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Research Article

Physiological and Biochemical Effects of Thermo-Priming on Wheat (*Triticum aestivum* L.) under Drought and Heat Stresses

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ABSTRACT

Seed priming is a physical method for increasing the stress tolerance of crops against stressful environmental conditions. Drought and high temperatures are important environmental factors that limit the growth and grain yield of wheat. The aim of our study is to determine the physiological (germination rate, root and shoot length, specific leaf area (SLA), relative water content (RWC), biomass, total chlorophyll amount (SPAD)), and biochemical (protein amount, hydrogen peroxide (H_2O_2) amount, catalase activity (CAT), ascorbate peroxidase activity (APX), glutathione reductase activity (GR)) changes that occur with thermo-priming in wheat seeds under drought stress (D) and heat stress (H). Our results showed that shoot lengths were drastically reduced with D, H, and HD compared to root lengths. Besides, combined stress protected RWC by 6.8% with 60 min thermo-priming compared to other stress treatments. Chlorophyll content decreased dramatically with D and H, while thermo-priming wasn't limited to that decrease. In addition, SLA was decreased with all stress treatments, while it healed only with 60 min thermo-priming (HDT60) by 12%. H_2O_2 was increased with drought stress, while reduced with all heat stress treatments. Among them, HDT60 was found to be more effective than the others. GR activities were increased with thermo-priming by 14-18%, with D and H by 5%. Additionally, GR activity was increased with 30 min thermo-priming (HDT30) in HD treatment by 5.8%, while only with HD by 3.2%. Consequently, HDT60 seemed to effectively on biochemical parameters in wheat seedlings against drought and heat stresses.

Keywords: *Drought stress, Heat stress, Osmotic stress, Thermo-priming, Wheat*

Kuraklık ve Isı Stresi altındaki Buğdayda Termo- Priming'in Fizyolojik ve Biyokimyasal Etkileri

ÖZET

Tohum priming, bitkilerin stresli çevre koşullarına karşı stres toleransını arttırmaya yönelik fiziksel bir yöntemdir. Kuraklık ve yüksek sıcaklıklar, buğdayın büyümesini ve tane verimini sınırlayan önemli çevresel faktörlerdir. Çalışmamızın amacı kuraklık stresi (D) ve sıcaklık stresi (H) altındaki buğday tohumlarında termo-priming ile meydana gelen fizyolojik (çimlenme oranı, kök ve gövde uzunluğu, spesifik yaprak alanı (SLA), bağıl su içeriği (RWC), biyokütle, toplam klorofil miktarı (SPAD)) ve biyokimyasal (protein miktarı, hidrojen peroksit (H_2O_2) miktarı, katalaz aktivitesi (CAT), askorbat peroksidaz aktivitesi (APX), glutatyon redüktaz aktivitesi (GR)) değişimleri belirlemektir. Sonuçlarımız, gövde uzunluklarının kök uzunluklarına kıyasla D, H ve HD ile çarpıcı şekilde azaldığını gösterdi. Ayrıca kombine stres, diğer stres uygulamalarına kıyasla 60 dakikalık termo-priming ile RWC %6,8 oranında korunmuştur. Klorofil içeriği, D ve H ile önemli ölçüde azalırken, termo-priming bu

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düşüşle sınırlı kalmadı. Ayrıca SLA tüm stres tedavilerinde azalırken sadece 60 dk termo-priming (HDT60) ile %12 iyileştirdi. H_2O_2 kuraklık stresi ile artarken, tüm ısı stresi uygulamaları ile azalmıştır. Bunlardan HDT60'ın diğerlerinden daha etkili olduğu tespit edildi. GR aktiviteleri termo-priming ile yaklaşık %14-18, D ve H uygulamaları ile %5 arttırıldı. Ek olarak, HD uygulamasında 30 dakikalık termo-priming (HDT30) ile GR aktivitesi %5,8 artarken, yalnızca HD ile %3,2 arttı. Sonuç olarak, HDT60'ın buğday fidelerinde kuraklık ve ısı streslerine karşı biyokimyasal parametreler üzerinde etkili olduğu görülmüştür.

Anahtar Kelimeler: *Kuraklık stresi, Isı stresi, Osmotik stres, Termo-priming, Buğday*

I. INTRODUCTION

The world population is expected to increase rapidly until 2050. For this reason, it is thought that the existing lands will be insufficient for growing grains in order to meet the increasing food needs in the future. The decrease in agricultural lands as a result of climate change, the increase in biotic and abiotic stress factors, and the ever-intensifying global climate change are among the important obstacles to agriculture and food production [1]. Wheat, an annual herbaceous plant, is used as a basic and strategic nutrient both in Turkey and in the world. Wheat ranks third place in grain production in the world with 762 million tons [2]. Today, wheat production is seriously affected by drought and heat stresses [3]. In the twentieth century, the average temperature was increased by 0.3 °C in the world at last 10 years and it is expected to increase by 1°C in 2025 and more than 3°C in 2100 [4]. These results herald more severe global climate changes in the future.

