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Screening of sunflower (*Helianthus annuus* L.) cultivars/hybrids for heat stress tolerance using growth and physiobiochemical indicators

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Abstract: High temperatures may cause scorching of the twigs and leaves along with visual symptoms of sunburn, leaf senescence, growth inhibition, and ultimately decreased plant growth and biomass. Sunflower (*Helianthus annuus* L.) is one of the important oil seed crops and it potentially fits in agricultural system and oil production sector of Pakistan. Hence, it is important to get the best variety which may able to tolerate high temperatures in hot and humid environments. For this purpose, we have conducted the present study using ten different cultivars of (Hysin-33, T-40, H-OI, Hysin-39, Suncross, Gulshin, FH-825, FH-797, FH-784, AGSUN-5270) of *H. annuus* grown in the growth chambers in two different temperatures, i.e. control (25 °C) and heat stress (45 °C). A pot experiment was conducted and contains nutritional sand medium in the controlled environment in the growth chamber for 4 weeks. Results from the present study showed that heat stress induced a significant ($p < 0.05$) decrease in shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content while increasing the production of reactive oxygen species (ROS) by increasing the concentration of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), which is manifested by increasing content of flavonoid and phenolic. Results also showed that Hysin-39, FH-825, and Hysin-33 showed better growth and development in heat-stressed environment and considered a heat-tolerant cultivar while Gulshin, Suncross, and AGSUN-5270 showed poor growth and development in the same stressed environment and were considered heat-sensitive cultivars. The overall trend of H. annuus cultivars grown under heat stress is as follows: Hysin-39 > FH-825 > Hysin-33 > T-40 > H-OI > FH-784 > FH-797> AGSUN-5270 > Suncross > Gulshin.

Key words: Edible crop, high temperature, oxidative damage, osmoprotectants, plant growth

1. Introduction

Plants are typically exposed to a broad myriad of biotic (Solanki et al., 2022; Al-Zaban et al., 2022) and abiotic stresses (Afridi et al., 2022 a, b; Salam et al., 2022; Ma et al., 2022), including feeding from wild animals and insects (Metayi et al., 2022), weed infestation, hail, mechanical injury, diseases, low soil fertility (Fahad et al., 2022; Yasin et al., 2023), temperature (Saeed et al., 2022), drought (Bibi et al., 2022; Dola et al., 2022; Farooq et al., 2022; Yasmeen et al., 2022), salinity (Faryal et al., 2022; Hussain et al., 2022; Saleem et al., 2022), and others that can diminish the plant photosynthetic area, and thus the attained total plant biomass or grain yield (Alsafran et al. 2021; Mohamed et al. 2020; Saleem et al. 2020; Khan et al., 2022). Climate change is the most challenging issue human kind has ever experienced, which poses risks for both the global ecological balance and economic security (Zainab et al., 2021; Amna et al., 2021; Mehmood et al., 2021). Rising global earth surface temperature is one of the most intriguing factors emerging from the changing climate and is the major environmental factor which affects plant growth, development, and yield (Kumar et al. 2013; Saleem et al. 2020; Saleem et al. 2020; Zhang et al. 2019; Akram et al., 2022; Saini et al., 2022). Globally, annual temperature is expected to rise by 1.8–4.0 °C at the end of the 21st century (Hassan et al. 2021). This increase raises the concern among scientists and governments, as temperature directly and indirectly affects life forms present on earth. Despite these happenstances, world food supply has to be augmented by more than 70% to meet the needs of ever booming population, which is expected to reach up to 9 billion humans by the year 2050 (ASHRAF et al. ; Lv et al. 2021;

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Walayat et al. 2021; Haider et al., 2022). Generally, heat stress is often defined as the rise in temperature beyond a threshold level for a period of time sufficient to cause irreversible damage to plant growth and development (Argosubekti 2020; Ma et al. 2022a, b, c; Polsky and von Keyserlingk 2017). Plants, being sessile organisms, cannot move to favorable conditions; thus, their activities are significantly affected by heat stress (Alatawi et al. 2022; Fahad et al. 2017; Kalyar et al. 2014; Ma et al. 2022d, e). Heat stress significantly affects plant activities including seed germination, growth, development, photosynthesis, reproduction, resulting in serious impacts on plant growth and ultimate yield of useful products (Killi et al. 2017; Venios et al. 2020). High temperatures damage the activity of proteins and the fluidity membrane lipids, thus affecting the activity of chloroplast- and mitochondria-based enzymes and membrane integrity (Lipiec et al. 2013; Ul Hassan et al. 2021). Stress conditions can disturb the dynamic equilibrium of reactive oxygen species (ROS) production and elimination under normal growth in plants (Adnan et al. 2022; Ahmad et al. 2022; Ahmad et al. 2022; Kamran et al. 2020; Saleem et al. 2020 a, b, c; Saleem et al. 2022) which promotes ROS accumulation, membrane lipid peroxidation, and disrupt the structure and function of cell membrane system (Akhtar et al. 2022; Alam et al. 2022; Imran et al. 2020; Rana et al. 2020; Saleem et al. 2020; Zaheer et al. 2020). Excessive reactive oxygen species (ROS) production causes oxidative stress, as reported for many crops under environmental stress, and is likely to be commenced by molecular oxygen excitation (O_2) to generate singlet oxygen or by electron transfer to O_2 and genesis of free radicals, i.e. O^{2-} and OH^- (Aziz et al. 2021; Hashmat et al. 2021; Javed et al. 2020; Manghwar et al. 2021; Rehman et al. 2019; Saleem et al. 2022; Wahab et al. 2022). Thus, it is imperative that we develop crops with improved heat tolerance by means of the screening of plant cultivars.

