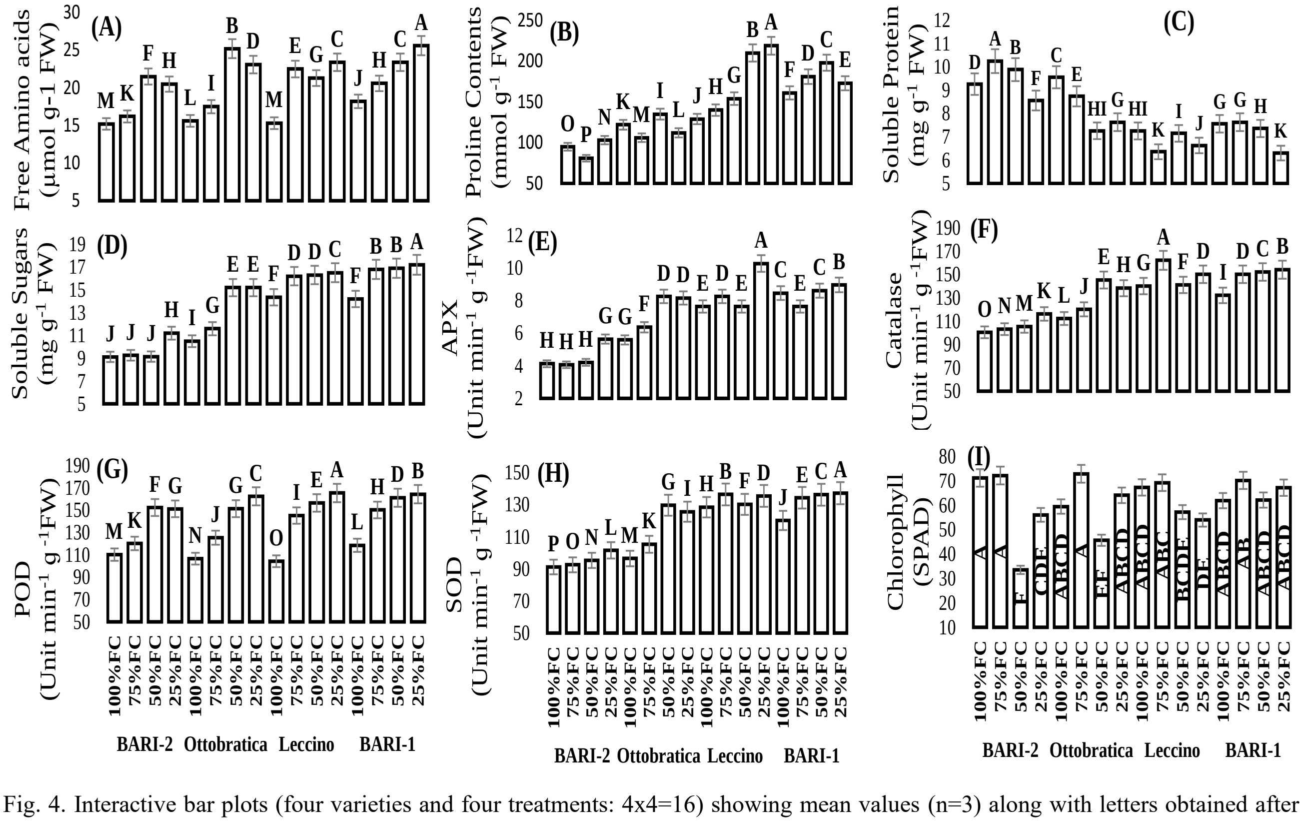

Fig. 4. Interactive bar plots (four varieties and four treatments: 4x4=16) showing mean values (n=3) along with letters obtained after the least significant difference test ($LSD_{0.05}$) of nine studied variables based on fresh weight.