Abiotic stress factors cause morphological, physiological, biochemical, and molecular changes in plants and reduce their yield by limiting their growth and development [5]. Similarly, drought and heat stresses limit grain production and yield in the world and in Turkey. Moreover, in many parts of the world heat stress can cause wheat, mustard, tomato, etc. It creates negative effects on the growth, metabolism, and productivity of various crops [6]. The state of water deficiency increases due to high temperature, which affects the growth, metabolism, and integrity of the plant. In addition, they increase the production of reactive oxygen species (ROS) produced at normal levels by damaging photosynthetic pigments, membrane lipids, proteins, and nucleic acids in plants [7]. ROS increase causes very serious damage to the cell and can lead to cell death. Therefore, many abiotic stress factors, including drought, also cause oxidative stress in the plant [8]. Oxidative stress, which is characterized by excessive ROS production, occurs during the reduction of oxygen to water during photosynthesis, oxidation of water in mitochondria, and electron transfer in chloroplasts [9]. ROS disrupts the stable structure of many biological molecules such as proteins, DNA, lipids, and carbohydrates. However, plants have (APX, Ascorbate peroxidase; CAT, Catalase; SOD, Superoxide dismutase; GR, Glutathione reductase) and non-enzymatic antioxidants (tocopherols, carotenoids, water-soluble reductants) to protect them from these harmful effects. Thus, they reduce the subcellular damage of ROS [10], [11]. Monitoring the antioxidant system in stress tolerance is suggested as a useful selection criterion to reduce stress-related yield loss, especially in agricultural products [12].

Priming is defined as the treatment that allows the seeds to take up water in an osmotic solution or water until the first stage of germination is completed, but does not allow the emergence of the rootlets [13], [14]. Priming, which is a physiological technique including hydration and drying of seeds to improve metabolic processes before germination, has been reported to increase the germination rate, seedling growth, and yield under biotic and abiotic stresses and can induce plant tolerance [15], [16]. In the thermo-priming technique, the seeds are kept in a dark environment at a high temperature for certain periods. Germination rates are positively affected by low or high-temperature treatments before planting [17], [18]. Thermo-priming treatments both positively affect seed germination and seedling emergence, and also help plant growth and development. In addition, it has been determined that it has positive effects on enzyme activity, plant growth, and metabolism [19]. In this context, priming has been reported to be a promising strategy for plants to cope with abiotic stresses, including high-temperature stress [20]. In wheat, seedling emergence rate [21], seedling shoot and root length, seedling root and shoot dry

weight [22], early flowering and harvesting, and nitrogen use efficiency [23] has also been reported to increase grain and straw yield, harvest index [21] and yield [24] in late sowing.

In this study, we focused on determining the physiological (germination rate, root and shoot length, specific leaf area (SLA), relative water content (RWC), biomass, the total chlorophyll amount (SPAD), hydrogen peroxide (H_2O_2) amount (spectrophotometric and histochemical staining)) and biochemical (protein amount, catalase activity (CAT), ascorbate peroxidase activity (APX), glutathione reductase activity (GR)) analyzes caused by thermo-priming in the drought sensitive wheat variety Ekiz.

II. MATERIAL and METHODS

A. PLANT MATERIALS, SEED STERILIZATION, PRIMING TREATMENT, and GROWTH CONDITION

Triticum aestivum L. is a type of bread wheat from the family *Poaceae*. In this study, wheat seeds of the drought-sensitive Ekiz culture variety were used, and the seeds were obtained from Konya Bahri Dagdas International Agricultural Research Institute. Surface sterilization of seeds was carried out with a 5% sodium hypochlorite solution. For thermo-priming (TP) treatment, the seeds were kept at 38±2°C for 30 min (T30) and 60 min (T60) [25]. At the end of the treatment, the seeds were washed with distilled water and their surfaces were dried at room temperature. The development of the seeds sown in petri dishes in the control and drought stress ((polyethylene glycol, 15% PEG 6000) groups were followed for 7 days in a growth chamber (24±2°C temperature, 16/8h day/night photoperiod) under controlled conditions. Heat stress (H) treatment of all groups (7-day-old seedlings) was carried out by keeping the seedlings in a plant growth chamber at 42°C for 3 hours.

Table 1. *The priming treatments groups in stressed seedlings (Control groups (C: Control, D: Drought, T30: Thermo-priming 30 min, T60: Thermo-priming 60 min, DT30: Drought + Thermo-priming 30 min, DT60: Drought + Thermo-priming 60 min), Stress groups (H:Heat stress, HD: Drought and Heat stress, HT30: Thermo-priming 30 min. + Heat stress, HT60: Thermo-priming 60 min. + Heat stress, HDT30: Thermo-priming 30 min. + Drought + Heat stress, HDT60: Thermo-priming 60 min. + Drought + Heat stress).*

Control Groups	Stress Groups
Control (C)	Heat stress (H)
Drought stress (D)	Drought and Heat stress (HD)
TP 30-min. (T30)	TP 30-min. + H (HT30)
TP 60-min. (T60)	TP 60-min. + H (HT60)
TP 30-min.+ D (DT30)	TP 30-min. + D and H (HDT30)
TP 60-min.+ D (DT60)	TP 60-min. + D and H (HDT60)

B. METHODS

B. 1. Physiological analyzes

The germination rate was determined after 7 days according to the formula (1) below.

$$Germination\ rate\ =\ Germination\ number\ \times \frac{100}{15} \quad (1)$$

The green part up to the root was determined as the shoot length (cm) and the root part as the root length (cm) of the wheat seedlings in all groups with the help of a ruler.

Biomass the weight of three seedlings from each group was determined by weighing on the precision scale (g plant^{-1}).