Sunflower (*Helianthus annuus* L.) is an important oilseed crop and the fourth largest oilseed crop in the world cultivated in more than 70 countries (ES et al. 2020; Killi et al. 2020). Efforts by the *H. annuus* breeders brought about tremendous increases in oil yield during the last century: 33% in 1940 which increased to 55% in 1968 as a result of direct selection by Russian scientists Pustovoit and Jdanov (Kalyar et al. 2014). In *H. annuus*, previous research has demonstrated that brief periods of heat stress during grain filling negatively impacts on many yield components, such as seed number and weight, oil yield and fatty acid composition (Catiempo et al. 2021; Hernández et al. 2018; Ismail et al. 2020; Killi et al. 2017). Its germination is very susceptible to changing field conditions and breeding for resistance to high temperatures is an important objective in many *H. annuus* programs (Akladious 2014; Catiempo et al. 2021; Kalyar et al. 2014). Moreover, it is widely grown commercially for the oil that is extracted from the seeds which is practically free of significant toxic compounds and contains four important fatty acids, namely, palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic (18:2) acids, which have potential health benefits (Abdel Razik et al. 2021; Farid et al. 2018; Killi et al. 2020). Environmental factors during all stages of growth and development can widely affect normal growth and development of the plant, photosynthetic efficiency, and biochemical responses (Ali et al. 2022a; Ali et al. 2022b; Ali et al. 2022c; Ali et al., 2022d; Imran et al. 2019; SALEEM et al. 2020; Saleem et al. 2019). The present study was conducted to find the responses of *H. annuus* plants to heat stress and to find out which variety can tolerate and survive under high temperature levels in a controlled environment. In this respect, we have used different cultivars of *H. annuus* to screen out in the heat-stressed environment and study various growth, physiological, and biochemical responses of the plant species. The results from the present study will increase our knowledge about (I) plant growth and yield (II) photosynthetic pigments and (III) oxidative stress and secondary metabolites in *H. annuus* cultivars grown under the heat-stressed environment. This is amongst the few studies which focus on the tolerance in different *H. annuus* cultivars grown in the high level of heat in the environment. The study will provide useful information to sort out suitable *H. annuus* cultivar for the sustainable use of it in the heat-stressed condition.

2. Materials and methods

2.1. Experimental setup and growth conditions

This experiment was conducted in plastic pots (10-cm-tall × 7-cm-wide) in botanical garden at Department of Botany, Government College University Faisalabad 38000, Pakistan (31° 24/N, 73° 04/E) during April 2019. Ten cultivars of *H. annuus* named as Hysin-33, T-40, H-OI, Hysin-39, Suncross, Gulshin, FH-825, FH-797, FH-784, AGSUN-5270 were collected from Ayub Agricultural Research Institute Faisalabad 38000, Pakistan. Before starting the pot experiment, the seeds were sterilized by 10% (v/v) of commercial bleach for 15 min and then washed thoroughly in distilled water. A hole was made in the bottom of plastic pot to avoid water logging and then plastic pots were filled with about 500 g of washed, air-dried, and contamination-free sand. Ten healthy and mature seeds of the *H. annuus* cultivar were sown in sand-filled plastic pots. The physiochemical and nutritional properties of the sand used in this experiment are presented in Table S1. After 1 week of seed sowing, uniform-sized rhizome was collected and all pots were distributed into two groups (i) controlled treatment (25 °C) and (ii) 45 °C temperature for heat stress. The sand

was also provided with the Hoagland's solution and the chemical composition of Hoagland's solution is presented in Table S2. All pots were placed in the growth chamber with 12 h light (13,000 lx) and 12 h dark. Although plants were sown separately, after 1 week of seed germination, all the plants were shifted into the pots for control and heat stress and kept under the stress treatment for 4 weeks. All plants were monitored daily and Cr-free water and other intercultural operations (such as weeding and thinning) were performed when needed. This experiment was designed as two-factor factorial, completely randomized (CRD) with four replications. All plants were harvested at the end of 4th week to measure various physiological and growth parameters.

2.2. Sampling and data collection for growth attributes

All plants were harvested in May, 2019 for different morphological and physiological attributes. All the plants were rooted-up from the pots, thoroughly washed with double distilled water, and divided into roots and shoots with the help of cutter. Leaf samples from each treatment group were picked after 1 month for chlorophyll, carotenoid, and other physiological measurements. The leaves, after being washed with distilled water, were placed in liquid nitrogen and stored at −80 °C for further analysis. Plant length (shoot and root) was measured using a measuring scale. Plant fresh weight (shoot and root) was determined by measuring the weight of plant with a digital weighting balance. Later, root and shoot were dried in an oven at 105 °C for 1 h, then at 70 °C for 72 h to determine their dry weight.

2.3. Determination of photosynthetic pigments

Leaves were collected for determination of chlorophyll and carotenoid contents. For chlorophylls, 0.1 g of fresh leaf sample was extracted with 8 mL of 95% acetone for 24 h at 4 °C in the dark. The absorbance was measured by a spectrophotometer (UV-2550; Shimadzu, Kyoto, Japan) at 646.6, 663.6, and 450 nm. Chlorophyll content was calculated by the standard method of Arnon (Arnon 1949).

2.4. Determination of oxidative stress biomarkers

The degree of lipid peroxidation was evaluated as malondialdehyde (MDA) contents. Briefly, 0.1 g of frozen leaves were ground at 4 °C in a mortar with 25 mL of 50 mM phosphate buffer solution (pH 7.8) containing 1% polyethene pyrrole. The homogenate was centrifuged at 10,000 × *g* at 4 °C for 15 min. The mixtures were heated at 100 °C for 15–30 min and then quickly cooled in an ice bath. The absorbance of the supernatant was recorded by using a spectrophotometer (xMark™ Microplate Absorbance Spectrophotometer; Bio-Rad, United States) at wavelengths of 532, 600, and 450 nm. Lipid peroxidation was expressed as l mol g^{-1} by using the formula: 6.45 (A532-A600)-0.56 A450. Lipid peroxidation was measured by using a method previously published by Health and Packer (Heath and Packer 1968).