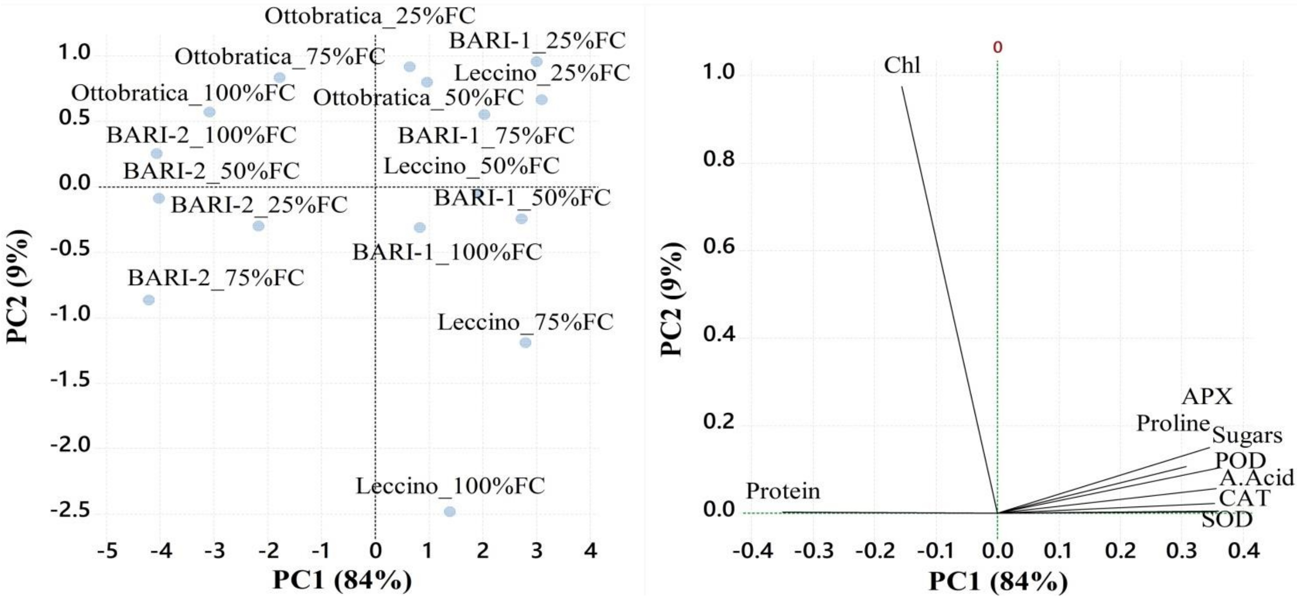

Fig. 5. Loading plot of variables (left) and score plot of observations (right) revealed through principal component analysis (PCA).

Selected biochemical parameters and antioxidant enzymes in various Olive cultivars expressed under interactive effects of variety and water deficit regimes: Interaction among four varieties and four treatments exhibited sixteen observations with various groups after $LSD_{0.05}$. Different biochemical parameters were affected significantly, as depicted in multiple mean comparisons. The least significant test ($LSD_{0.05}$) is used to sort out significantly affecting treatment (factor); t-test-based pairwise comparison was performed among interactive effects of varieties and treatments to identify the significant differences. Thirteen distinct groups of total free Amino Acids, sixteen of Proline, twelve in the amount of total soluble Protein, ten in total soluble Sugars, eight in APX activity, fifteen in value of Catalase and POD activity, sixteen in SOD activity and nine distinct groups in the value of Chlorophyll contents (SPAD) were recognized. Olive cultivars differently expressed the behavior of different biochemical parameters under water deficit levels under study. No regular trend under the desired deficit levels can be found for all cultivars (Fig. 4A-H).

The highest value of Proline contents, APX, and POD activity was shown by 25% Leccino, and the highest value of Catalase was demonstrated by 75% of the same genotype. The lowermost values of these parameters were confined to various deficit levels, mostly 100% FC in BARI-2 except proline (75% FC) in the similar cultivar. In comparison, 100% FC gripped lowest POD movement in Leccino. A significant difference was not found from 100% to 50% FC in BARI-2 for APX activity. The maximum amounts of TFA, TSS, and SOD activity were shown by 25% Bari-1. Here also, 100% FC in BARI-2 gripped the lowest rank for these parameters, and no significant difference was established by the same deficit level in Leccino as well as 100% to 50% FC in BARI-2. The highest rate of total soluble Protein was demonstrated by 75% Bari-2, and the lowest place was occupied by 25% BARI-1 and 75% Leccino, with no significant difference between them. 75% FC revealed the highest value of Chlorophyll contents in Ottobratica, and any significant difference was not seen between 100% and 75%FC in BARI-2 as compared to Ottobratica, while the lowest level was expressed by 50% FC in BARI-2 in terms of SPAD values (Fig. 4A-H).