Specific leaf area (SLA) was calculated using the leaf photos of wheat seedlings in the Image J program. After the samples are dried at 70°C for 24 h and recorded their wieght. SLA is calculated by the formula (2) [26]:

$$\mathrm{SLA} = Area\ (cm^2)\ /\ Dry\ weight(mg^{-1}) \quad (2)$$

The leaves whose wet weight (FW) was determined for the relative water content (RWC) were kept in a plastic container containing pure water for 4 h between filter papers, and their turgor weights (TW) were recorded. After this process, the dry weights (DW) of the leaves were determined after they were dried in an oven at 70°C for 24 h. RWC was calculated by applying these values to the formula (3) [27]:

$$\mathrm{RWC} = (\mathrm{FW} - \mathrm{DW})\ /\ (\mathrm{TW} - \mathrm{DW})\ \times\ 100 \quad (3)$$

Chlorophyll content of the leaf samples was recorded with the aid of a chlorophyllmeter (Minolta, SPAD-502) [28]. Experimental data were obtained from different leaves of the seedlings with 15 replicates..

B. 2. Biochemical analyzes

Total protein content was homogenized leaf tissue with 50 mM NaP buffer (pH 7.8, 1 mM EDTA) and centrifuged for protein anlaysis. 0.1 g of Coomassie Brilliant Blue G 250 was mixed in a tube with protein reagent containing ethanol (50 mL) and ortho-phosphoric acid (100 mL). The absorbance values determined at 595 nm in the spectrophotometer were used to calculate the total protein content (mg g^{-1}) on the standard graph [29].

Ascorbate peroxidase activity (APX) was measured according to [30]. The reaction mixture contained 0.05 M Na-phosphate buffer (pH 7), 0.5 mM ascorbate, 0.1 mM EDTA.Na_2, 1.2 mM H_2O_2, and 0.1 mL enzyme extract in a final assay volume of 1 mL. Ascorbate oxidation was assessed at 290 nm. The concentration of oxidized ascorbate was calculated using extinction coefficient. One unit of APX was defined as 1 mmol mL^{-1} ascorbate oxidized per min.

Glutathione reductase activity (GR) was measured according to [31]. The reaction mixture contained 0.025 mM Na-phosphate buffer (pH 7.8), 0.5 mM GSSG, 0.12 mM NADPH.Na_4 and 0.1 mL enzyme of extract in a final assay volume of 1 mL. NADPH oxidation was determined at 340 nm. The activity was calculated using the extinction coefficient for GSSG. One unit of GR was defined as 1 mmol mL^{-1} GSSG reduced per min.

Catalase activity (CAT) was measured by measuring the initial rate of disappearance of H_2O_2 [32]. The reaction mixture contained 3% H_2O_2 and 0.1 mM EDTA in 0.05 M Na-phosphate buffer (pH 7) and 70 µl enzyme extract in a final assay volume of 1 mL. The decrease in H_2O_2 was measured as a decline in optical density at 240 nm, and the activity was calculated as µmol H_2O_2 consumed per min. Thermo Scientific Genesys Ones UV-Vis spectrophotometer was used during all spectrophotometric analyses in this study.

Hydrogen peroxide amount (H_2O_2), a mixture of plant tissue (0.1 g), 3 ml of H_2SO_4 and cold acetone was homogenized with homogenization buffer and centrifuged. Supernatants were determined at 550-800 nm (µg/ml) spectrophotometric with reading buffer containing H_2SO_4, purified water, ferrous ammonium sulfate, xylenol orange, sorbitol, and ethanol (e-FOX) [33].

For histochemical staining localization of H_2O_2, leaves were immersed in a solution containing 1 mg/ml 3′,3′-diaminobenzidine (DAB) at 25°C for 12 h. The incubated leaves were decolorized by immersion

in boiling ethanol (90%) for 15 min to visualize the reddish-brown spots of H_2O_2. Then stained leaves were photographed against a contrasting background for proper visual [34].

B. 3. Statistical analysis

The results were given as means ± standard error of five replicates. The compiled data were subject to an ANOVA (ONE-WAY) and the differences between the means were compared by the Tukey test to assess the effect of thermo-priming on physiological and biochemical analysis in *T. aestivum (*cv. Ekiz) during heat and drought stresses. Those comparisons with $P \leq 0.05$ were taken as significantly different. The data were analyzed by using SPSS 22.0 software [35].

III. RESULTS

All drought treatments (D, DTs, HD, HDTs) reduced the shoot length of cv. Ekiz by at least 55-64% and root length by 16-23% compared to the control (C). In contrast, shoot and root lengths were less affected by heat stress (HTs) with thermo-priming (TP) treatments compared to drought stress (Figure 2 (a)). Interestingly, it was determined that there was not statistical difference in root and shoot lengths between DT and HDT treatments. On the other hand, seed germination was determined at the control plant level in all stress treatments except DT60 treatment (Figure 1).

