Assiut University Journal of Multidisciplinary Scientific Research (AUNJMSR)
Faculty of Science, Assiut University, Assiut, Egypt.
Printed ISSN 2812-5029
Online ISSN 2812-5037
Vol. 2 (1): 20- 42 (2025)
https://aunj.journals.ekb.eg

Thriving under Heat Stress: Exploring Ecophysiological and Agronomic Dynamics of Sugar Beet (*Beta vulgaris* L.) Across Varied Thermal Regimes

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ARTICLE INFO

Article History:
Received: 2024-11-09
Accepted: 2024-12-18
Online: 2025-1-20

Keywords:
Heat stress, Environmental changes, Heat shock proteins, Thermal tolerance, Oxidative stress, Antioxidant enzymes, Agronomical traits

ABSTRACT

The study aimed to investigate the effects of heat stress on sugar beet growth and physiology. Two field tests were conducted at temperatures of 42 °C (heat stress) and 23 °C (moderate-temperature) in different two seasons. The results showed that sugar beet leaves grown under heat stress conditions exhibited higher levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) compared to moderate temperature conditions in both seasons. Additionally, the activity of antioxidant enzymes, such as superoxide dismutase (SOD) and ascorbate peroxidase (APX), significantly increased in response to high temperatures. However, catalase (CAT) activity was lower in the second season's high-temperature samples compared to moderate-temperature samples. The polypeptide patterns of the high-temperature samples showed the presence of new polypeptides (M.Wts of 123, 110, 103, 77, 51, 39, and 31 kD), while moderate-temperature samples did not have these polypeptides. Agronomic traits revealed lower values of root weight, top fresh weight, root fresh yield, top fresh yield, and sucrose % at high-temperature conditions. Furthermore, gross sugar yield was lower, while sugar loss% and α-amino-N concentration were higher in the high-temperature conditions. Multivariate analysis was conducted to examine correlations between biochemical and developmental traits under heat stress.

INTRODUCTION

Global warming and the current unpredictable weather would seriously impair almost every stage of a plant's life, including vegetative growth, reproduction, and eventually output [1, 2, 3]. Every plant has a specific range of ideal temperatures within which it can grow to the fullest extent of its genetic potential [4]. As a result, crops grown in the warm season would require a different optimal temperature range than crops grown in the cold season [5]. The yields of the majority of important feed, food, and fiber crops would decline sharply once the temperature exceeded 30 °C, as previously reported [6], and it has been hypothesized that heat stress during the summer would result in a 20 to 36% yield decline [7]. As a result, scientists have been paying more attention to heat stress recently, and significant efforts have been made to address the problems that high-temperature stress on many crops presents [8]. Heat stress causes reactive oxygen production (ROS), stomatal closure, destruction of photosynthetic pigments, hormone and other physiological changes in plant tissues. Heat stress is typically accompanied by photooxidative stress [9]. These physiological modifications have detrimental effects on water content, photosynthetic efficiency, and other processes [2]. Hydrogen peroxide (H_2O_2), superoxide radicals, and lipid peroxidation all produce more ROS under heat stress, which worsens membrane damage [10, 11]. Plants, which are sessile organisms, have fascinating molecular, biochemical, and physiological mechanisms for adjusting to dramatic environmental changes [1, 12]. The basic physiological functions of plants, such as the metabolism of carbohydrates, phytohormones, the antioxidant system, and the osmotic adjustment system, are severely impacted by high temperatures [13]. Additionally, plants adapt to heat stress by changing a variety of complex molecular processes, including non-coding RNAs, changes in regulatory and functional genes, and DNA or protein epigenetic modification [14]. Transcription factors resembling heat shock factors (HSFs) are critical role in the plant's response to heat stress. The expression of heat shock proteins (HSPs) and the heat shock response (HSR) are mostly regulated by heat shock transcription factors [14, 15]. Plant HSR is characterized by the quick reprogramming of gene expression, which produces a specific class of HSPs [15, 16]. According to Jacob et al. [16], the HSPs are the main metabolism enzymes that keep the cellular proteome in homeostasis and play a significant role in the signal transduction chain facilitating plant response to a variety of stress conditions. Proteins are denaturized as a result of heat stress, which causes proteotoxic stress [15]. HSFs typically exist in a monomeric state, and inhibitory associations with HSPs like HSP70 suppress their activities [17]. According to their molecular weights, HSPs are classified into five classes: small heat shock proteins, HSP60, HSP70, HSP90 and HSP100 [3, 12, 18]. HSPs serve as molecular chaperones.Exposure to environmental stressors or high metabolic load inhibits the production or activation of HSPs and antioxidant proteins [19]. They are known as stress proteins and are indicators of cellular stress [20, 21]. An edible plant belonging to the Amaranthaceae family is the sugar beet (*Beta vulgaris* L.). According to Ganapati et al. [22], it is found in Asia Minor, the Mediterranean, and Europe. Environmental factors such as heat, a lack of water, a lack of nutrients, cold, and salinity frequently limit the production of sugar beet. By reducing the rates of photosynthesis and canopy expansion, these conditions had a direct impact on plant growth, development, and overall crop productivity [23]. Regrettably, global warming has raised the average global temperature and altered the climate in many regions, including those with sugar

beet plantations, which has a negative impact on the productivity of the crop. Climate change will, however, have less of an impact on sugar beet cultivation if some cultivars can be demonstrated to be tolerant to heat stress. As a result, sugar beet cultivation during the summer and in tropical areas is of interest [24]. Furthermore, the need to breed cultivars for high-temperature conditions is urgent due to the maximum temperature in semi-arid regions [25]. For sugar beet plants to transpire and photosynthesis to occur without stomatal restriction in Uber Egypt (high temperature), there needs to be enough moisture available on a daily basis to meet atmospheric demand. These ideal circumstances, however, are frequently not met, either as a result of insufficient rainfall or limited irrigating options. Heat is frequently present in dry conditions, and in some cases, damaging temperatures can result from temperatures that are higher than what is ideal for growth and physiological processes. Despite a sizeable portion of sugar beet being planted in hot climates around the world, little research and development has focused on sugar beet's ability to withstand heat stress. The purpose of the current study was to investigate the biochemical and physiological mechanisms underlying sugar beet heat tolerance and how they affect crop yield. To do this, we compared the variations in MDA and H_2O_2 content as stress indicators, some antioxidant enzymes activity, including SOD, CAT and APX as well as the induction of HSPs in sugar beet leaves grown under heat stress and moderate conditions in two consecutive seasons.

2. MATERIALS AND METHODS

2.1. Plant material and Experimental design

During the 2018/2019 and 2019/2020 growing seasons, two field experiments were conducted in two farms at two provinces varying in their average temperatures: Assiut in the middle of Upper Egypt and Qena in the Southern part of Upper Egypt. These are the Agronomy Department Farm, Faculty of Agriculture, Assiut University, Assiut (moderate temperature), and a private farm in Qena (high temperature), Egypt, to examine the response of sugar beet to different heat conditions in both regions. This experimental design was performed at the soil was treated with 31 kg of P_2O_5 fed.$^{-1}$ as calcium superphosphate (15.5% P_2O_5) during soil preparation. Nitrogen fertilizer was applied at 100 kg N fed.$^{-1}$ as ammonium nitrate (33.3% N). The potassium fertilization was applied as potassium sulphate (48% K_2O) at a rate of 50 kg fed.$^{-1}$ in a single dosage.

Each plot had an area of 10.5 m^2 (3.5 m × 3 m length: width) with six ridges spaced 50 cm apart and a length of 3.5 m. This design was carried out twice; at 2018/2019 and 2019/2020. Hence, multi-germ sugar beet seed balls of the commercial cultivar in Egypt that is called Gollia were seeded at the beginning of september and at the beginning of November at a level of 2-3 balls hill^{-1}. The plants were reduced to one plant hill^{-1} (at the fourth true leaf stage) after 21 days from seeding. The Upper Egypt cultivated sugar beet using the advised cultural practices. In both seasons, the irrigation of sugar beet was discontinued two weeks prior to harvest.

2.2. Analytical methods

Three plant leaves from each plot were sampled 190 days after planting. Assiut samples were taken in March during the 2018/2019 and 2019/2020 seasons, respectively, when the temperature was 23.14 and 23.23. While Qena samples were taken in May during the 2018/2019 and 2019/2020 seasons, respectively, when the temperature was 42.17 °C and 42.20 °C. To prepare the leaves for further analysis, they were only briefly rinsed with deionized water, gently blotted with filter paper, and then immediately frozen in liquid nitrogen and kept at -80 °C.