Principal component analysis (PCA): The first principal component (PC1) represents 84% of the total variation. The PC1 was strongly correlated with eight of the variables. The PC1 increased with increasing Sugars, SOD, CAT, POD, TFA, APX, and Proline, and decreasing Protein scores. This suggests that these eight variables vary together. If one increases, the left ones try to uplift as well, except protein which is negatively correlated and tends to show the opposite behavior to that of the other seven variables. This component can be viewed as a measure of the quality of Sugars, SOD, CAT, POD, Amino Acids, APX, Proline, and decreasing Protein (recall that declining values for protein means increasing stress level). Furthermore, we noted that PC1 was strongly correlated with the sugars and SOD. It may be stated based upon the correlation of 0.99 that PC1 is primarily a measure of the sugars and SOD, followed by CAT and POD with a correlation of 0.98, respectively (Table 2). It would follow that parameters with high eigenvalues tend to have more variation explanation about the olive cultivar behavior against water deficit conditions. At the same time, parameters with small values would have very few of these types of information. The second principal component (PC2) represents 9% of the total variability. The PC2 explained variation in chlorophyll contents. This component can be viewed as a measure of how water deficit affects the amount of chlorophyll among various olive cultivars. The association of cultivars under different field capacity conditions was classified by PC1 and PC2 (Fig. 5).

Table 2. Eigenvalues of six principal components (a) and eigenvectors of first two principal components (b) along with their correlation with variables and percentage contribution in variability.

a)	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	7.56	0.85	0.34	0.11	0.08	0.04
Proportion	0.84	0.10	0.04	0.01	0.01	0.00
Cumulative	0.84	0.93	0.97	0.98	0.99	1.00
b)	**Eigenvectors**		**Correlation**		**% Contribution**	
Variable	**PC1**	**PC2**	**PC1**	**PC2**	**PC1**	**PC2**
Total soluble proteins (TSP)	-0.35	0.00	**-0.96**	0.00	12.32	0.00
Total free amino acids (TFA)	0.35	0.02	**0.97**	0.02	12.53	0.04
Total soluble sugars (TSS)	0.36	0.10	**0.99**	0.10	12.89	1.08
Proline	0.31	0.11	**0.84**	0.10	9.43	1.14
Superoxide dismutase (SOD)	0.36	0.00	**0.99**	0.00	12.96	0.00
Peroxidase (POD)	0.36	0.06	**0.98**	0.05	12.68	0.31
Catalase	0.36	0.00	**0.98**	0.00	12.68	0.00
Ascorbate peroxidase (APX)	0.35	0.15	**0.95**	0.14	11.97	2.28
Chlorophyll	-0.16	0.98	-0.43	**0.90**	2.43	95.06

Discussion

The current research evaluated the natural adaptability potential of Olive cultivars of diverse geographical origins to water scarcity at new agroclimatic zone. Four selected cultivars were grown under hotter and dryer climates and subjected to four water deficit levels. The effects of Olive cultivars, water deficit levels, and their interactions were estimated on selected parameters. ROS are produced as a result of dehydration's disturbance of the respiratory biosynthetic route (Gómez *et al*., 2019; Nadarajah, 2020). ROS (secondary focus) made in excess are detrimental to organelles since they induce lipid peroxidation and damage proteins and nucleic acids. These include superoxide radicals (O2), hydroxyl radicals ($OH^{\bullet}$), singlet oxygen ($1O_2$), and hydrogen peroxide (H_2O_2) (Raza *et al*., 2019). To prevent oxidative damage, plants contain both enzymatic and non-enzymatic ROS-scavenging strategies. Enzymatic antioxidants include CAT, SOD, and POD whereas, non-enzymatic antioxidant system components include ascorbic acid, carotenoids, flavonoids, glutathione, proline, α-tocopherol, and phenolic substances, lower oxidative damage by working with antioxidant enzymes and reducing ROS activity directly (Meena *et al*., 2019; Nadarajah, 2020; Bandurska, 2022). A wheat genotype that displayed osmotic adjustment brought on by the buildup of proline and soluble sugars and improved training of enzymatic and non-enzymatic antioxidants demonstrated a reduced yield-drop under drought circumstances. These modifications enable quick recovery following re-watering and the preservation of high photosynthetic CO_2 assimilation during drought, which are essential for the ultimate production (Abid *et al*., 2018). Osmotic adjustment, which entails the buildup of organic osmotic substances (proline, glycine-betaine, soluble proteins, and carbohydrates) in leaves and roots, is one of the downstream mechanisms involving ABA (dehydration avoidance strategy), that are important for maintaining tissue hydration (Claeys & Inzé, 2013; Takahashi *et al*., 2018). Increasing soluble and active osmotic substances, such as low-molecular-weight proteins (Ingram & Bartels, 1996), carbohydrates (Vijn & Smeekens, 1999), proline (Sairam & Srivastava, 2002; Bacelar, 2006), is thought to be one of the tolerance strategies used by plants under drought stress (Nadarajah, 2020). Leccino and 25% FC had the most proline, whereas BARI-2 and 100% FC contained the least. The maximum concentration of proline was discovered at 25% FC in BARI-1 and the lowest concentration at 75% FC in BARI-2. Olive cultivars exhibit high proline concentration in their leaves due to drought stress; Meski and Chemlali (Ennajeh *et al*., 2006), Bladi, Mary, Roghani, Zard and Mission (Arji & Arzani, 2008), Chetoui, Chemlali and Zalmati (Boughalleb & Mhamdi, 2011) and Konservolia (Elhami *et al*., 2015). Our findings were consistent with those studies that TFA, TSP and proline contents varied according to cultivars and levels of stress.