To estimate H_2O_2 content of plant tissues (root and leaf), 3 mL of sample extract was mixed with 1 mL of 0.1% titanium sulfate in 20% (v/ v) H_2SO_4 and centrifuged at 6000 × *g* for 15 min. The yellow color intensity was evaluated at 410 nm. The H_2O_2 level was computed by extinction coefficient of 0.28 $mmol^{-1}$ cm^{-1}. The contents of H_2O_2 were measured by the method presented by Jana and Choudhuri (Jana and Choudhuri 1981).

2.5. Determination of secondary metabolites

Plant ethanol extracts were prepared for the determination of some secondary metabolites. For this purpose, 50 mg of dry plant material was homogenized with 10 mL ethanol (80%) and filtered through Whatman No. 41 filter paper. The residue was reextracted with ethanol, and the 2 extracts were pooled together to a final volume of 20 mL. The determination of flavonoids (Pękal and Pyrzynska 2014) and phenolics (Bray and Thorpe 1954) was performed from the extracts.

2.6. Statistical analysis

The normality of data was analyzed using IBM SPSS software (Version 21.0. Armonk, NY, USA: IBM Corp) through a multivariate post hoc test, followed by a Duncan's test in order to determine the interaction among significant values. Thus, the differences between treatments were determined by using ANOVA, and the least significant difference test ($p < 0.05$) was used for multiple comparisons between treatment means where significant, Tukey's HSD post hoc test was used to compare the multiple comparisons of means. The analysis showed that the data in this study were almost normally distributed. The graphical presentation was carried out using Origin-Pro 2017. The Pearson correlation coefficients between the measured variables of *H. annuus* were also calculated. The plots of principal component analysis on *H. annuus* parameters were carried out using the RStudio software.

3. Results

3.1. Plant growth and photosynthetic measurements

The present experiment was conducted to analyze the effect of heat stress in various cultivars of *H. annuus* to study various growth and photosynthetic parameters. The results regarding various growth parameters of *H. annuus* cultivars are presented in Figure 1 while results regarding photosynthetic pigments are presented in Figure 2. The results from the present findings show that the heat stress induced a significant ($p < 0.05$) reduction in root length, shoot length, root fresh weight, shoot fresh weight, root dry weight, shoot dry weight, chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid content in all cultivars of

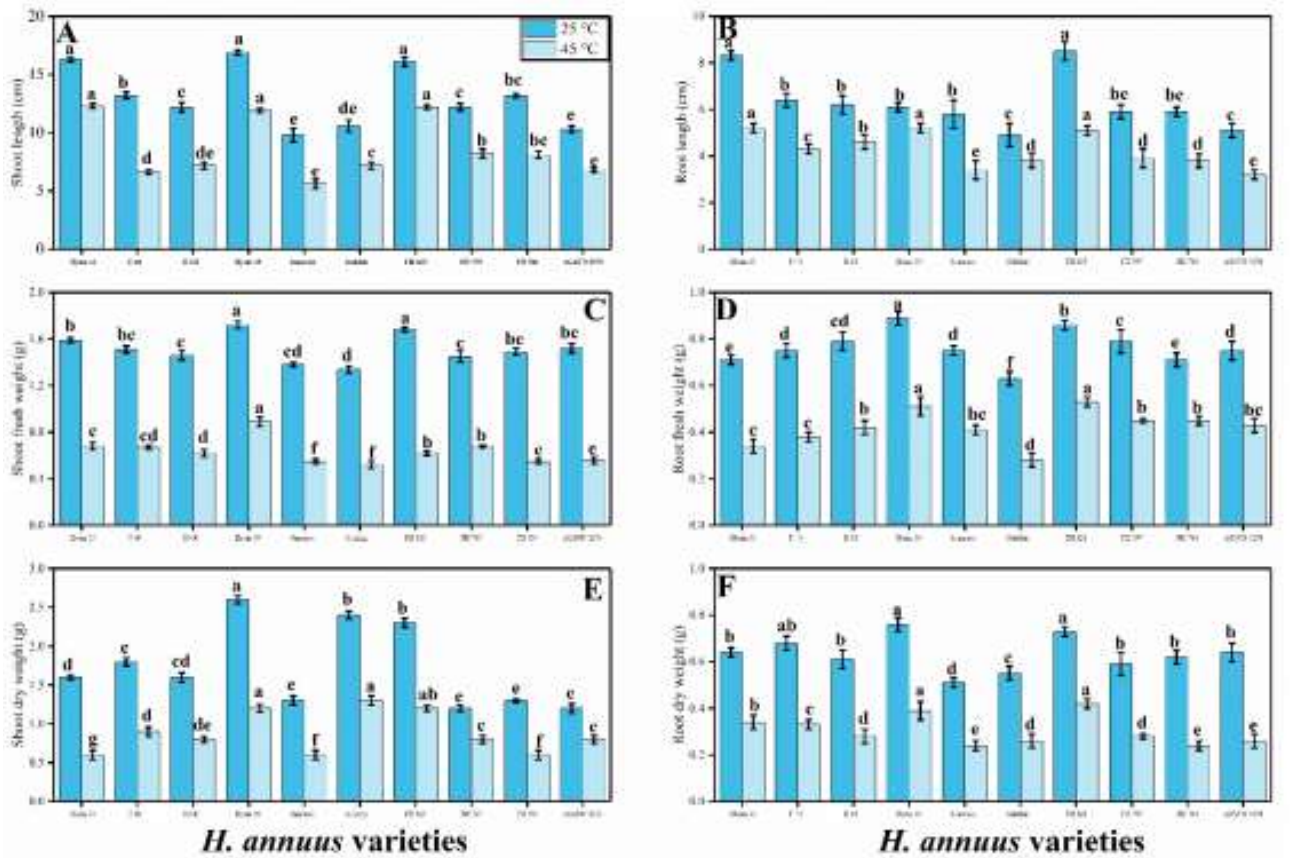

Figure 1. Response of morphological traits such as shoot length (A), root length (B), shoot fresh weight (C), root fresh weight (D), shoot dry weight (E), and root dry weight (F) of *H. annuus* (Hysin-33, T-40, H-OI, Hysin-39, Suncross, Gulshin, FH-825, FH-797, FH-784, and AGSUN-5270) cultivars grown under control (25 °C) and heat-stressed (45 °C) condition. Data presented in the figure are the means of four replicates of just one harvest along with standard deviation (SD; n = 4). Two-way ANOVA was performed, and means differences were tested by HSD ($p < 0.05$). Different lowercase letters on the error bars indicate significant difference between the *H. annuus* cultivars.