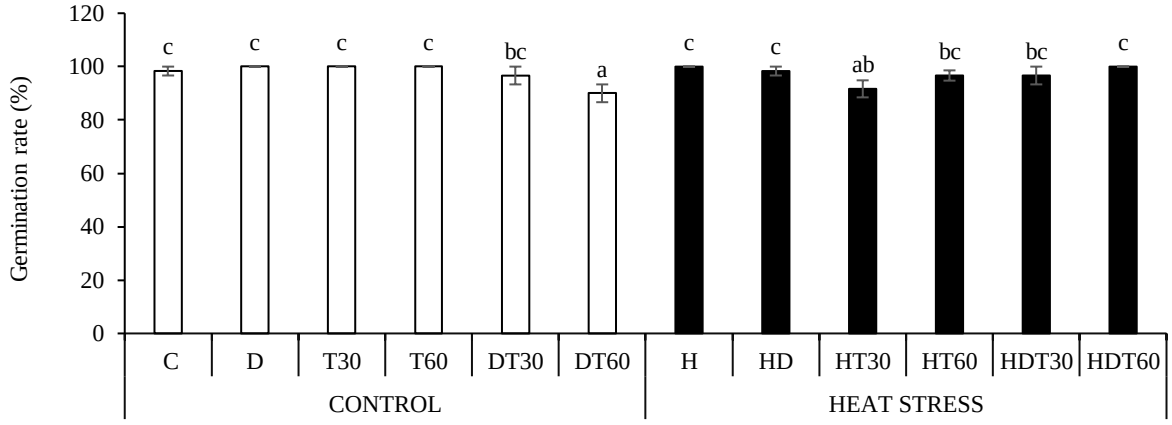
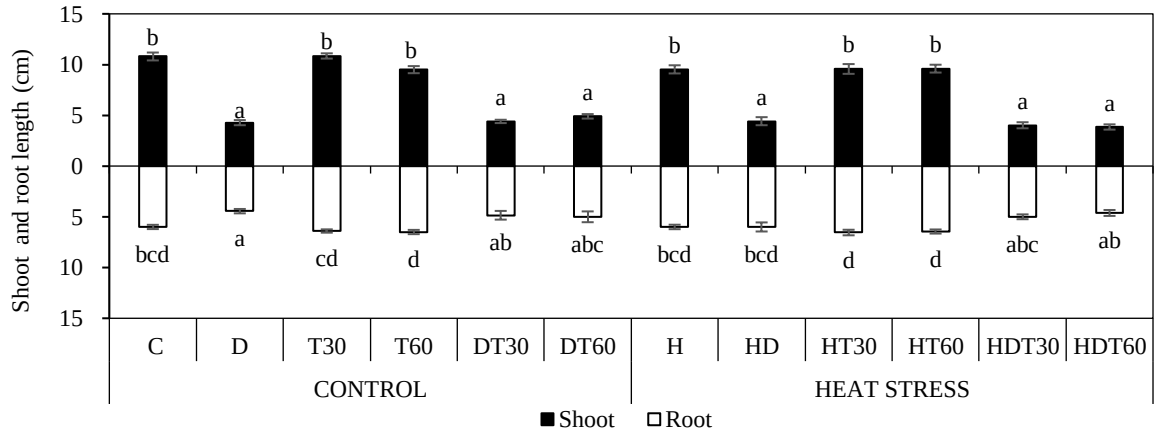
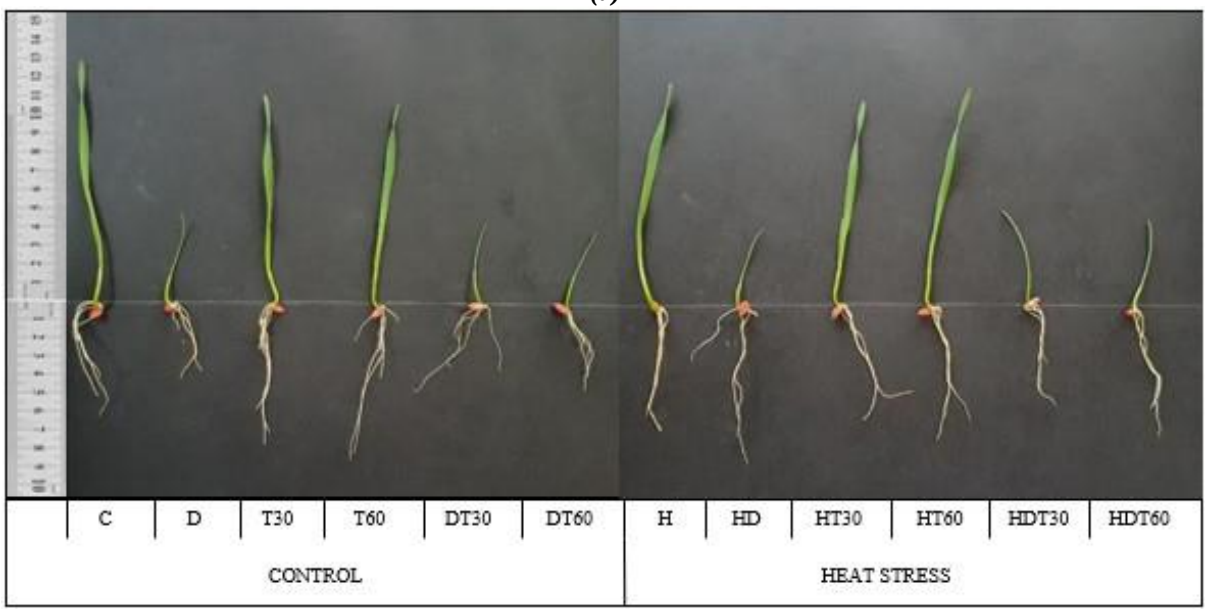