The quantity of soluble sugars increased greatly depending on the cultivar type; BARI-2 reached the most significant amount at 25% FC irrigation, while BARI-2 at 100% FC irrigation produced the least amount of soluble sugars. When cells are under drought stress, a buildup of soluble sugars, including suitable osmotic substances, lowers their water potential in favour of more water remaining to maintain cell turgor (Boughalleb & Mhamdi, 2011). In several plant species, soluble sugars' accumulations appeared to correlate with drought resistance. Black poplar (Regier *et al*., 2009), mangos (Elsheery & Cao, 2008), and other woody plants have all been shown to accumulate soluble sugars more actively than sensitive cultivars due to increased drought tolerance. Our results agreed with those of (Boughalleb and Mhamdi, 2011), who had made similar observations on the accumulation of carbohydrates in water-stressed olive plants (Ahmadipour *et al*., 2018). The quantity of chlorophyll dramatically decreased in all cultivars during water deficit circumstances. Cultivar differences in chlorophyll content and degrees of stress were also observed. The overall chlorophyll content of cultivars varied significantly; Ottobratica had the highest concentration, while Leccino had the lowest. BARI-2, which was watered at 50% FC, had the least chlorophyll, while Ottobratica, which was irrigated at 75% FC, had the most. While 25% of FC had the lowest level of chlorophyll and 100% of FC had most significant level, loss of chlorophyll is a standard indicator of oxidative stress (Brito *et al*., 2002). According to (Guerfel *et al*., 2009), the Chl (a + b) content of the olive cultivars Chemlali and Chetoui was decreased. Similar outcomes were seen in our study, where chlorophyll loss occurred under water stress but varied by cultivar.

Under water stress, reactive oxygen species (ROS) build-up, and lipid peroxides cause cell damage. Under such conditions, oxidative stressors serve as secondary stress and decrease the stability of cell membranes, the photosynthetic process, and ultimately plant production. Under this regard, POD, SOD, APX, and CAT enzymes mitigate the adverse effects of ROS, enhancing the integrity of cell membranes and promoting plant development in such conditions. In this experiment, the interaction between cultivar and water stress significantly negatively impacted leaf POD, SOD, APX, and CAT activity (p 0.05). Water stress generated more significant and more excellent leaf peroxidase activity in BARI-1, Leccino, Ottobratica, and BARI-2, respectively, and the reaction to water deficit was cultivar dependent. The outcomes of this experiment demonstrated that the activity of the enzymes above, was decreased at 100% FC irrigation while increased at 25% FC irrigation. During normal conditions, there were significant differences in the activity of POD, APX, SOD, and CAT amongst cultivars. BARI-1 had the most fabulous POD and APX, followed by Leccino, Ottobratica, and BARI-2, respectively. Leccino outperformed BARI-1 in terms of SOD and CAT, while BARI-2 had the lowest position. Species or cultivars under drought stress will have substantially varied antioxidant levels and antioxidant enzyme activity (Reddy *et al*., 2004). According to Liu *et al*., (2011), there is a clear correlation between raising the level of oxidative stress and raising the activity of antioxidant enzymes in tolerant cultivars compared to sensitive ones to reduce the harmful effects of oxidative stressors. Our findings corroborated other studies which found that water stress enhanced the POD activity of olive (Amini, 2014; Elhami *et al*., 2015), walnut (Yadollahi *et al*., 2010), GF 677 peach and almond rootstock (Mashayekhi *et al*., 2015), sweet cherry rootstock (Sivritepe *et al*., 2008), and banana (Chai *et al*., 2005).