2.2.1. Malondialdehyde measurement

The quantity of lipid peroxidation was assessed using the Narwal et al. [26] method by measuring the formation of malondialdehyde (MDA) with thiobarbituric acid (TBA). One and a half mL of trichloroacetic acid (0.1%) was used to homogenize 0.1 g of freshly sampled leaf tissue. Two milliliters of 20% TCA with 0.5% TBA were added to one milliliter of the homogenate that was centrifuged at 10,000 rpm for ten minutes. After heating the extract to 95ºC for 30 minutes in a water bath, it was quickly chilled in an ice bath. Next, the extract underwent a 10-minute, 10,000 rpm centrifugation. A UV/Vis spectrophotometer was used to measure the absorbance of the supernatant at 600 nm and 532 nm.

2.2.2. Hydrogen peroxide measurement

Mukherjee and Choudhuri [27] used a modified method to assess the hydrogen peroxide content of sugar beet leaves. Cold acetone was used to extract 0.1 g of leaf samples. After adding 1 ml of 0.1% titanium dioxide to 20% (v:v) H_2SO_4 in an aliquot of the extracted solution (3 ml), the mixture was centrifuged at 6000 rpm for 15 minutes. At a wavelength of 415 nm, the yellow color supernatant's intensity was measured. From a standard curve plotted with known H_2O_2 concentrations, the concentration of H_2O_2 was calculated and expressed as mg/g FW.

2.2.3. Extraction of antioxidant enzymes

The method of Cakmak and Marschner [28] followed in this study to extract the enzymes. In liquid N_2, 0.5 g of leaf tissue was pulverized into a finely ground powder. The leaf tissue was then dissolved in 5 milliliters of a buffer solution (pH = 7.8) that contained 100 mM potassium phosphate, 0.1 mM ethylene-diamine tetra-acetic acid, and 0.1 g polyvinyl-pyrrolidone. Then, the homogenate was centrifuged at 4 °C (18,000 rpm for 10 min). Finally, the supernatants were used to test the enzyme activity.

2.2.4. Activity of superoxide dismutase (SOD, EC 1.15.1.1)

Cakmak and Marschner [28] method that used epinephrine autoxidation was adopted to evaluate the activity of superoxide dismutase. Two milliliters of the reaction media were used for this step. This medium included 100 L of enzyme extract, 200 L of 0.5 mM EDTA, and 25 mM Na-carbonate buffer at pH = 10.2. 100 L of 15 mM epinephrine (dissolved in 10 mM HCl, pH of 2.4) was added to begin the process. With the aid of a UV-Vis spectrophotometer, the autoxidation of adrenaline was measured at 480 nanometers.

2.2.5. Activity of catalase (CAT, EC 1.11.1.6)

By observing the decreased absorbance at 240 nm brought by H_2O_2, catalase activity was spectrophotometrically assessed [29]. Aliquots of 500 µL of enzyme extract and 2.4 mL of the 50 mM potassium phosphate buffer (pH 7) made up the reaction medium. 100 mL of H_2O_2 (10 mM) was added to start the reaction.

2.2.6. Activity of ascorbate peroxidase (APX, EC 1.11.1.11)

Zhang and Kirkham [29] method was used to estimate the ascorbate peroxidase's performance. The rate of H_2O_2-dependent AsA oxidation was followed using the UV-Vis spectrophotometer in a reaction mixture containing 50 mM potassium phosphate buffer (pH 7), 0.1 mM Na_2–ETDA, 5 mM H_2O_2, 0.5 mM ascorbic acid (AsA), and 50 µL enzyme extract.

2.2.7. Heat shock proteins (SDS-PAGE)

The proteins from the leaves were separated using SDS-PAGE with the discontinuous buffer technique. Electrophoresis was performed at the Molecular Biology Research and Studies Institute at Assiut University in Assiut, Egypt. Briefly, leaf polypeptides were solubilized using 65 gM Tris-HCI sample buffer (pH 6.8), 1% (v/v) 2-mercaptoethanol, 10% (v/v) glycerol, and 2% (w/v) SDS. Leaf were homogenized for 1 minute at the highest setting in a Brinkman polytron. These homogenates were centrifuged at 7,500 g for five minutes at 23 °C. The supernatant was then added to the gel sample wells after two minutes of boiling. During electrophoresis, a 35 mAmp continuous current was applied. Gels were stained for protein detection for 30 min in a solution that included 20% (v/v) methanol and 7% glacial acetic acid, 0.2% (w/v) Coomassie blue, 50% (v/v) methanol and 7% (v/v) glacial acetic acid, and 0.2% (w/v) acetic acid. Co-electrophoresis was used to determine molecular weights in conjunction with protein standards.

2.3. Measured yield parameters

The plants were harvested 200 days after seeding in order to determine the top and root yields (ton fed^{-1}) for each subplot. Each plot's ten guarded plants were randomly selected to collect the fresh weights of the top and roots (g $plant^{-1}$). From each plot, 25 kg of roots were chosen at random and submitted to the beet laboratory of the Abo-Korkas sugar factory to test for various parameters related to root quality, including sucrose%, sugar loss percentage, gross sugar yield percentage, and amino-N concentration (mmol 100 g^{-1}beet paste).

Using an autoanalyzer, the amounts of sodium (Na), potassium (K), and alpha-amino nitrogen (-amino-N) were determined by the guidelines supplied by Horwitz and Latimer [30]. The concentrations were calculated in millimoles per 100 grams of beet paste. Fresh samples of sugar beet roots were tested for sucrose concentration using the Saccharometer and the Le Docte technique [31]. Reinefeld et al. [32] stated that the following calculation was used to determine the percentage of sugar loss:

Sugar loss percentage = 0.29 + 0.343 (K+Na) + 0.094 α-amino-N

Gross sugar yield (ton $fed.^{-1}$) equal root yield (ton $fed.^{-1}$) × Sugar loss %.

2.4. Statistical analysis

The mean and standard error values of each trait were calculated from three replicates (n = 3) and represented in figures as columns with vertical bars, respectively. The results of the current study were subjected to the statistical analysis using statistical software package SPSS version 25.0 [33]. The independent sample T-test was the most suitable test for estimating the traits differences between the Assiut and Qena samples. The difference level at $p \leq 0.05$ was considered significant and represented on figures by different letters that show a significance in difference between the two temperature conditions. Cluster analysis is used to determine the similarity and dissimilarity of variable trends (correlation coefficient) using PAST software [34].

3. RESULTS & DISCUSSIONS

3.1. Physiological traits

3.1.1. Stress indicators

Enzymes that are sensitive to varying degrees of high-temperature impact different metabolic pathways. According to Asada [35], heat stress may, like other abiotic stresses, decouple metabolic pathways and enzymes, which can lead to the accumulation of undesirable and dangerous ROS like hydroxyl radical ($OH^{\bullet}$), singlet oxygen ($^1O_2$), superoxide radical ($O_2^{\bullet}$), and hydrogen peroxide (H_2O_2), which are the primary causes of oxidative stress. Heat-induced oxidative stress results in protein denaturation, which causes membrane lipid peroxidation and disrupts the stability of cell membranes [36, 37].

The results of the current study showed marked induction in MDA content in sugar beet leaves collected from high-temperature conditions (42 °C) compared to moderate-temperature conditions (23 °C) samples in both studied seasons (Fig. 1). The concentration of MDA in sugar beet leaves collected from high-temperature conditions was 18.7 and 29% higher than those of moderate-temperature conditions samples in S1 and S2, respectively. In the same context, H_2O_2 concentration in sugar beet leaves collected from high-temperature conditions was slightly higher than that in sugar beet leaves grown under moderate-temperature conditions in both studied seasons. The H_2O_2 concentration of sugar beet leaves in high temperature conditions was 11.9% and 5.4% higher than those of moderate-temperature conditions samples in S1 and S2, respectively (Fig. 1).