H. annuus compared to the plants grown in the normal temperature, i.e. 25 °C. Our results also advocated that the Hysin-39, FH-825, and Hysin-33 cultivars showed more tolerance and resistance to the heat stress, compared to the other cultivars of *H. annuus* under the same environmental condition. In contrast, Gulshin, Suncross, and AGSUN-5270 showed poor growth and biomass in controlled and also heat-stressed environment compared to other cultivars of *H. annuus* which were studied in this experiment. Similarly, Hysin-39, FH-825, and Hysin-33 showed higher contents of chlorophyll-a, chlorophyll-b, total chlorophyll, and carotenoid while Gulshin, Suncross, and AGSUN-5270 showed lower photosynthetic contents compared to all other varieties of *H. annuus*, studied in this experiment. The overall trend for the plant growth and biomass and photosynthetic pigments of studied cultivars of *H. annuus* in the heat-stressed environment are as follows: Hysin-39 > FH-825 > Hysin-33 > T-40 > H-OI > FH-784 > FH-797> AGSUN-5270 > Suncross > Gulshin.

3.2. Oxidative stress and osmoprotectants

Oxidative stress biomarkers such as malondialdehyde (MDA) content and hydrogen peroxide (H_2O_2) contents were also measured in the present study in different cultivars of *H. annuus* when grown in the heat-stressed condition (45 °C). The results regarding the contents of MDA in various cultivars of *H. annuus* is presented in Figure 3A and data regarding the content of H_2O_2 is presented in Figure 3B. Based on the findings of the present study, the content of MDA and H_2O_2 were increased significantly ($p < 0.05$) in all plants grown in the heat-stressed condition, compared to those plants which were grown in the normal temperature, i.e. 25 °C (Figures 3A and 3B). We also advocated that the heat-sensitive cultivars (Gulshin, Suncross, and AGSUN-5270) showed more concentration of MDA and H_2O_2 compared to other cultivars of *H. annuus*. Contrastingly, heat-tolerant cultivars (Hysin-39, FH-825, and Hysin-33) of *H. annuus* showed lower concentration of MDA and H_2O_2 in the