Since plants use certain enzymes like CAT and POD to deal with drought-stress situations, their activity level may indicate how well-tolerated a stress state is by a plant. POD, SOD, APX, and CAT activity on assessed olive cultivars were examined in this study, and the findings showed that irrigation water volume significantly impacted all enzyme activity during drought treatments compared to control (100% FC). All of the aforementioned enzymes showed a fairly significant pattern of variation, which was strongly connected with the deficiency levels. According to our findings, during water stress, 100%, 75%, 50%, and 25% of FC, respectively, had enhanced activity of POD, SOD, APX, and CAT. Plants with these kinds of enzymes can defend themselves against oxidative damage (Lima *et al*., 2002). In the present study, BARI-1, Leccino, Ottobratica, and BARI-2, all exhibited rising levels of the aforementioned enzyme-activity in addition to deficiency levels, so they also shown a higher level of water stress resistance. The functions of the aforementioned enzymes support protective plant cell metabolite processes, which are crucial for cell survival against oxidative stressors (Jiang & Huang, 2001). Our experiment's findings were consistent with those found in olive (Boughalleb & Mhamdi, 2011; Fouad *et al*., 2014), mulberry (Reddy *et al*., 2004), sweet cherry rootstock (Sivritepe *et al*., 2008), and banana (Chai *et al*., 2005), in which drought stress elevated the POD, SOD, APX, and CAT activities. All of the biochemical characteristics that were considered had varying effects and varied greatly between cultivars, including chlorophyll concentration. BARI-1, Leccino, Ottobratica, and BARI-2 contain the most significant concentrations of TFA and TSS, respectively. Regarding Proline, Leccino outperformed BARI-1, with BARI-2 coming in last. In comparison to other cultivars, BARI-2 and Ottobratica had the most significant protein and chlorophyll concentrations.

Conclusions

Promoting olive farming under the new agroclimatic site was essential to ascertain the olive cultivars among BARI-1, Leccino, Ottobratica, and BARI-2 that are compatible with semi-tropical-desert climates. The results demonstrated that cultivars contrasted in their ability to withstand drought. Except protein contents, the pattern of variation among all of the aforementioned indicators was extremely significant and strongly associated with the shortfall levels. TSP reduced as the water shortage rose, but all other metrics increased as the water stress increased. Between 75% and 50% FC levels, there was no discernible difference in the amount of chlorophyll present. Leccino was shown to have the greatest Proline, POD, and APX activity in 25% FC, but the same cultivar had 75% FC of the most CAT activity. 25% FC in Bari-1 demonstrated the highest TFA, TSS, and SOD activity. In Ottobratica, TSP levels were maximum at 75% FC, chlorophyll levels were highest at 75% FC in Bari-2.

Recommendations

According to assessments, all evaluated olive cultivars were tolerant to water deficit with variable degrees. Ottobratica, Leccino, Bari-1, and Bari-2 were each more tolerant than the others repectively. Therefore, it was suggested that Leccino and BARI-1 may be exlpoited, when there is more scarcity of water at the study area and similar climates.

Acknowledgments

We extend our obligations to CEFORT, BARI, Chakwal, Pakistan, for providing authenticated plant (Olive) material. We are also very thankful to the departments of Agronomy and Entomology, Faculty of Agriculture and Environment for extending their wire houses for setting up the parts of experiments in secure environments. Without these facilities, our study could not be completed in such aa excellent manner.

References

Abid, M., S. Ali, L.K. Qi, R. Zahoor, Z. Tian, D. Jiang, J.L. Snider and T. Dai. 2018. Physiological and biochemical changes during drought and recovery periods at tillering and jointing stages in wheat (*Triticum aestivum* L.). *Sci Rep*., 8(1): 1-15.