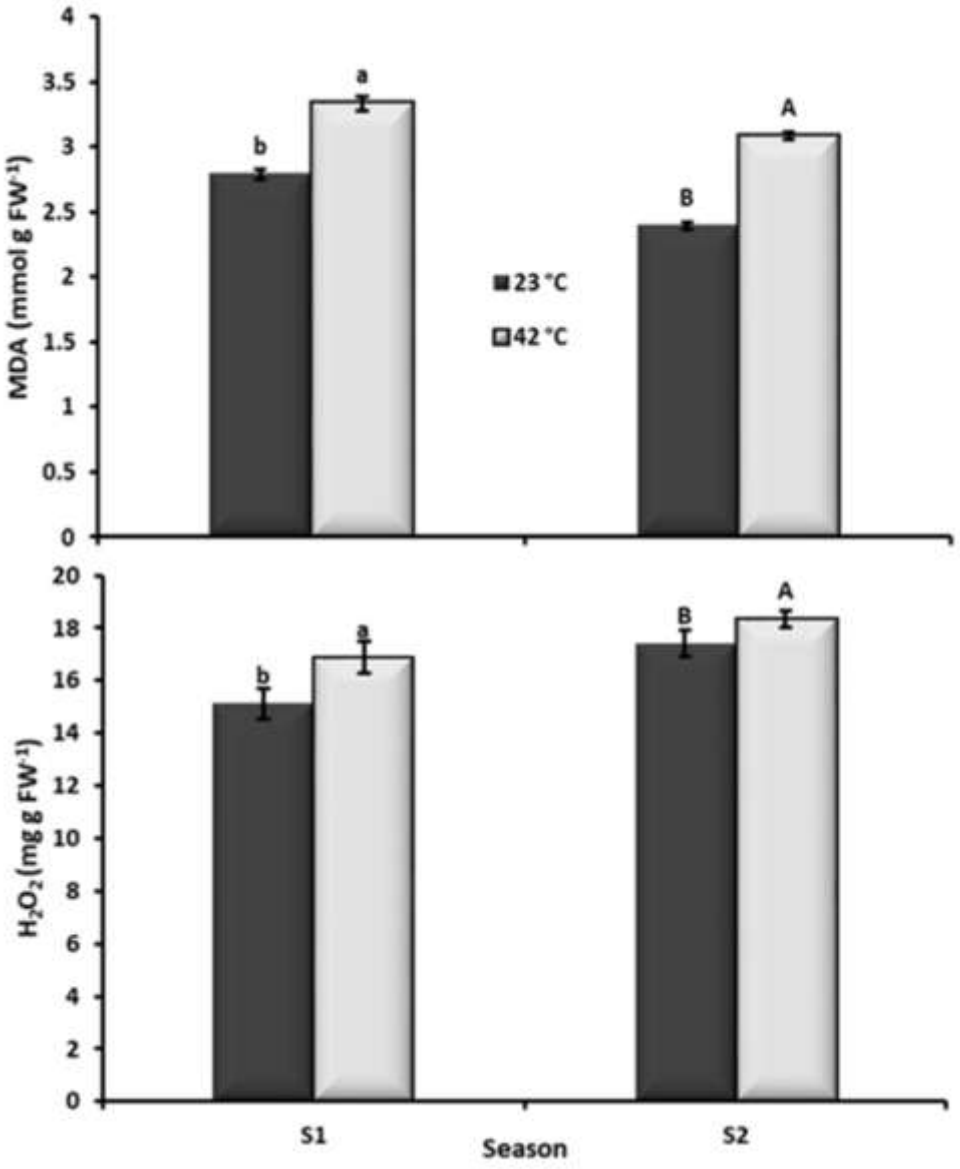

Fig. 1 Malondialdehyde (MDA) and Hydrogen peroxide (H_2O_2) contents in sugar beet leaves grown during 2018/2019 (S1) and 2019/2020 (S2) seasons under high (42°C) and moderate (23 °C) temperatures. Letters on bars refer to the difference ($P < 0.05$) or similarity ($P > 0.05$) between two temperatures.

Wheat leaves under heat stress elevated their MDA levels [38]. Rice and tobacco were found to exhibit activated membrane lipid peroxidation and membrane damage in response to high temperature stress [39, 40]. Oxidative stress was evident in populations of *Lolium perenne* those were subjected to 36 °C (moderate heat stress) and 40 °C (severe

heat stress). Higher H_2O_2 levels served as evidence on this stress that is strongly coupled with cell membrane integrity and lipid peroxidation [41].

3.1.2. Antioxidant Enzymes

The antioxidant defense mechanism plays a significant role in heat stress tolerance. It was found that heat-tolerant cultivars of wheat significantly increased their antioxidant enzymatic activities at all stages of growth in response to heat stress, whereas susceptible cultivars significantly decreased their antioxidant enzyme activities under the same conditions [42]. The current study's findings demonstrated a notable improvement in SOD and APX activity in sugar beet leaves in high-temperature conditions samples at both seasons in compared with the corresponding moderate-temperature conditions samples (Fig. 2). However, no significant differences were recorded between CAT activity in the leaves of sugar beet plants grown under high and moderate-temperature conditions in S1. SOD activity was 3.3 and 3.7 folds of those in moderate-temperature conditions samples in S1 and S2 respectively. CAT activity in leaves of sugar beet collected from high-temperature conditions plants was 45% lower than that of moderate-temperature conditions samples in S2. The most pronounced induction was recorded for APX activity in sugar beet leaves of high-temperature conditions samples during both seasons compared with moderate-temperature conditions. APX activity was 3.9 and 1.5 folds of those in moderate-temperature conditions samples in S1 and S2, respectively (Fig. 2).

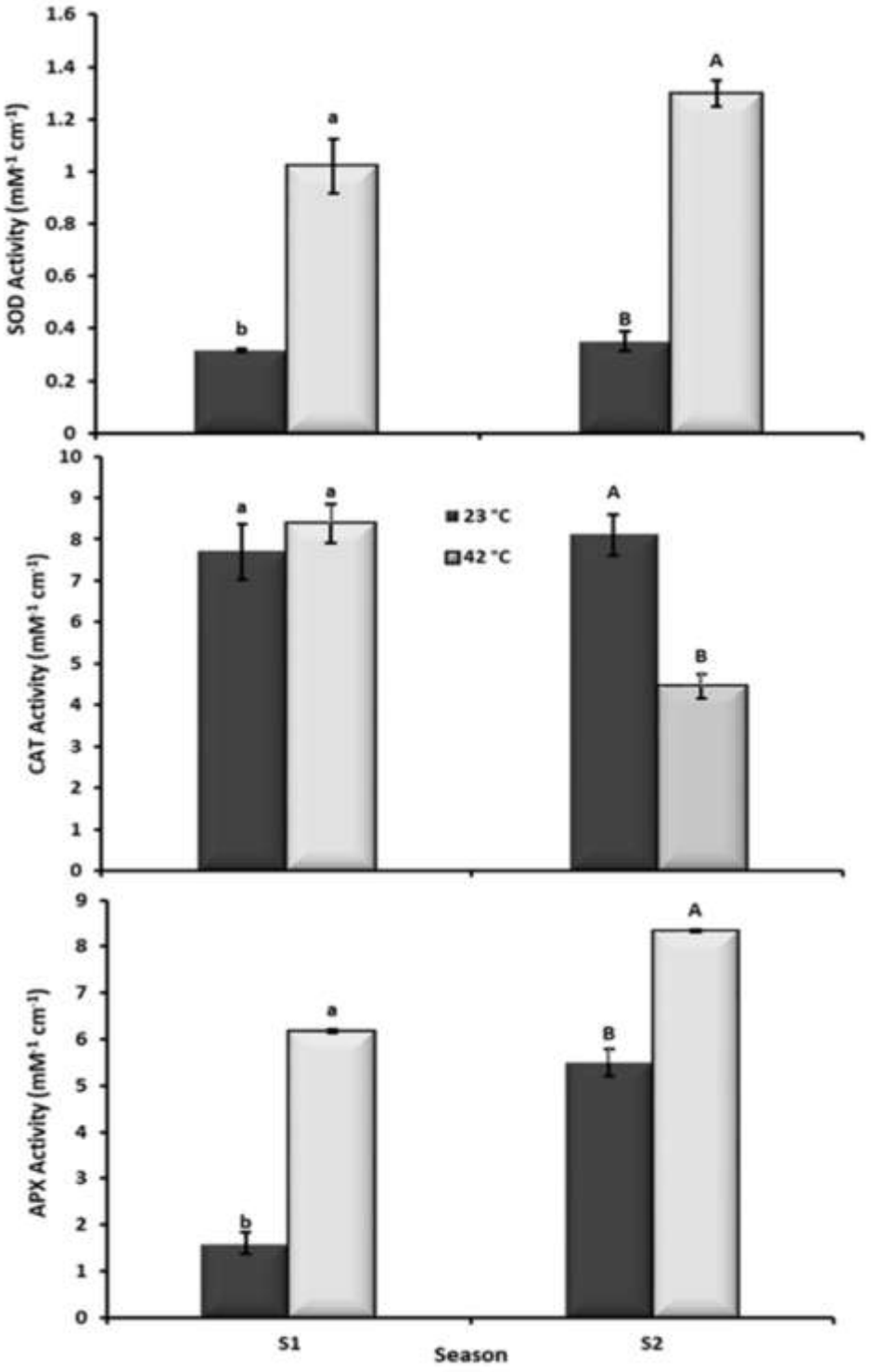

Fig. 2 Superoxid dismutase (SOD), Catalase (CAT) and Ascorbate peroxidase (APX) activity in sugar beet leaves grown during 2018/2019 (S1) and 2019/2020 (S2) seasons under high (42 °C) and moderate (23 °C) temperatures. Letters on bars refer to the difference ($P < 0.05$) or similarity ($P > 0.05$) between two temperatures.