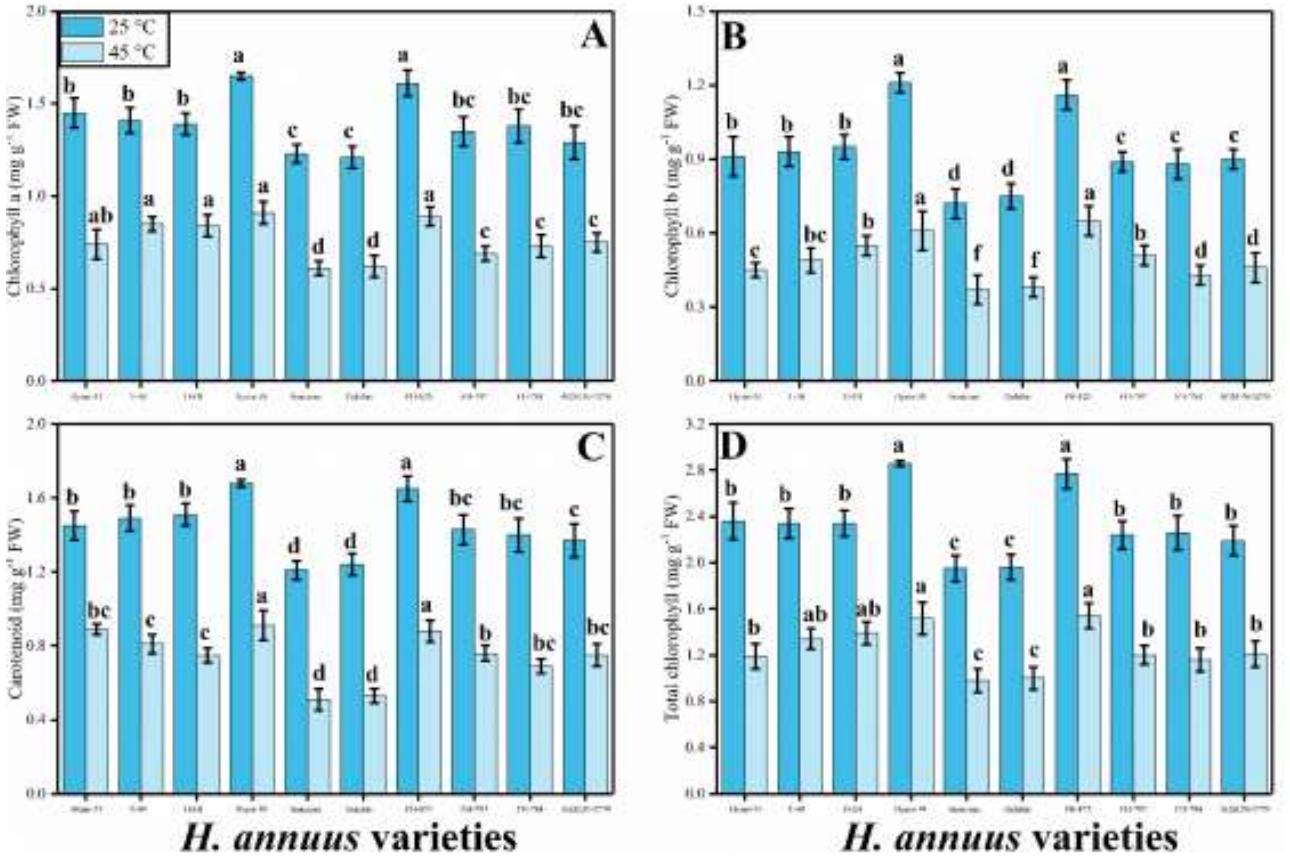

Figure 2. Response of photosynthetic pigment such as chlorophyll a (A), chlorophyll b (B), carotenoids (C), and total chlorophyll (D) of *H. annuus* (Hysin-33, T-40, H-OI, Hysin-39, Suncross, Gulshin, FH-825, FH-797, FH-784, and AGSUN-5270) cultivars grown under control (25 °C) and heat-stressed (45 °C) conditions. Data presented in the figure are the means of four replicates of just one harvest along with standard deviation (SD; n = 4). Two-way ANOVA was performed, and means differences were tested by HSD ($p < 0.05$). Different lowercase letters on the error bars indicate significant difference between the *H. annuus* cultivars.

membranous bounded organelles of the cellular body, compared to the other cultivars of *H. annuus*.

Phenolic and flavonoid content was increased in all cultivars of *H. annuus* when grown in the heat-stressed condition (45 °C) (Figures 3C and 3D). Phenolic and flavonoid contents was found to be more in heat-tolerant cultivars (Hysin-39, FH-825, and Hysin-33) compared to the heat-sensitive cultivars (Gulshin, Suncross, and AGSUN-5270).

3.3. Correlation analysis between various growth parameters of *H. annuus* cultivars

A Pearson's correlation graph was also illustrated to depict a correlation between various growth and physiological parameters studied in this experiment grown in the heat-stressed condition (Figure 4). Malondialdehyde contents were positively correlated with hydrogen peroxide content while negatively correlated with shoot length, shoot fresh weight, total chlorophyll content, chlorophyll a content, carotenoid content, root dry weight, root length, shoot dry weight, flavonoid, chlorophyll b content, root fresh weight, and phenolic content. Similarly, hydrogen peroxide contents were positively correlated with malondialdehyde contents while negatively correlated with shoot length, shoot fresh weight, total chlorophyll content, chlorophyll a content, carotenoid content, root dry weight, root length, shoot dry weight, flavonoid, chlorophyll b content, root fresh weight, and phenolic content. This relationship showed a close connection between oxidative stress and growth in various varieties of *H. annuus* composition.

3.4. Principal component analysis

The loading plots of PCA depicting a relationship between growth and composition of *H. annuus* cultivars are presented in Figure 5. Dim1 and Dim2 occupied most potion in PCA components with Dim1 concise with 95.9% while dim2 concise with 1.6% in the whole database. All the parameters were dispersed successfully in the dataset in two portions. Malondialdehyde contents and hydrogen peroxide contents were positively correlated in the database. While shoot length, shoot fresh weight, total chlorophyll content, chlorophyll a content, carotenoid