Figure 1. *The effects of drought and heat stress of thermo-priming treatments on germination of 7d old wheat seedlings (C: Control, D: Drought, T30: Thermo-priming 30 min, T60: Thermo-priming 60 min, DT30: Drought + Thermo-priming 30 min, DT60: Drought + Thermo-priming 60 min; H: Heat stress (42°C for 3 h), HD: Drought and Heat stress, HT30: Thermo-priming 30 min. + Heat stress, HT60: Thermo-priming 60 min. + Heat stress, HDT30: Thermo-priming 30 min. + Drought + Heat stress, HDT60: Thermo-priming 60 min. + Drought+ Heat stress) (Means values followed by different letters are significantly different at P <0.05).*



(a)



(b)

Figure 2. *The effects of drought and heat stress of thermo-priming treatments on shoot and root length of 7d old wheat seedlings* ***(a)****. The effects of thermo-priming treatments on seedling growth of wheat* ***(b)*** *(C: Control, D: Drought, T30: Thermo-priming 30 min, T60: Thermo-priming 60 min, DT30: Drought + Thermo-priming 30 min, DT60: Drought + Thermo-priming 60 min; H: Heat stress (42°C for 3 h), HD: Drought and Heat stress, HT30: Thermo-priming 30 min. + Heat stress, HT60: Thermo-priming 60 min. + Heat stress, HDT30: Thermo-priming 30 min. + Drought + Heat stress, HDT60: Thermo-priming 60 min. + Drought+ Heat stress) (Means values followed by different letters are significantly different at P <0.05).*

Specific leaf area was drastically reduced (55-69%) with all D treatments compared to control. However, it was determined that TP treatment prevented the decrease in SLA only under heat stress (Figure 3). Interestingly, TP treatment was found to reduce SLA by 12% in DT30 and 3% in DT60 compared to D. Combined stress decreased SLA by 68% in HD, 60% in HDT30, and 55% in HDT60 compared to H (Figure 3).

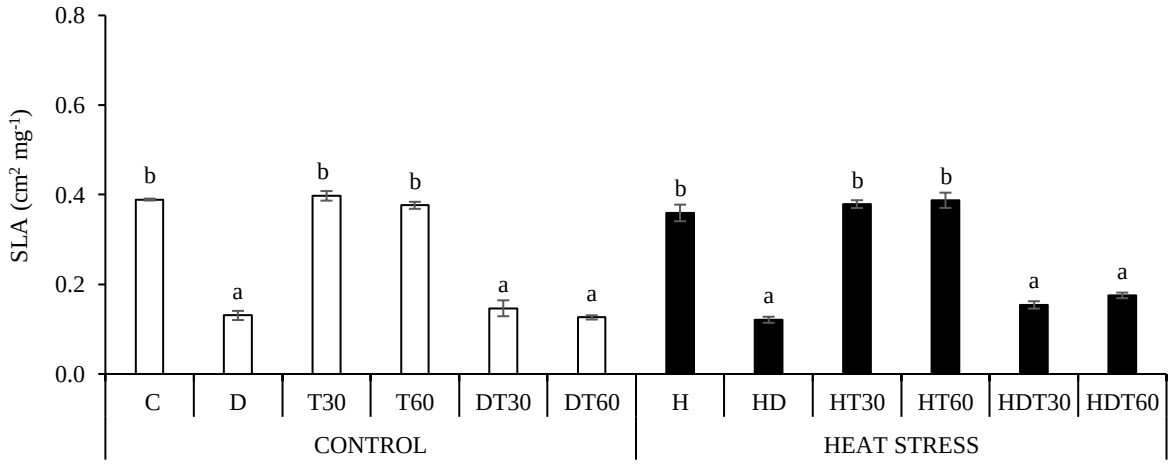


Figure 3. *The effects of thermo-priming treatments on specific leaf area (SLA) (cm² mg^{-1}) of 7d old wheat seedlings under drought and heat stress. (C: Control, D: Drought, T30: Thermo-priming 30 min, T60: Thermo-priming 60 min, DT30: Drought + Thermo-priming 30 min, DT60: Drought + Thermo-priming 60 min; H: Heat stress (42°C for 3 h), HD: Drought and Heat stress, HT30: Thermo-priming 30 min. + Heat stress, HT60: Thermo-priming 60 min. + Heat stress, HDT30: Thermo-priming 30 min. + Drought+ Heat stress, HDT60: Thermo-priming 60 min. + Drought + Heat stress) (Means values followed by different letters are significantly different at P <0.05).*

The relative water content was reduced by 10%, 20% and 30% in D, DT30 and DT60 compared to the control, respectively. On the other hand, RWC increased by 2% with T60 treatment. Heat stress treatment reduced RWC by 2% in HD compared to H. On the contrary, it increased by 1%, 4% and 6% in HT30, HT60 and HDT60, respectively (Figure 4).