Following the results of the present study, Chakraborty and Pradhan [43] observed that CAT, SOD, and APX showed increased activity when subjected to 38-40 °C. Hasanuzzaman et al. [44] also observed that high-temperature (38 °C) caused a rise in the antioxidant enzyme activities, including APX. Different antioxidant enzyme activities are temperature sensitive, and stimulation occurs at different temperature ranges, but the activities of these enzymes are stimulated with increasing temperature [44]. Although differences in enzymatic activity were found for various antioxidant enzymes, wheat variety tolerance demonstrated a correlation with the quantity of antioxidants. According to Almeselmani et al. [45], in the genotypes of wheat under study, several antioxidant enzymes showed a positive correlation with photosynthetic pigment concentrations and a negative correlation with the membrane injury index during the growth stages. According to Yoshimura et al. [46], plants mostly withstand temperature shocks thanks to ROS-scavenging processes.

3.1.3. Induction of heat shock proteins

Heat shock proteins (HSPs) play a vital role as defense systems against heat stress. Stress-related proteins are synthesized as a stress-tolerance strategy because of molecular changes that occur instantly after exposure to high temperatures and the perception of signals. These changes affect gene expression and transcript accumulation [18]. According to Key et al. [47], the reported induction temperatures for HSPs synthesis in laboratory-grown plants range from 38°C to 41°C. In the current study, the temperature in Qena (high-temperature conditions) was warmer in both seasons than in Assiut (moderat-temperature conditions). At the time the samples were collected, the temperature in Assiut was 23.14 °C, and in Qena it was 42.17 °C and 42.20 °C in S1 and S2, respectively. The protein of the sugar beet leaves were raised in high and moderate temperature conditions and were separated on one-dimensional SDS-polyacylamide slab gels (Fig. 3). This study found that at least seven new polypeptides accumulated in roughly 58.4% of the total matrix polypeptides when the polypeptide patterns of high temperature conditions samples (42 °C) were compared. The polypeptides' apparent molecular weights (Fig. 3, lanes A and B) were 123, 110, 103, 77, 51, 39, and 31 kD. The sugar beet leaves collected from moderate temperature conditions (23 °C) in both seasons, however, did not contain these polypeptides (Fig. 3, lanes C and D). The apparent M.Wt. and banding pattern of the accumulated polypeptides in the high temperature conditions samples strictly resemble the M.Wt for HSPs from other plant species that have been reported [48, 49, 50]. The production of HSPs enables plants to withstand and adapt to extreme abiotic stress. The synthesis of a particular range of molecules contributed to the tolerance to heat or other abiotic stress is triggered by this molecular mechanism, which also causes an increase in the synthesis of normal structural and functional proteins [51].

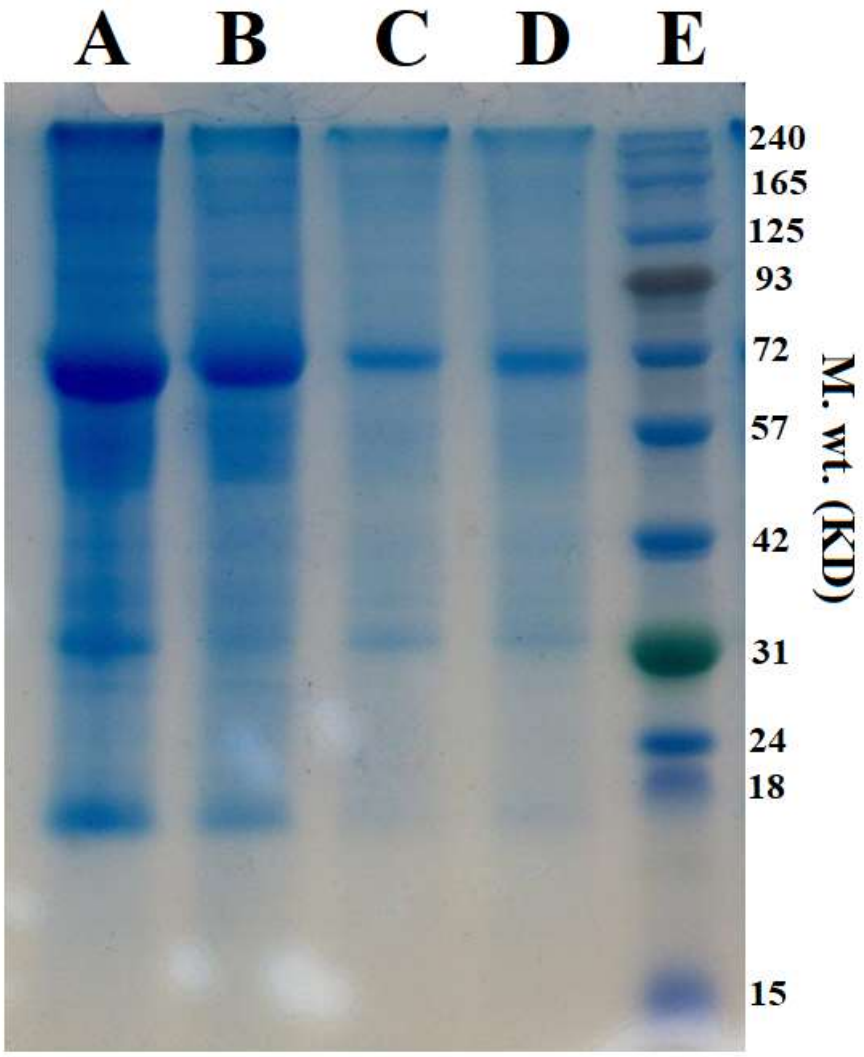

Fig. 3 SDS-polyacrylamide slab gel of polypeptide banding patterns of sugar beet leaves grown during 2018/2019 (S1) and 2019/2020 (S2) seasons under high (42 °C) and moderate (23°C) temperatures. Lane A, leaf polypeptides of high temperature plants collected in S1; lane B, leaf polypeptides of high temperature plants collected in S2; lane C, leaf polypeptides of low temperature plants collected in S1; lane D, leaf polypeptides of low temperature plants collected in S2 and lane E, standards M.Wt. Arrows and associated numbers in the right margin indicate the M.Wt. of the standards.

Numerous studies have demonstrated that ROSs play a critical role, such as molecular signals, relating plant responses to environmental stresses and even developmental stimuli, even though ROSs are unquestionably a direct cause of cellular damage on a variety of levels [52]. The chloroplast and mitochondria's ROS/redox signaling networks play significant roles in how well plants adapt to abiotic stresses. By regulating crucial processes like translation, transcription, energy metabolism, and protein phosphorylation,

these signals facilitate the intricate interaction between different cellular components and the homeostasis of organelles under stress [53]. According to Königshofer et al. [54], the production of ROS aids in the heat signal's transmission and the expression of genes that control the synthesis of heat shock proteins. The induction of HSPs in this study was correlated with a slight induction of H_2O_2 accumulation in high-temperature conditions samples compared with moderate-temperature conditions plants.

3.2. Agronomy traits

Elevated temperatures have raised global concerns about crop yields and food security due to their severe detrimental impact on crop production. Even a relatively minor increase of 1.5°C in temperature can significantly and negatively affect crop yields [55]. Higher temperatures had an impact on crop yield, mostly through altering phenological development processes [56]. The current study's findings showed that, in Qena, the first and second seasons of heat stress were characterized by the lowest mean values of root weight (845, 862 g plant^{-1}), top fresh weight (262, 274 g plant^{-1}), root fresh yield (32.67, 33.24-ton fed.$^{-1}$), top fresh yield (7.79, 9.47-ton fed.$^{-1}$), and sucrose% (17.42, 17.73%). On the other hand, the highest mean values of sugar loss (%) were 2.42, 2.51%, and α-amino-N concentration was 1.73, 1.62 (mmol 100 g^{-1} beet paste), but the lowest mean values of gross sugar yield were 0.68, 0.72-ton fed^{-1}, Fig. 4.