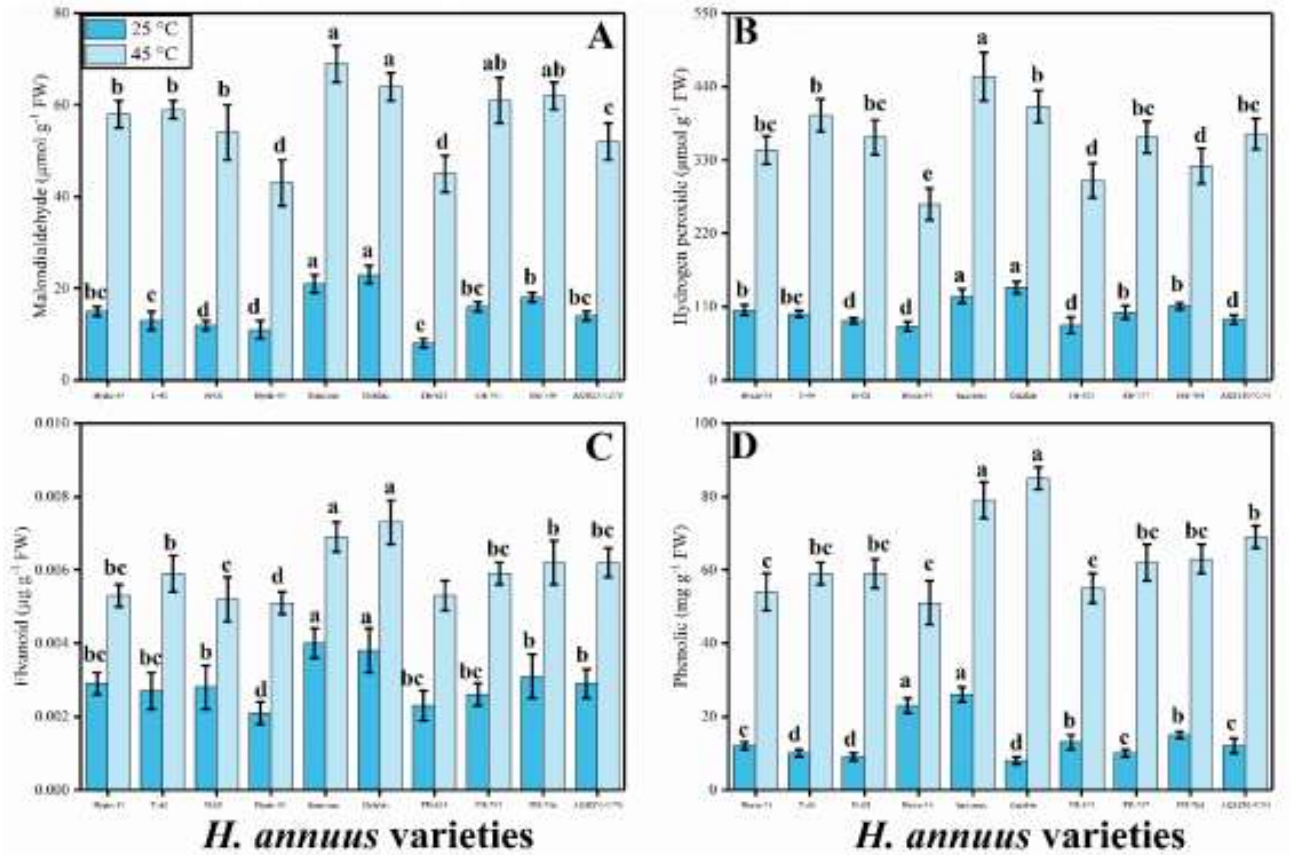

Figure 3. Response of oxidative stress and osmo-protectants like malondialdehyde (A), hydrogen peroxide (B), flavonoids (C), and phenolics (D) of *H. annuus* (Hysin-33, T-40, H-OI, Hysin-39, Suncross, Gulshin, FH-825, FH-797, FH-784, and AGSUN-5270) cultivars grown in the control (25 °C) and heat-stressed (45 °C) condition. Data presented in the figure are the means of four replicates of just one harvest along with standard deviation (SD; n = 4). Two-way ANOVA was performed, and means differences were tested by HSD ($p < 0.05$). Different lowercase letters on the error bars indicate significant difference between the *H. annuus* cultivars.

content, root dry weight, root length, shoot dry weight, flavonoid, chlorophyll b content, root fresh weight, and phenolic content were negatively correlated in the database compared with all others studied variables.

4. Discussion

Increased temperatures caused by global warming threaten agricultural production, as warmer conditions can inhibit plant growth and development or even destroy crops in extreme circumstances (Ismail et al. 2020; Kalyar et al. 2014; Ul Hassan et al. 2021). Temperature is one of the major environmental factors which affect plant growth, development, and yield and is persistently above optimal for plant growth, which may induce heat stress and reduce yields (Shehzad et al. 2021; Wu et al. 2018). A significant reduction in the growth and net assimilation rate was observed in *Oryza sativa* (Agarie et al. 1998), *Triticul aestivum* (ZAFAR et al. 2021), *Brassica napus* (Wu et al. 2018), and *H. annuus* (Ismail et al. 2020) under heat stress. Raja et al. (2020) reported a significant reduction in the plant growth and biomass accumulation along with early leaf senescence in *Solanum lycopersicum* under heat stress. The results from the present study revealed that the heat stress induced a significant ($p < 0.05$) reduction in root length, shoot length, root fresh weight, shoot fresh weight, root dry weight and shoot dry weight in all cultivars of *H. annuus*, compared to the plants grown in the control treatment (Figure 1). Temperature stress reduced *Oryza sativa* yield by reducing the performance of its growth and yield traits (Agarie et al. 1998). A major effect of the heat stress is commonly noticed in *Solanum lycopersicum* as it influences meiosis, fertilization, and growth of fertilized embryo ultimately causing a noticeable reduction in the yield (Raja et al. 2020). For the successful production of *H. annuus* cultivars, when cultivated in the temperature stress condition, development and selection of tolerant varieties through screening is crucial. Hence, for the better growth and development of *H. annuus* for crop production and natural resources, it is necessary to cultivate the most suitable variety of *H. annuus*. However, the resistance or

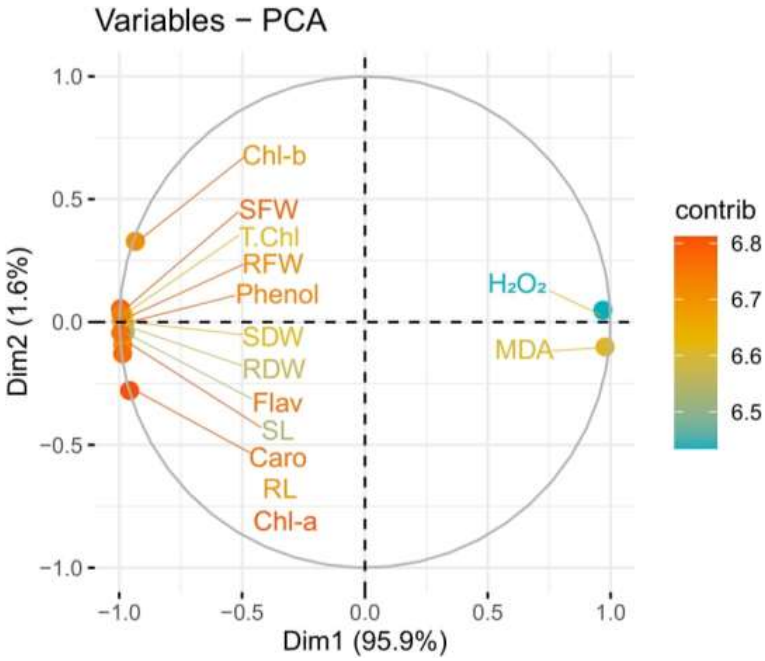

Figure 4. Pearson's correlation between various growth and physiological attributes in various varieties of *H. annuus* (Hysin-39, FH-825, Hysin-33, T-40, H-OI, FH-784, FH-797, AG-5270, Suncross and Gulshan), grown in the heat-stressed condition. Different abbreviations used in the figure are as follows: H_2O_2 (hydrogen peroxide content), SL (shoot length), SFW (shoot fresh weight), T-Chl (total chlorophyll content), Chl-a (chrlophyll a content), Caro (carotenoid content), RDW (root dry weight), RL (root length), SDW (shoot dry weight), Flav (flavonoid content), Chl-b (chrlophyll b content), RFW (root fresh weight), and phenol (phenolic content).