Aebi, H. 1984. Catalase in vitro. *Meth. Enzymol.,* 105(C): 121-126.

Ahmadipour, S., I. Arji, A. Ebadi and V. Abdossi. 2018. Physiological and biochemical responses of some olive cultivars (*Olea europaea* L.) to water stress. *Cell. Mol. Biol.*, 64(15): 20-29.

Akram, A., A.K. Wallyat and A.S. Bashir. 1986. Desertification processes in Cholistan desert.

Amini, Z. 2014. Effects of water deficit on proline content and activity of antioxidant enzymes among three olive (*Olea europaea* L.) cultivars. *J. Plant Res.* (*Iranian J. Biol.*)., 27(2): 156-67.

Arji, I. and K. Arzani. 2008. Effect of water stress on some biochemical changes in leaf of five Olive (*Olea europaea* L.) cultivars. *Acta Hortic.*, 79(1): 523-6.

Atlas, G.S. 2021. Solar Radiation Map Bahawal[pur, Punjab, Pakiatan 29.425843, 071.783981, Synergis: The World Bank 1818 H Street NW, Washington DC 20433, USA.

Baccari, S., O. Elloumi, A. Chaari-Rkhis, E. Fenollosa, M. Morales, N. Drira, F. Ben Abdallah, L. Fki and S. Munné-Bosch. 2020. Linking leaf water potential, photosynthesis and chlorophyll loss with mechanisms of photo-and antioxidant protection in juvenile olive trees Subjected to severe drought. *Front. Plant Sci*., 11: 614144.

Bacelar, E.L.V.A. 2006. "Ecophysiological responses of olive (*Olea europaea* L.) to restricted water availability Ecophysiological responses of olive (*Olea europaea* L.) to restricted water availability Limitations, damages and drought resistance mechanisms" Universidade De Trás-Os-Montes E Alto Douro Vila Real, pp. 1-149.

Bandurska, H. 2022. Drought stress responses: coping strategy and resistance. *Plants*, 11(7): 9-22.

Bates, L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil*, 39(1): 205-207.

Boughalleb, F. and M. Mhamdi. 2011. Possible involvement of proline and the antioxidant defense systems in the drought tolerance of three olive cultivars grown under increasing water deficit regimes. *Agric. J.*, 6(6): 378-91.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72(1-2): 248-254.

Brito, C., L.T. Dinis, H. Ferreira, J. Moutinho-Pereira and C.M. Correia. 2020. Foliar pre-treatment with Abscisic acid enhances olive tree drought adaptability. *Plants*, 9(3): 341.

Brito, G., A. Costa, H.M. Fonseca and C.V. Santos. 2002. Response of *Olea europaea* ssp. maderensis in vitro shoots exposed to osmotic stress. *Sci Hortic*., 97(3-4): 411-17.

Buysse, J. and R. Merckx. 1993. An improved colorimetric method to quantify sugar content of plant tissue. *J. Exp. Bot.*, 44(10): 1627-1629.

Chai, T.T., N.M. Fadzillah, M. Kusnan and M. Mahmood. 2005. Water stress-induced oxidative damage and antioxidant responses in micropropagated banana plantlets. *Biol Plant.,* 49(1): 153-6.

Cima, R.D.E.L. and F. Urbano. 2008. Selection of Suitable Areas for Olive Growing in Pakistan Selection of Suitable Areas. Nova Arti Grafiche Signa, Firenze, Italy.

Claeys, H. and D. Inze. 2013. The agony of choice: how plants balance growth and survival under water-limiting conditions. *Plant Physiol.*, 162(4): 1768-1779.

Cramer, G.R., K. Urano, S. Delrot, M., Pezzotti and K. Shinozaki. 2011. Effects of abiotic stress on plants: a systems biology perspective. *BMC Plant Biol*., 11(1): 1-14.

Elhami, B., F. Zaare-Nahandi and S. Jahanbakhsh-Godehkahriz. 2015. Effect of sodium nitroprusside (SNP) on physiological and biological responses of olive (*Olea europaea* cv. Conservolia) under water stress. *Int. J. Biosci.*, 6(4): 148-56.

Elsheery, N.I. and K.F. Cao. 2008. Gas exchange, chlorophyll fluorescence, and osmotic adjustment in two mango cultivars under drought stress. *Acta Physiol Plant.*, 30(6): 769-77.