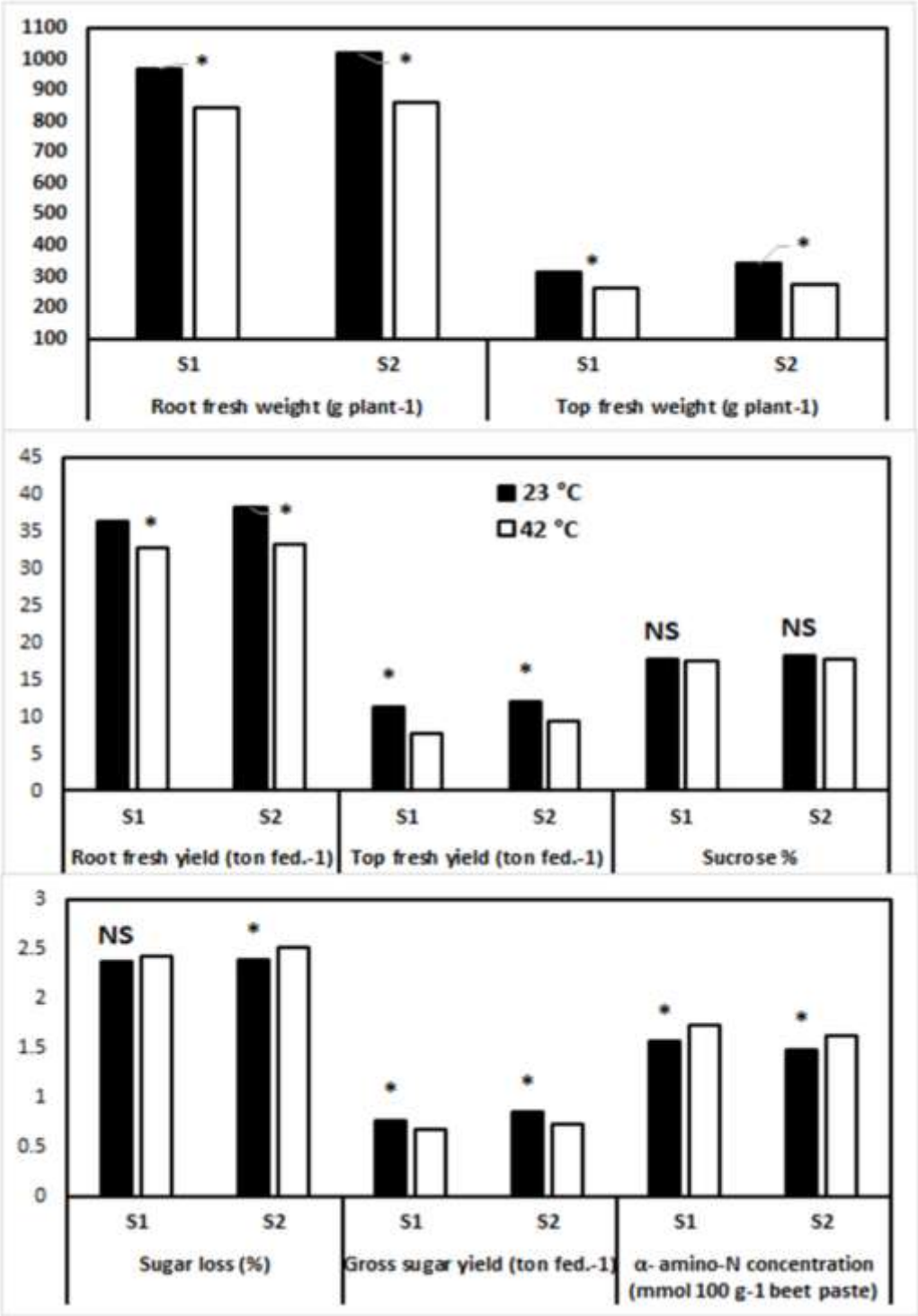

Fig. 4. Effect of heat stress (moderate and high temperature conditions) on yield and its traits of sugar beet grown in 2018/2019 (S1) and 2019/2020 (S2) seasons. NS = non-significant ($P > 0.05$) and * = significant differences ($P \leq 0.05$)

In both growth seasons, sugar beet plants exhibit a reduction in the top-root proportion in response to heat stress. Growing season conditions can be defined by the limitations imposed by stress on the crop at different phases of its development. The production of the yield component that is most actively increasing during the stress period is delayed by heat stress. In heat stress, reduced assimilatory capacity [57], which is caused by decreased photosynthesis by changed membrane stability [58], and increased maintenance respiration costs [59] are the main contributors to loss of productivity. According to Murakami et al. [60], increased temperature causes photosynthesis to be inhibited, particularly PSII activity, through a decrease in electron transport activity. According to Mohammadidian et al. [61] and Kumar et al. [62], the quantum efficiency of photosystem II (Fv/Fm), which measures the health and nutrient status of plants, is correlated with chlorophyll concentration and positively correlated with CO_2 fixation and dry matter production. Both maize [63] and barely [64] have records of these occurrences.

High temperatures (33–40 °C) severely impacted light capture, water use efficiency, biomass, gain yield, and harvest index in maize, whereas heat at the stage of flowering produced a bigger yield loss than at the grain filling period [56]. Crop quality and performance are impacted by rising temperatures. Barley's grain quality attributes were considerably altered by heat stress. While the concentrations of total non-structural starch, carbohydrates, fructose, raffinose, lipids, and aluminum were declined in barley grain, several concentrations of the proteinogenic amino acid and maltose content developed [65]. Okra exposed to high temperatures showed damage in pod quality indicators like fiber content and Ca pectate breakdown [66].

3.3. Multivariate analysis

The PCA biplot provides a providing a useful tool for analyzing and interpreting data. Visual representation of the correlations among the different beet root variables and their affinities to moderate (Assiut) and high (Qena) temperatures shown in Fig. 5A. The positions of the variables in the plot indicate their relationships with each other on the one hand and their affinities to heat stress on the other hand. By examining the scatter of points in the biplot, it is possible to identify that these variables are closer to each other on the plot have a stronger correlation (significant positive correlations). In comparison, those that are further apart have a weaker correlation. The positive correlations are shown among sucrose contents, growth traits, and yield traits (e.g. fresh and dry plant yields). On the other hand, oxidative stress enzymes (APX and SOD) were positively coupled with H_2O_2. However, they collectively are going in opposite direction with the previously mentioned growth variables (strong negative correlations).