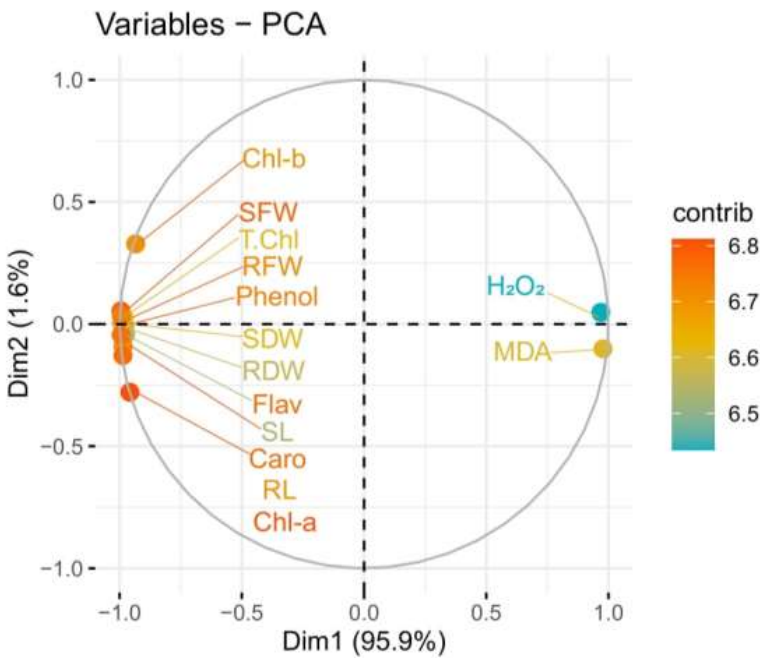

Figure 5. Loading plots of principal component analysis (PCA) on different studied parameters of *H. annuus* (Hysin-39, FH-825, Hysin-33, T-40, H-OI, FH-784, FH-797, AG-5270, Suncross, and Gulshan) varieties grown in the heat-stressed condition. Different abbreviations used in the figure are as follows: H_2O_2 (hydrogen peroxide content), SL (shoot length), SFW (shoot fresh weight), T-Chl (total chlorophyll content), Chl-a (chrlophyll a content), Caro (carotenoid content), RDW (root dry weight), RL (root length), SDW (shoot dry weight), Flav (flavonoid content), Chl-b (chrlophyll b content), RFW (root fresh weight), and phenol (phenolic content).

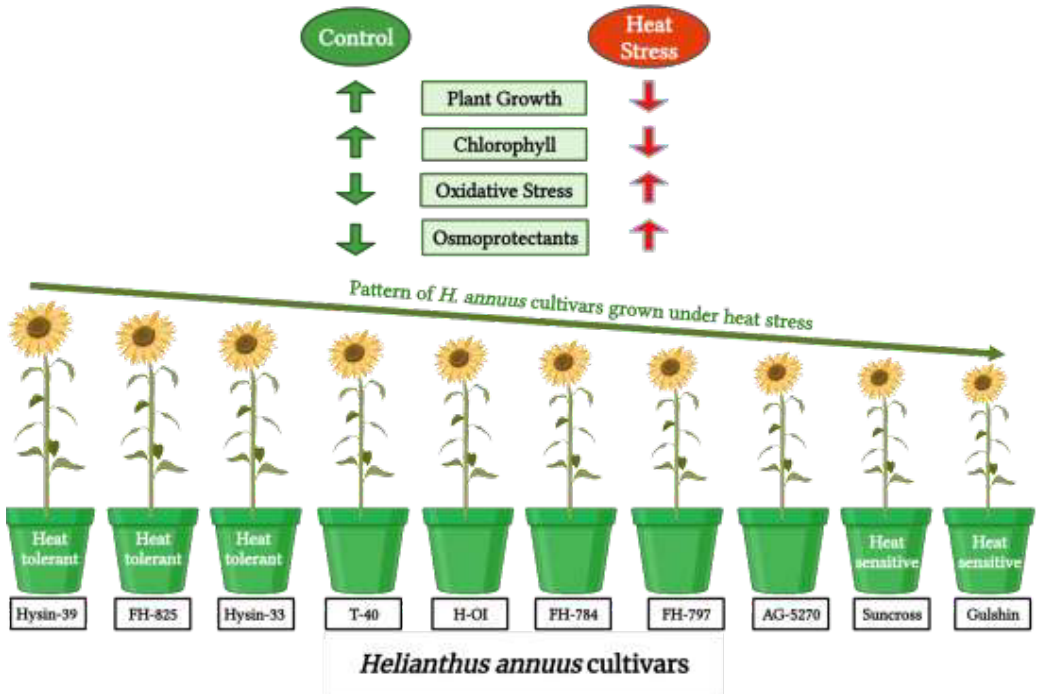

Figure 6. Schematic presentation interpreting the heat stress in different *H. annuus* (Hysin-33, T-40, H-OI, Hysin-39, Suncross, Gulshin, FH-825, FH-797, FH-784, AGSUN-5270) cultivars. The heat stress inhibited the plant growth characteristics and higher ROS concentration was accumulated in the organs of *H. annuus* cultivars. The current study demonstrated the heat stress in different *H. annuus* cultivars and oxidative damage induced by the high temperature in membrane bounded organelles of the plant. The results also depicting that the Hysin-39, FH-825, and Hysin-33 cultivars showing better growth while Gulshin, Suncross, and AGSUN-5270 showing reduction in growth and biomass compared to all other cultivars of *H. annuus* in heat-stressed environment.

tolerance mechanism of a plant depends on its specific physiological and biochemical activities (Mwadzingeni et al. 2016; Saleem et al. 2020). Therefore, a preliminary experiment was conducted to expose heat-sensitive varieties and heat-resistant varieties to the temperature stress condition.