Ennajeh, A.M., H. Khemira, M.B. Mimoun and R.M.V. Hellali. 2006. Defense mechanisms against water deficit in two olive (*Olea europaea* L.) cultivars 'Meski' and 'Chemlali.' *J Hortic Sci Biotechnol*., 81(1): 99-104.

Fouad, M.O., A. Essahibi, B. Laila and A. Qaddoury. 2014. Effectiveness of arbuscular mycorrhizal fungi in the protection of olive plants against oxidative stress induced by drought. *Spanish J Agric Res.,* 12(3): 763-71.

Giannopolitis, C.N. and S.K. Ries. 1977. Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiol.,* 59(2): 309-314.

Gómez, R., P. Vicino, N. Carrillo and A.F. Lodeyro. 2019. Manipulation of oxidative stress responses as a strategy to generate stress-tolerant crops. From damage to signaling to tolerance. *Crit. Rev. Biotechnol.*, 39(5): 693-708.

Google Maps. 2022. https://www.google.com/ maps/@ 29.3796775,71.7537149,14z (Accessed: 30 September 2022)

Guerfel, M., O. Baccouri, D. Boujnah, W. Chaïbi and M. Zarrouk. 2009. Impacts of water stress on gas exchange, water relations, chlorophyll content and leaf structure in the two main Tunisian olive (*Olea europaea* L.) cultivars. *Sci Hortic.*, 119(3): 257-63.

Herzog, V. 1973. Determination of the activity of peroxidase. *Anal. Biochem*., 55: 554-562.

Hideg, É., M.A. Jansen and A. Strid. 2012. UV-B exposure, ROS, and stress: inseparable companions or loosely linked associates?. *Trends Plant Sci.,* 18(2): 107-115.

Imakumbili, M.L.E. 2019. Making water stress treatments in pot experiments: An illustrated step-by-step guide. *Sokoine University of Agriculture*. Morogoro, Tanzania, pp. 1-17.

Ingram, J. and D. Bartels. 1996. The molecular basis of dehydration tolerance in plants. *Annu Rev Plant Biol.*, 47(1): 377-407.

Jiang, Y. and B. Huang. 2001. Drought and heat stress injury to two cool-season turfgrasses in relation to antioxidant metabolism and lipid peroxidation. *Crop Sci.*, 41(2): 436-42.

Khalil, H.A. and D.O. El-Ansary. 2020. Morphological, physiological and anatomical responses of two olive cultivars to deficit irrigation and mycorrhizal inoculation. *Eur. J. Hort. Sci*., 85(1): 51-62.

Lambers, H., F.S. Chapin III and T.L. Pons. 2008. *Plant Physiol. Ecol*. 2nd edn. Springer Science Busines Media, LLC: New York, NY, USA.

Lima, A.L.S., F.M. DaMatta, H.A. Pinheiro, M.R. Totola and M.E. Loureiro. 2002. Photochemical responses and oxidative stress in two clones of Coffea canephora under water deficit conditions. *Environ Exp Bot.,* 47(3): 239-47.

Liu, Y., K. Guo, D. Fan, G. Li, Y. Zheng, L. Yu and R.C.L. Yang. 2011. Effect of drought on pigments, osmotic adjustment and antioxidant enzymes in six woody plant species in karst habitats of southwestern China. *Environ Exp Bot.*, 71(2): 174-83.

Mashayekhi, M., M.E. Amiri and F. Habibi. 2015. Study of the biochemical responses and enzymatic activity of GF677 (peach and almond hybrid) rootstock to *In vitro* salinity stress. *Iran J. Hort Sci.*, 29(2): 207-15.

Meena, M., K. Divyanshu, S. Kumar, P. Swapnil, A. Zehra, V. Shukla, M. Yadav and R.S. Upadhyay. 2019. Regulation of L-proline biosynthesis, signal transduction, transport, accumulation and its vital role in plants during variable environmental conditions. *Heliyon*, 5(12): p.e02952.

Meteoblue, 2022a. Climate Change Bahawalpur-meteoblue. https://www.meteoblue.com/en/climate-change/bahawalpur_pakistan_1183880 (Accessed: 19 September 2022).