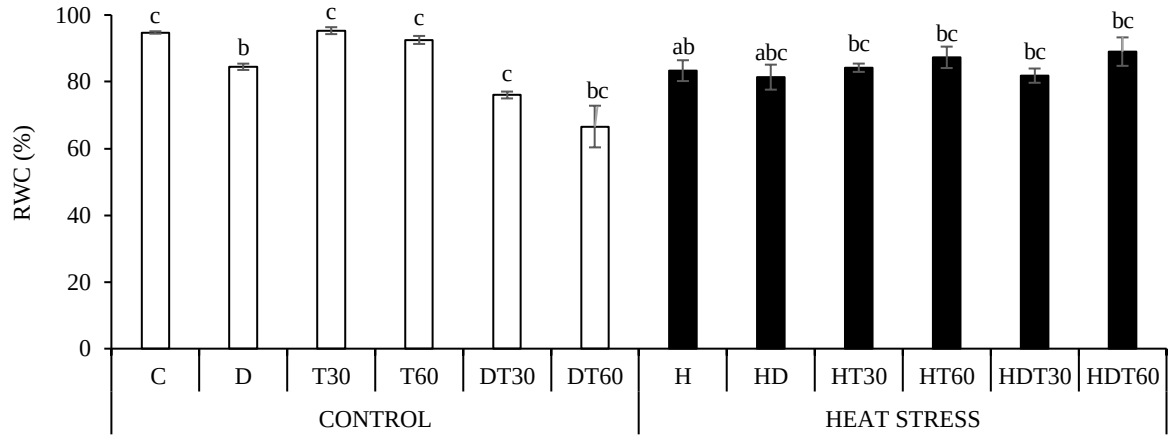


Figure 4. *The effects of thermo-priming treatments on the relative water content (RWC) of 7d old wheat seedlings under drought and heat stress. (C: Control, D: Drought, T30: Thermo-priming 30 min, T60: Thermo-priming 60 min, DT30: Drought + Thermo-priming 30 min, DT60: Drought + Thermo-priming 60 min; H: Heat stress (42°C for 3 h), HD: Drought and Heat stress, HT30: Thermo-priming 30 min. + Heat stress, HT60: Thermo-priming 60 min. + Heat stress, HDT30: Thermo-priming 30 min. + Drought + Heat stress, HDT60: Thermo-priming 60 min. + Drought + Heat stress) (Means values followed by different letters are significantly different at P <0.05).*

Biomass was reduced by 50% in all D treatments of the trial compared to the control. However, biomass decreased by 8% at most with all treatments except drought stress (Figure 5).

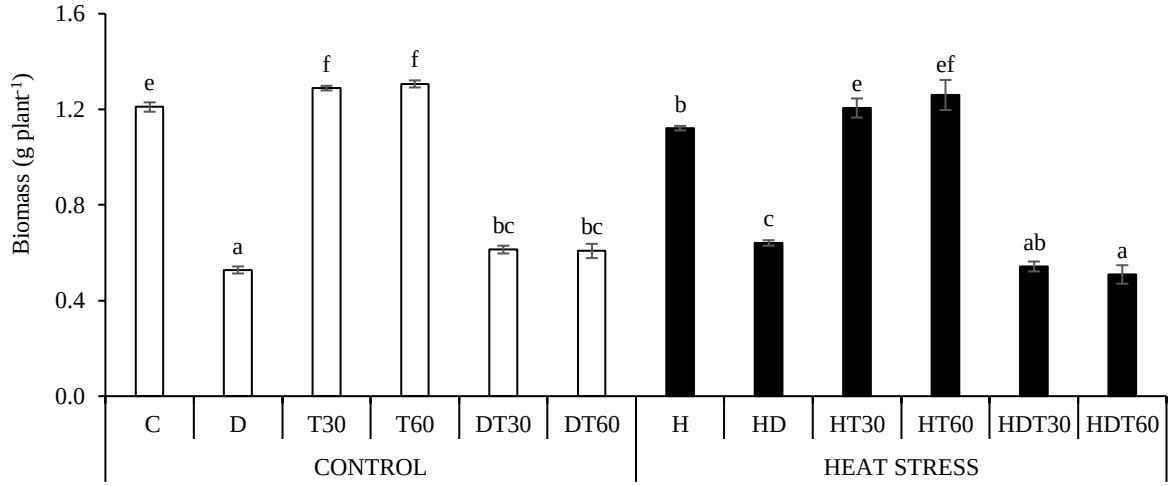


Figure 5. *The effects of thermo-priming treatments on biomass in 7d old wheat seedlings under drought and heat stress. (C: Control, D: Drought, T30: Thermo-priming 30 min, T60: Thermo-priming 60 min, DT30: Drought + Thermo-priming 30 min, DT60: Drought + Thermo-priming 60 min; H: Heat stress (42°C for 3 h), HD: Drought and Heat stress, HT30: Thermo-priming 30 min. + Heat stress, HT60: Thermo-priming 60 min. + Heat stress, HDT30: Thermo-priming 30 min. + Drought + Heat stress, HDT60: Thermo-priming 60 min. + Drought + Heat stress) (Means values followed by different letters are significantly different at P <0.05).*

While the total chlorophyll content decreased by 37% with D treatment compared to the control, it reduced approximately 2 twice with drought damage in wheat seedlings (DT30 and DT60) grown from thermo-priming seeds. On the other hand, total chlorophyll decreased by 59% with heat and drought stress (HD) compared to control, while thermo-priming treatments (HDT30 and HDT60) decreased by

54% and 43%, respectively (Figure 6). Accordingly, it can be said that the decrease in the total amount of chlorophyll in thermo- primed seeds is limited by drought and heat stresses.