High temperatures damage the activity of proteins and the fluidity membrane lipids, thus affecting the activity of chloroplast and mitochondria based enzymes and membrane integrity (Ali et al. 2020; Mfarrej et al.). Both severe heat stress and long-term exposure to moderate high temperatures can result in cellular damage and cell death (Killi et al. 2020; Mfarrej et al.). During photosynthesis in higher plants, sunlight is trapped and converted into biological energy by chloroplasts. These organelles serve as metabolic centers and play key roles in sensing heat stress and instigating appropriate physiological adaptive responses (Killi et al. 2020). Photosynthesis-associated processes including electron transport, CO_2 assimilation, photophosphorylation, chlorophyll (Chl) biosynthesis, thylakoid membrane fluidity and photochemical reactions are sensitive to heat stress (Killi et al. 2020; Wang et al. 2010). Heat stress-induced damage to chloroplasts leads to the inactivation of heat-sensitive proteins such as Rubisco activase (RCA) and the down-regulation of important chloroplast components, thereby leading to decreased photosynthetic efficiency, redox imbalance and possible cell death (Prasad et al. 2008; Shalaby et al. 2021; Ul Hassan et al. 2021). Hence, similar reports were also observed in the present study that increased temperature stress caused a significant ($p < 0.05$) decrease in the contents of chlorophyll-a, chlorophyll-b, total chlorophyll, and carotenoid in the plants grown in the high temperature environment (Figure 2). That different trends were observed in different cultivars of *H. annuus* in the same environment might be due to the response of specific genes which are involved in the protein biosynthesis (Figure 2).

Abiotic stresses are considered a primary source of injury to the cell membrane, frequently attributing to lipid peroxidation (Nawaz et al., 2022; Naz et al., 2022; Ali et al., 2022). As a result of metal accumulation, a large number of active free oxygen radicals are formed, which may be the main cause of cell membrane lipid peroxidation, and also harm the functioning and structure of the cell membrane

(AFZAL et al. 2021; Akhtar et al. 2022; Ali et al. 2020; Rana et al. 2020; REHMAN et al. 2020; Saleem et al. 2020; Saleem et al. 2020; Yaseen et al. 2020). Excessive reactive oxygen species (ROS) production causes oxidative stress, as reported for many crops under heat stress treatment, and is likely to be commenced by molecular oxygen excitation (O_2) to generate singlet oxygen or by electron transfer to O_2 and genesis of free radicals, i.e. O^{2-} and OH^- (Ali et al. 2022; Aziz et al. 2021; Hussain et al. 2022; Ismail et al. 2020; Kalyar et al. 2014; Lipiec et al. 2013; Saleem et al. 2021). In general, the tolerance of the plant to heat stress is characterized by minimal damage to photosynthetic machinery and increased biosynthesis of the protective compounds (Poór et al. 2021; Shehzad et al. 2021). The same results have been found in the present study that heat stress induced a significant ($p < 0.05$) increase in the concentration of MDA, H_2O_2 which were also manifested by phenolic and flavonoid contents (Figure 3). However, the plants produce a variety of secondary metabolites such as flavonoids and phenolics that improve tolerance against environmental stress condition (AFZAL et al. 2021; Alam et al. 2021; Imran et al. 2021; Saleem et al. 2021, Ali et al., 2022). The schematic presentation of *H. annuus* cultivars grown in the heat-stressed environment is presented in detail in Figure 6.

5. Conclusion

The present study demonstrated the effect of heat stress on plant growth and biomass, photosynethtic pigments, and response of oxidative stress biomarkers and some secondary metabolites in different cultivars of *H. annuus* grown in the heat-stressed environment (45 °C). Results showed that the heat stress decreased plant growth and biomass and photosynthetic pigments while increased the concentration of MDA and H_2O_2 which was manifested by the content of phenolic and flavonoid. However, we have also concluded that Hysin-39, FH-825, and Hysin-33 are heat-tolerant cultivars of *H. annuus* while Gulshin, Suncross, and AGSUN-5270 are heat-sensitive cultivars of *H. annuus* in temperature stressed environments. The overall trend for the plant growth and biomass and photosynthetic pigments of studied cultivars of *H. annuus* in the heat-stressed environment are as follows: Hysin-39 > FH-825 > Hysin-33 > T-40 > H-OI > FH-784 > FH-797> AGSUN-5270 > Suncross > Gulshin. Hence, further study is needed to be conducted at molecular level to understand their mechanism more briefly.

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Table S1. Physical and nutritional properties of the sand used in this experiment.

Physical properties	
Organic matter	>70% of total solids
Density	350 kg/m^3
pH	7.6
Electrical conductivity	20 mS/m
Organic nitrogen	1400 mg/L
Nutrients	**g/m^3**
Nitrogen (NO_3-N + NH_4-N)	150
Phosphorus (P)	75
Potassium (K)	160
Magnesium (Mg)	250
Calcium (Ca)	1600
Sulfur (S)	85
Copper (Cu)	2.5
Zinc (Zn)	1.8
Molybdenum (Mo)	2.7
Iron (Fe)	5.6

Table S2. Chemical composition and proportions of Hoagland's solution used in the hydroponic.

Proportions	**Salts**
269.76	$Ca(NO_3)_2 \cdot 4H_2O$
35.15	KH_2PO_4
48.80	K_2SO_4
167.68	$CaCl_2 \cdot 2H_2O$
324.5	$MgSO_4 \cdot 7H_2O$
2.8818	$MnCl_2 \cdot 4H_2O$
0.1472	$(NH_4)6Mo_7O_{24} \cdot 4H_2O$
1.8304	H_3BO_3
0.0704	$ZnSO_4 \cdot 7H_2O$
13.33	Na_2EDTA
9.96	$FeSO_4 \cdot 7H_2O$
0.0629	$CuSO_4 \cdot 5H_2O$