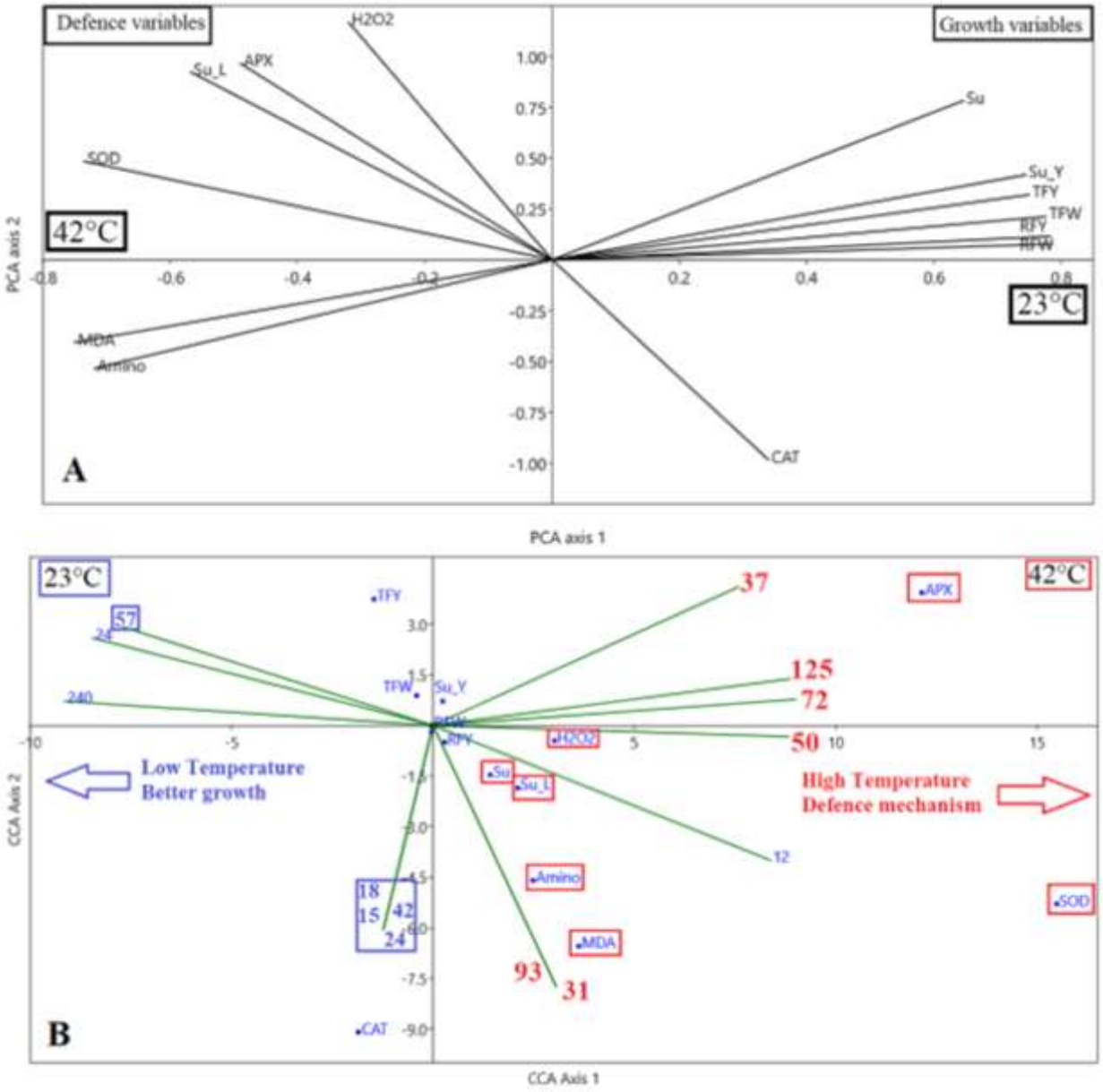

Fig. 5. A. Princible component analysis (PCA) bi-plot computes all possible correlations among estimated variables in sugar beet with their temperatures affinities. B. Canonical correspondence analysis (CCA) triple-blot illustrate all possible regressions of sugar beet variables according to SDS-PAGE bands produced under both temperatures regemes. Numbers of each band (in KD) refer to the nearest marker-bands to the accumulated sugar beet proteins. RFW = root fresh weight, RFY = root fresh yield, TFW = Top fresh weight, TFY = Top fresh yield, SuY = sugar yield, Su = sucrose (%), SuL = sugar loss (%), CAT = catalase, SOD = superoxide dismutase, APX = Ascorbate peroxidase, H_2O_2 = hydrogen peroxide, MDA = Malonaldehyde, Amino = α-amino-N.

Depending on the particular enzymes involved, the degree and duration of heat stress, as well as the physiological and genetic traits of the sugar beet plants, there may be a variety of specific mechanisms explaining the negative correlation between sugar beet enzymes and growth parameters under heat stress. Under constant stress from a variety of environmental factors, including heat, plants fight to survive. A plant may withstand heat stress to some degree by internal physiological changes as well as frequently through the production of signals that alter metabolism. In order to maintain cell turgor through osmotic adjustment, organize proteins and cellular structures, and modify the antioxidant system to restore cellular redox balance and homeostasis, plants respond to this stress by altering their metabolism in a variety of ways [67]. Heat stress modifies the expression of genes that directly defend against heat stress at the molecular level [68]. These comprise the genes that express detoxifying enzymes, regulatory proteins, transporters, and osmoprotectant expression [69]. Under circumstances like heat stress, alterations in gene expression that modify physiological and biochemical functions eventually result in the development of heat tolerance as either acclimation or, in the best scenario, adaptation [70].

A triple-blot graph that depicts every potential association is produced by canonical correspondence analysis (CCA, Fig. 5B). Using the multiple regression analysis techniques, it was used to demonstrate the correlations between the morpho-agro-physiological sugar beet variables (dependent variables) and the SDS-PAGE protein bands (intermediate variables) created under two temperature regimes (independent variables). The scatter plot's data point placements indicate the direction and strength of the associations between the protein bands and the sugar beet factors. A positive correlation could be suggested by the plot's data points being close to one another, while a negative correlation might be suggested by the dots being farther apart. As a result, it is making it possible to comprehend how various temperature regimes affect these variables better.

Obtained results revealed a strong associated between APX, SOD enzyme and H_2O_2 on the one hand and unique sugar beet protein bands recorded near to marker bands (37, 50, 72 and 125 KD) on the other hand. Also, there is good fidelity between α-amino-N and MDA with marker bands (31 and 93 KD). Notably, all these regressions were greatly influenced by the high temperature (42°C) of the Qena site in Upper Egypt. On the contrary, growth indicators (fresh and dry wt.) and protein bands near the 57 KD marker were greatly associated together and preferred the lower temperature (23°C) of the Assiut site. Also, the CAT enzyme was strongly associated with protein bands near to 15, 18, 24 and 42 KD and 23°C.

The activity of the enzymes SOD, APX, and CAT was enhanced in the wheat cultivar and showed better tolerance to heat stress and projection against ROS production [71]. Chakraborty and Pradhan [43] observed that catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD) showed an initial increase before declining to 50°C. The molecular understanding of heat shock proteins and antioxidant genes linked to heat in *Lolium perenne* [72]. Heat stress increased plant damage and oxidative stress indicators like H_2O_2 and MDA, but it also highly expressed the genes for heat shock proteins like HSP70 and antioxidants like SOD, CAT, and APX, which were linked to the

detoxification process. The interactome map (a compilation of all molecular interactions in cells, especially in relation to protein-protein interactions) was depicted by Rahman et al. [72]. This implies that genes and the expected partner functions are strongly correlated. Therefore, HSP genes and antioxidants may be important in abiotic stress.

Plants frequently display alterations in their growth indicators and antioxidant enzyme activities under stress (biotic or environmental stressors). These two vital responses (antioxidant enzymes and growth) may show an adverse relationship that may be explained by the plant's attempt to counteract the negative impacts of stress [73], like heat stress.

Stress can interfere with regular physiological functions, reducing the amount of photosynthesis, reducing the absorption of nutrients, and changing the balance of hormones. A decrease in the general growth and development of plants can be caused by several reasons. However, plants have a defense mechanism that activates their antioxidant systems to combat damage caused by stress. Antioxidant enzymes, such as APX, CAT, and SOD are essential for scavenging ROS, which are produced because of oxidative stress triggered by stress. These enzymes shield plant cells from oxidative damage and aid in the maintenance of cellular redox equilibrium.

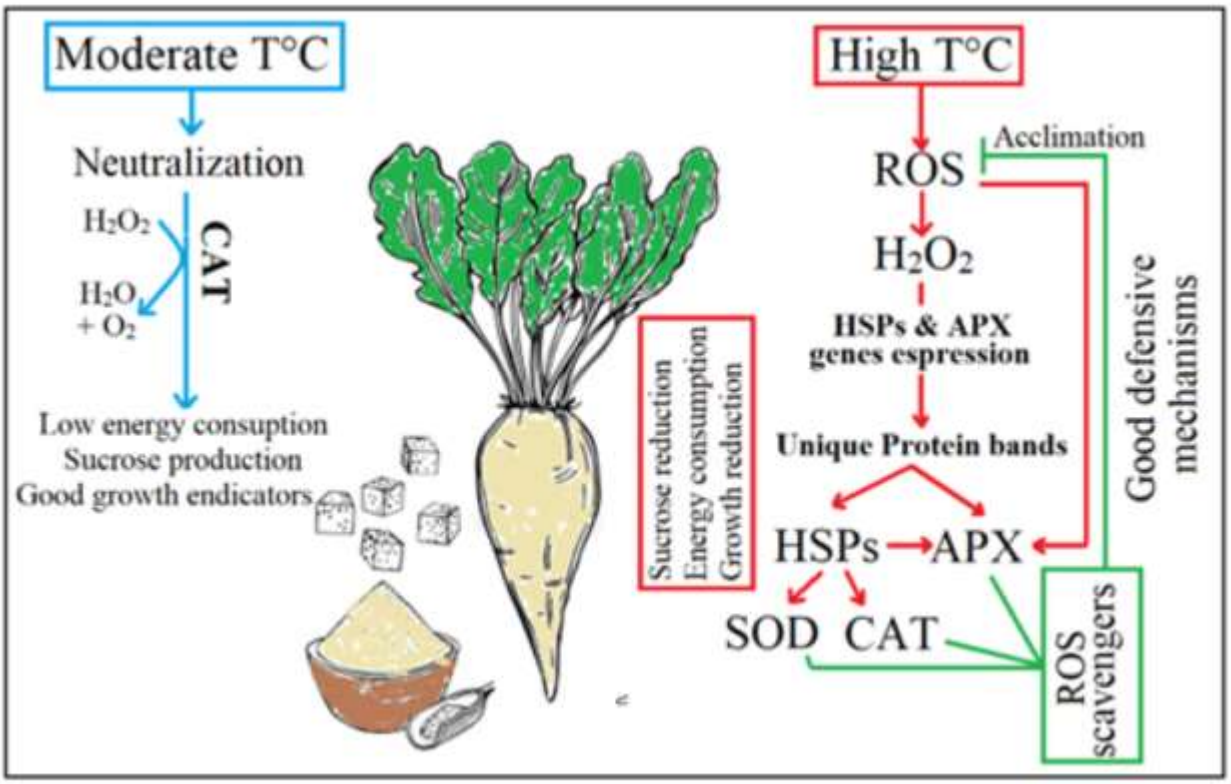

Fig. 6 All possible relations among assessed morphological and physiological variables under heat stress regimes.

The reason for the inverse relationship between antioxidant enzyme activity and growth parameters is that plants devote more of their resources to stress adaptation than to

development. To counteract oxidative stress and reduce cellular damage, a stressed plant focuses its energy and resources on activating and upregulating its antioxidant enzyme systems. This shift in resource allocation from growth-related processes to stress response mechanisms can result in reduced growth parameters [74].