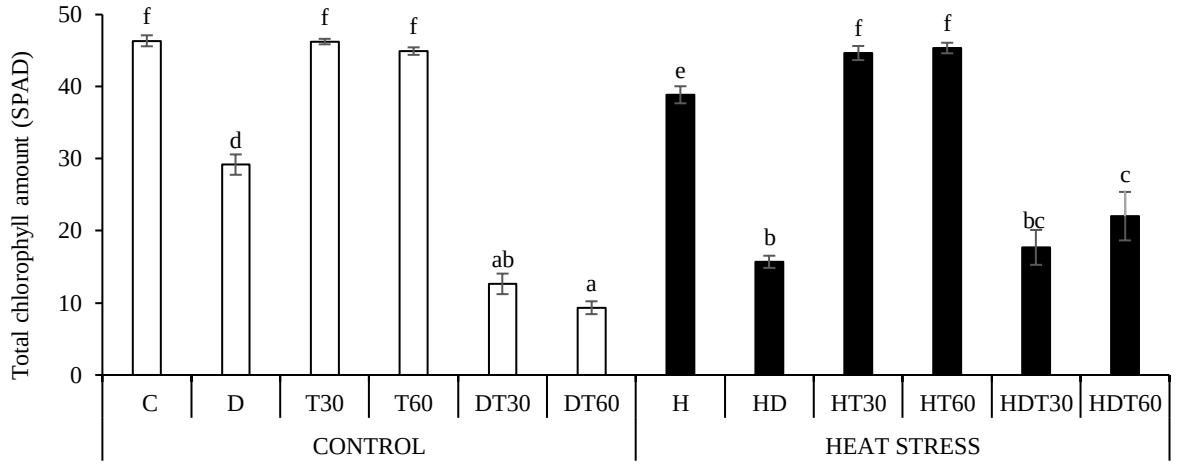


Figure 6. *The effects of thermo-priming treatments on total chlorophyll amount (SPAD) in 7d old wheat seedlings under drought and heat stress. (C: Control, D: Drought, T30: Thermo-priming 30 min, T60: Thermo-priming 60 min, DT30: Drought + Thermo-priming 30 min, DT60: Drought + Thermo-priming 60 min; H: Heat stress (42°C for 3 h), HD: Drought and Heat stress, HT30: Thermo-priming 30 min. + Heat stress, HT60: Thermo-priming 60 min. + Heat stress, HDT30: Thermo-priming 30 min. + Drought + Heat stress, HDT60: Thermo-priming 60 min. + Drought + Heat stress) (Means values followed by different letters are significantly different at P <0.05).*

The amount of protein increased significantly with all treatments compared to the control. The highest increase was determined as 55% in T60. Similarly, heat stress was found to increase protein content by 43%, 46% and 44%, respectively, with HT60, HDT30 and HDT60 treatments compared to control (C) (Figure 7).

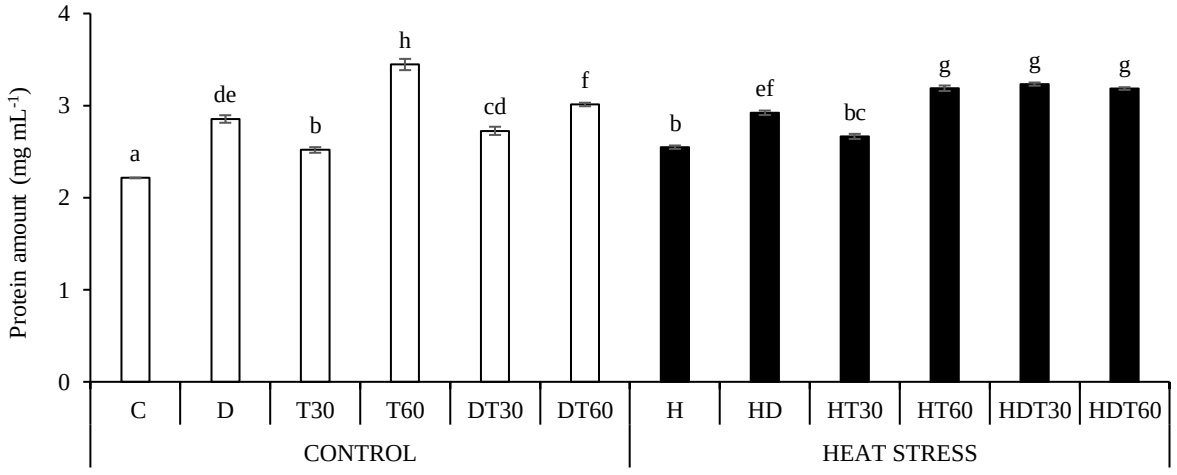


Figure 7. *The effects of thermo-priming treatments on protein content in 7d old wheat seedlings under drought and heat stress. (C: Control, D: Drought, T30: Thermo-priming 30 min, T60: Thermo-priming 60 min, DT30: Drought + Thermo-priming 30 min, DT60: Drought + Thermo-priming 60 min; H: Heat stress (42°C for 3 h), HD: Drought and Heat stress, HT30: Thermo-priming 30 min. + Heat stress, HT60: Thermo-priming 60 min. + Heat stress, HDT30: Thermo-priming 30 min. + Drought + Heat stress, HDT60: Thermo-priming 60 min. + Drought + Heat stress) (Means values followed by different letters are significantly different at P <0.05).*

Ascorbate peroxidase activity increased 33% with D treatment compared to control, while it decreased significantly in thermo-priming treatments under normal condition. However, thermo-priming + drought treatments (DT30 and DT60) increased APX activities by 1.5 and 2.5 times, respectively, compared to the control (C). Similarly, 30 and 60 min of thermo-priming + heat stress treatments (HDT30 and

HDT60) increased the APX activities 2 and 3 times, respectively, compared to the control (H) under heat stress condition (Figure 8).