Meteoblue, 2022b. Observed historical climate and weather data for Bahawalpur-. https://www.meteoblue.com/en/ weather/ historyclimate/climateobserved/bahawalpur_pakistan_1183880 (Accessed: 19 September 2022)

Nadarajah, K.K. 2020. ROS Homeostasis in Abiotic Stress Tolerance in Plants. *Int. J. Mol. Sci.*, 21(15): 5208.

Nakano, Y. and K. Asada. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.*, 22(5): 867-80.

Pakistan topographic map, elevation, relief, 2022. https://en-us.topographic map.com/maps/tlia/Pakistan/ (Accessed: 30 September 2022)

Petridis, A., I. Herios, G. Amouris, S. Koundouras and A. Giannakoula. 2012. Effect of water deficit on leaf phenolic composition, gas exchange, oxidative damage and antioxidant activity of four Greek olives (*Olea europaea* L.) cultivars. *Plant Physiol. Biochem.*, 60: 1-11.

Raza, A., A. Razzaq, S.S. Mehmood, X. Zou, X. Zhang, Y. Lv and J. Xu. 2019. Impact of climate change on crops adaptation and strategies to tackle its outcome: A review. *Plants*, 8(2): 34.

Reddy, A.R., K.V. Chaitanya and M. Vivekanandan. 2004. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *J. Plant Physiol.*, 161(11): 1189-202.

Regier, N., S. Streb, C. Cocozza, M. Schaub, P. Cherubini, S.C. Zeeman and B. Frey. 2009. Drought tolerance of two black poplar (*Populus nigra* L.) clones: contribution of carbohydrates and oxidative stress defence. *Plant Cell Environ.*, 32(12): 1724-36.

Rojas, O. 2020. Agricultural extreme drought assessment at global level using the FAO-Agricultural Stress Index System (ASIS). *Weather Clim. Extremes*, 27: 100184.

Sairam, R.K., K.V. Rao and G. Srivastava. 2002. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Sci.*, 163(5): 1037-46.

Sircelj, H., M. Tausz, D. Grill and F. Batic. 2005. Biochemical responses in leaves of two apple tree cultivars subjected to progressing drought. *J. Plant Physiol.*, 162(12): 1308-1318.

Sivritepe, N., U. Erturk, C. Yerlikaya, I. Turkan, M. Bor and F. Ozdemir. 2008. Response of the cherry rootstock to water stress induced in vitro. *Biol Plant.*, 52(3): 573-6.

Sofo, A., B. Dichio, C. Xiloyannis and A. Masia. 2005. Antioxidant defences in olive trees during drought stress: Changes in activity of some antioxidant enzymes. *Funct. Plant Biol.*, 32(1): 45-53.

Statistix8.1, 2003. User’s Manual. Analytical Software, Tallahassee.

Sukhera, M.B. and M.A.R. Pasha. 1987. Solar radiation maps for Pakistan. *Solar & Wind Tech.*, 4(2): 229-238.

Taiz, L. and E. Zeiger. 2010. Plant Physiology. 4th edn. Sinauer Associates Inc.: Sunderland, MA, USA.

Takahashi, F., T. Kuromori, H. Sato and K. Shinozaki. 2018. Regulatory gene networks in drought stress responses and resistance in plants. *Adv. Exp. Med. Biol.*, 1081: 189-214.

Tombesi, S., A. Tombesi, M. Molfese, M. Cipolletti and T. Visco. 2019. Minitab, Available at https:// www. minitab.com.

Valente, S., B. Machado, D.C. Pinto, C. Santos, A.M. Silva and M.C. Dias. 2020. Modulation of phenolic and lipophilic compounds of olive fruits in response to combined drought and heat. *Food Chem*., 329: 127191.

Vijn, I. and S. Smeekens. 1999. Fructan: more than a reserve carbohydrate? *Plant Physiol.*, 120(2): 351-360.

Yadollahi B., N. Lotfi, K. Vahdati, R. Amiri and D.A.K. Hassani. 2010. Morphological and physiological responses to water stress for seedlings of different walnut genotypes. *Acta Hortic.*, 86(1): 253-62.

Yucel, C.K., M. Bor and P. Ryser. 2014. Interspecific diversity in root antioxidative enzyme activities reflect root turnover strategies and preferred habitats in wetland graminoids. *Ecol. Evol.*, 4(6): 841-850.

(Received for publication 5 August 2022)