In comparison to moderate heat, the sugar beet plant's membrane stability index (MDA) and stress indicator (H_2O_2) content slightly increased under heat stress conditions. Additionally, remarkable increases in the antioxidant enzyme activity of SOD and APX were seen. Low molecular weight polypeptides ranging from 31 to 123 kD were only found in Qena samples (high-temperature), suggesting that sugar beet may synthesize HSPs in response to elevated leaf temperature. Heat stress had a negative impact on some agronomic traits, such as root and top fresh weight and root and top fresh yield. The physiological parameters, antioxidant enzyme activities, and product quality parameters all showed positive correlations. Whereas sucrose (%), sugar loss (%), gross sugar yield, and α-amino-N concentration in sugar beet were the same in both regions, this showed that Qena plants' adaptability (high-temperature samples) to heat stress was augmented by the accumulation of heat shock proteins and the improved activity of antioxidant enzymes. The experiment's results can aid in a better understanding of the various mechanisms relating to sugar beet adaptability to heat stress conditions and aid in the development of cultivars with higher heat stress tolerance. Overall, the findings demonstrated the adverse effects of heat stress on sugar beet physiology and agronomic traits, which shown as possible pathways in Figure 6.

ABBREVIATIONS

MDA: Malondialdehyde; H_2O_2 Hydrogen peroxide; SOD: Superoxide dismutase; CAT: Catalase; APX: Ascorbate peroxidase; ROS: Reactive oxygen species; HSPs: Heat shock proteins; HSFs: Heat shock factors; HSR: Heat shock response; TBA: Thiobarbituric acid; TCA: Trichloroacetic acid.

REFERENCES

[1] N. Ohama, H. Sato, K. Shinozaki, K. Yamaguchi-Shinozaki. Transcriptional regulatory network of plant heat stress response. Trends Plant Sci. 22(1) (2017) 53–65.
[2] Y. Chen, C. Deng, Q. Xu. Integrated analysis of the metabolome, transcriptome and miRNome reveals crucial roles of auxin and heat shock proteins in the heat stress response of loquat fruit. Sci Hortic. 294 (2022) 110764.
[3] G. Ramakrishna, A. Singh, P. Kaur, S.S. Yadav, S. Sharma, K. Gaikwad. Genome wide identification and characterization of small heat shock protein gene family in pigeon pea and their expression profiling during abiotic stress conditions. Int J Biol Macromol. 197 (2022) 88–102.
[4] S. Haider, J. Iqbal, S. Naseer, T. Yaseen, M. Shaukat, H. Bibi, Y. Ahmad, H. Daud, N.L. Abbasi, T. Mahmood. Molecular mechanisms of plant tolerance to heat stress: current landscape and future perspectives. Plant Cell Rep, 40(12) (2021) 2247–2271.

[5] J.L. Hatfeld, J.H. Prueger. Temperature extremes: Effect on plant growth and development. Weather Clim Extrem. 10 (2015) 4–10.
[6] W. Schlenker, M.J. Roberts. Nonlinear temperature effects indicate severe damages to U.S. crop yields under climate change. Proc Nat Acad Sci. 106(37) (2009) 15594–15598.
[7] N.V. Fedoroff, D.S. Battisti, R.N. Beachy. Radically rethinking agriculture for the 21st century. Science. 327 (2010) 833–834.
[8] A. Ullah, F. Nadeem, A. Nawaz. Heat stress effects on the reproductive physiology and yield of wheat. J Agro Crop Sci. 208 (2022) 1–17.
[9] O. Hernandez, C.A. Torres, M.A. Moya-Leon, M.C. Opazo, I. Razmilic. Roles of the ascorbate-glutathione cycle, pigments and phenolics in postharvest 'sunscald' development on 'Granny Smith' apples (*Malus domestica* Borkh.). Postharvest Biol Technol. 87 (2014) 79–87.
[10] H.L. Liu, Z.X. Lee, T.W. Chuang. Effect of heat stress on oxidative damage and antioxidant defense system in white clover (*Trifolium repens* L.). Planta. 254 (2021) 103
[11] A.I. Kokorev, M.A. Shkliarevskyi, Y.E. Kolupaev. Involvement of Nitric Oxide in the Implementation of the Stress-Protective Action of Cadaverine on Wheat Seedlings under Hyperthermia and Its Relations to Different Signal Messengers. Russ J Plant Physiol. 69 (2022) 68.
[12] M. Liu, T. Sun, C. Liu. Integrated physiological and transcriptomic analyses of two warm- and cool-season turfgrass species in response to heat stress. Plant Physiol Biochem. 170 (2022;) 275–286.
[13] B. Huang, D. Michelle, Y. Jiang. Research advances in mechanisms of turfgrass tolerance to abiotic stresses: from physiology to molecular biology. Crit Rev Plant Sci. 33 (2014) 141–189.
[14] J. Zhao, Z. Lu, L. Wang, B. Jin. Plant responses to heat stress: physiology, transcription, noncoding RNAs, and epigenetics. Int J Mol Sci. 22(1) (2021) 117.
[15] S. Haider, A. Raza, J. Iqbal, M. Shaukat, T. Mahmood. Analyzing the regulatory role of heat shock transcription factors in plant heat stress tolerance: a brief appraisal. Mol Biol Rep. 49(6) (2022) 5771–5785.
[16] P. Jacob, H. Hirt, A. Bendahmane. The heat-shock protein/ chaperone network and multiple stress resistance. Plant Biotechnol J. 15(4) (2017) 405–414.
[17] S. Fragkostefanakis, S. Röth, E. Schleif, K.D. Scharf. Prospects of engineering thermotolerance in crops through modulation of heat stress transcription factor and heat shock protein networks. Plant Cell Environ. 38(9) (2015) 1881–1895.
[18] X. Pan, Y. Zheng, K. Lei. Systematic analysis of Heat Shock Protein 70 (*HSP70*) gene family in radish and potential roles in stress tolerance. BMC Plant Biol. 24 (2024) 2.
[19] M.G. Santoro. Heat shock factors and the control of the stress response. Biochem Pharmacol. 59 (2000) 55–63.
[20] A.A.A. Al-Ghzawi, M.B. Al-Zghoul, S. Zaitoun, I. M. Al-Omary, N. A. Alahmad. Dynamics of heat shock proteins and heat shock factor expression during heat stress in daughter workers in pre-heat-treated (rapid heat hardening) *Apis mellifera* mother queens. J Therm Biol. 104 (2022) 103194.

[21] A. Nazir, D.K. Saxena, D. Kar Chowdhuri. Induction of hsp70 in transgenic Drosophila: biomarker of exposure against phthalimide group of chemicals. Biochim Biophys Acta. 1621 (2003) 218–225.
[22] R.K. Ganapati, M.M. Rahman, R.C. Kabiraj, R. Sen, M.S. Islam. Screening of water stress tolerant sugar beet (*Beta vulgaris* L.) genotypes. Int J Sustain Crop Prod. 11(3) (2016) 29–35.
[23] S.I. Zandalinas, R. Mittler, D. Balfagón, V. Arbona, A. Gómez-Cadenas. Plant adaptations to the combination of drought and high temperatures. Physiol Plant. 162(1) (2018) 2–12.
[24] E.S. Ober, A. Rajabi. Abiotic stress in sugar beet. Sugar Tech. 12(3–4) (2010) 294–298.
[25] F. Karandish, S.S. Mousavi, H. Tabari. Climate change impact on precipitation and cardinal temperatures in different climatic zones in Iran: analyzing the probable effects on cereal water-use efficiency. Stoch Environ Res Risk Assess. 31(8) (2017) 2121–2146.
[26] S.S. Narwal, R. Bogatek, B.M. Zagdanska, D.A. Sampietro, M. Vattuone. Plant Biochemistry; Studium Press LLC: Houston, TX, USA. (2009).
[27] S.P. Mukherjee, M.A. Choudhuri. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings. Physiol Plant. 58 (1983) 166–170.
[28] I. Cakmak, H. Marschner. Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. Plant Physiol. 98 (1992) 1222–1227.
[29] J. Zhang, M.B. Kirkham. Antioxidant responses to drought in sunflower and sorghum seedlings. New Phytol. 132(3) (1996) 361-73.
[30] Horwitz W and Latimer GW. Official Methods of Analysis of AOAC International. 18th Edition, Association of Official Analytical Chemistry International, Maryland. (2005).
[31] A. Le Docte. Commercial determination of sugar in the beet root using the Sacks Le Docte process. Inter Sugar J. 29 (1927) 488–492.
[32] E. Reinefeld, A. Emmerich, G. Baumgarten, C. Winner, U.M. Beiß. Zur Voraussage des Melassezuckersaus Rübenanalysen. Zucker. 27 (1974) 2–15.
[33] IBM Corp. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp. (2017).
[34] Hammer, Ø., Harper, D.A.T., and P. D. Ryan. PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeont Electr. 4(1) (2001) 9pp.
[35] K. Asada. Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol. 141 (2006) 391–396.
[36] M. Rodríguez, E. Canales, O. Borrás-Hidalgo. Molecular aspects of abiotic stress in plants. Biotechnol Apl. 22(1) (2005) 1–10.
[37] D. Camejo, A. Jiménez, J.J. Alarcón, W. Torres, J.M. Gómez, F. Sevilla. Changes in photosynthetic parameters and antioxidant activities following heat–shock treatment in tomato plants. Func Plant Biol. 33(2) (2006) 177–187.
[38] M. Savicka, N. Škute. Effects of high temperature on malondialdehyde content, superoxide production and growth changes in wheat seedlings (*Triticum aestivum* L.). Ekologija. 56(1) (2010) 26–33.

[39] A.R. Mohammed, L. Tarpley. Effects of high night temperature and spikelet position on yield-related parameters of rice (*Oryza sativa* L.) plants. Eur J Agron. 33 (2010) 117–123.
[40] W. Tan, Q.W. Meng, M. Brestic, K. Olsovska, X. Yang. Photosynthesis is improved by exogenous calcium in heat-stressed tobacco plants. J Plant Physiol. 168 (2011) 2063–2071.
[41] W.S. Soliman, M. Fujimori, K. Tase, S.I. Sugiyama. Oxidative stress and physiological damage under prolonged heat stress in C3 grass *Lolium perenne*. Grassland Sci. 57 (2011) 101–106.
[42] M. Almeselmani, P.S. Deshmukh, R.K. Sairam. High temperature stress tolerance in wheat genotypes: role of antioxidant defense enzymes. Acta Agron Hung. 57 (2009) 1–14.
[43] U. Chakraborty, D. Pradhan. High temperature-induced oxidative stress in *Lens culinaris*, role of antioxidants and amelioration of stress by chemical pre-treatments. J Plant Interact. 6 (2011) b43–52.
[44] M. Hasanuzzaman, K. Nahar, M. Fujita. Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages. In: P. Ahmad, M.M. Azooz, M.N.V. Prasad (ed) Ecophysiology and Responses of Plants under Salt Stress, Springer, New York, NY, USA, pp 25–87. (2013).
[45] M. Almeselmani, P.S. Deshmukh, R.K. Sairam, S.R. Kushwaha, T.P. Singh. Protective role of antioxidant enzymes under high temperature stress. Plant Sci. 171 (2006) 382–388.
[46] K. Yoshimura, K. Miyao, A. Gaber. Enhancement of stress tolerance in transgenic tobacco plants overexpressing Chlamydomonas glutathione peroxidase in chloroplasts or cytosol. Plant J. 37 (2004) 21–33.
[47] J.L. Key, C.Y. Lin, Y.M. Chen. Heat shock proteins of higher plants. Proc Natl Acad Sci USA 78 (1981) 3526-3530.
[48] J.J. Burke, J.L. Hatfield, R.R. Kleini, J.E. Mulle. Accumulation of Heat Shock Proteins in Field-Grown Cotton. Plant Physiol. 78 (1985) 394–398.
[49] C. Lenne, R. Douce. A Low Molecular Mass Heat-Shock Protein 1s Localized to Higher Plant Mitochondria. Plant Physiol. 105 (1994) 1255–1261.
[50] T. Jinn, Y. Chen, C. Lin. Characterization and physiological function of class I low Molecular-mass, heat-shock protein complex in soybean. Plant Physiol. 108 (1995) 693-701.
[51] G.J. Lee, E. Vierling. A small heat shock protein cooperates with heat shock protein 70 systems to reactivate a heat-denatured protein. Plant Physiol. 122 (2000) 189–198.
[52] T.S. Gechev, F. Van Breusegem, J.M. Stone, I. Denev, C. Laloi. Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. Bioessays. 28 (2006) 1091–1101.
[53] R. Mittler, S. Vanderauwera, N. Suzuki. ROS signaling: the new wave? Trends Plant Sci. 16 (2011) 300–309.
[54] H. Königshofer, H.W. Tromballa, H.G. Löppert. Early events in signaling high-temperature stress in tobacco BY2 cells involve alterations in membrane fluidity and enhanced hydrogen peroxide production. Plant Cell Environ. 31 (2008) 1771–1780.

[55] J. Warland, A.W. McKeown, M.R. McDonald. Impact of high air temperatures on Brassicacae crops in southern Ontario. Can J Plant Sci. 86(4) (2006) 1209–1215.
[56] X. Zhang, J. Cai, B. Wollenweber, F. Liu, T. Dai, W. Cao, D. Jiang. Multiple heat and drought events affect grain yield and accumulations of high molecular weight glutenin subunits and glutenin macropolymers in wheat. J Cereal Sci. 57: (2013) 134–140.
[57] R.K.M. Hay, J.R. Porter. The Physiology of Crop Yield. Blackwell, Oxford, UK. (2006).
[58] Y. Zhang, M.A.R. Mian, J.H. Bouton. Recent molecular and genomic studies on stress tolerance of forage and turfgrasses. Crop Sci. 46 (2006) 497–511.
[59] M.P. Reynolds, C.S. Pierre, A.S.I. Saad, M. Vargas, A.G. Condon. Evaluating potential genetic gains in wheat associated with stress-adaptive trait expression in elite genetic resources under drought and heat stress. Crop Sci. 47 (2007) 172–189.
[60] Y. Murakami, M. Tsuyama, Y. Kobayashi, H. Kodama, K. Iba. Trienoic fatty acids and plant tolerance of high temperature. Science 287(5452) (2000) 476–479.
[61] R. Mohammadian, S.Y. Sadeghian, M. Moghaddam, H. Rahimian. Evaluation of drought tolerance indices in determining sugar beet genotypes under early season drought conditions. J Sugar Beet. 18 (2003) 29-51.
[62] M. Kumar, V. Govindasamy, J. Rane. Canopy temperature depression (CTD) and canopy greenness associated with variation in seed yield of soybean genotypes grown in semi-arid environment. S Afr J Bot. 113 (2017) 230–238.
[63] M. Cicchino, J.I.R. Edreira, M. Uribelarrea, M.E. Otegui. Heat stress in field grown maize: response of physiological determinants of grain yield. Crop Sci. 50(4) (2010) 1438–1448.
[64] P Högy, C. Poll, S. Marhan, E. Kandeler, A. Fangmeier. Impacts of temperature increase and change in precipitation pattern on crop yield and yield quality of barley. Food Chem. 136 (2013) 1470–1477.
[65] F. Vasseur, F. Pantin, D. Vile. Changes in light intensity reveal a major role for carbon balance in Arabidopsis responses to high temperature. Plant Cell Environ. 34 (2011) 1563–1576.
[66] J.I.R. Edreira, M.E. Otegui. Heat stress in temperate and tropical maize hybrids: Differences in crop growth, biomass partitioning and reserves use. Field Crops Res. 130 (2012) 87–98.
[67] A. Janska, P. Marsik, S. Zelenkova, J. Ovesna. Cold stress and acclimation: What is important for metabolic adjustment? Plant Biol. 12 (2010) 395–405.
[68] V. Chinnusamy, J. Zhu, T. Zhou, J.K. Zhu. Small RNAs: Big Role in Abiotic Stress Tolerance of Plants. In: Jenks MA, Hasegawa PM, Jain SM (ed) Advances in Molecular Breeding toward Drought and Salt Tolerant Crops. Springer, Dordrecht, The Netherland, pp 223–260. (2007).
[69] J. Krasensky, C. Jonak. Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. J Exp Bot. 63(4) (2012) 1593–1608
[70] A.A. Moreno, A. Orellana. The physiological role of the unfolded protein response in plants. Biol Res. 44 (2011) 75–80.
[71] K. Balla, S. Bencze, T. Janda, O. Veisz. Analysis of heat stress tolerance in winter wheat. Acta Agron Hung. 57 (2009) 437–444.

[72] M.A. Rahman, J.H. Woo, Y. Song, S.H. Lee. Heat Shock Proteins and Antioxidant Genes Involved in Heat Combined with Drought Stress Responses in Perennial Rye Grass. Life 13;12(9) (2022) 1426.
[73] M. Imtiaz, S. Tu, Z. Xie, D. Han, M. Ashraf, M.S. Rizwan MS. Growth, V uptake, and antioxidant enzymes responses of chickpea (*Cicer arietinum* L.) genotypes under vanadium stress. Plant Soil. 390 (2015) (17-27)
[74] A.K. Shanker, B. Venkateswarlu. Abiotic stress response in plants-physiological, biochemical and genetic perspectives. Agricultural and Biological Sciences. Intech: Rijeka, Croatia. (2011).