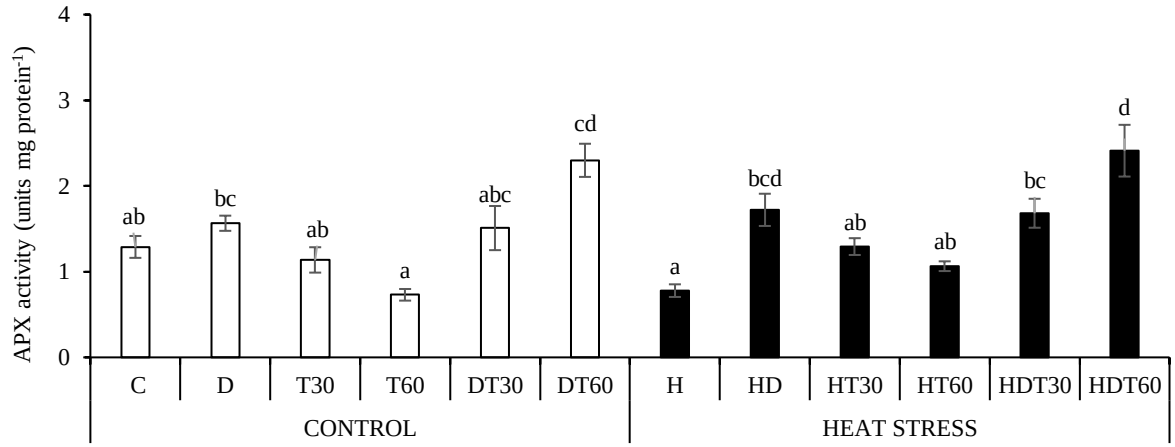


Figure 8. *The effects of thermo-priming treatments on APX activity in 7d old wheat seedlings under drought and heat stress. (C: Control, D: Drought, T30: Thermo-priming 30 min, T60: Thermo-priming 60 min, DT30: Drought + Thermo-priming 30 min, DT60: Drought + Thermo-priming 60 min; H: Heat stress (42°C for 3 h), HD: Drought and Heat stress, HT30: Thermo-priming 30 min. + Heat stress, HT60: Thermo-priming 60 min. + Heat stress, HDT30: Thermo-priming 30 min. + Drought + Heat stress, HDT60: Thermo-priming 60 min. + Drought + Heat stress) (Means values followed by different letters are significantly different at P <0.05).*

Glutathione reductase activities in thermo-priming treatments (T30 and T60) increased by 14-18%, respectively, compared to the control, while increased by 5% with drought and heat stress. In addition, GR activity increased by 4% in DT30, and by 3.3% in HD. In general, all treatments decreased GR activity under heat stress conditions, except HDT30 (Figure 9).

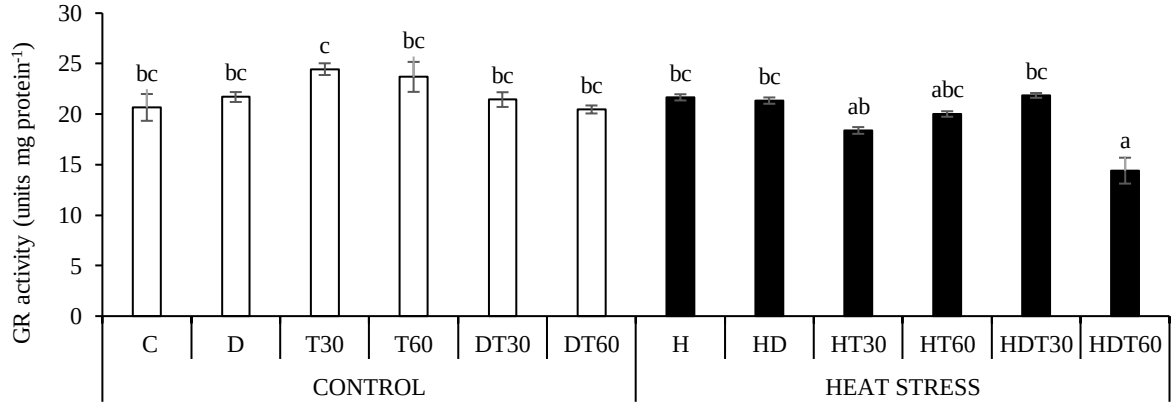


Figure 9. *The effects of thermo-priming treatments on GR activity in 7d old wheat seedlings under drought and heat stress. (C: Control, D: Drought, T30: Thermo-priming 30 min, T60: Thermo-priming 60 min, DT30: Drought + Thermo-priming 30 min, DT60: Drought + Thermo-priming 60 min; H: Heat stress (42°C for 3 h), HD: Drought and Heat stress, HT30: Thermo-priming 30 min. + Heat stress, HT60: Thermo-priming 60 min. + Heat stress, HDT30: Thermo-priming 30 min. + Drought + Heat stress, HDT60: Thermo-priming 60 min. + Drought + Heat stress) (Means values followed by different letters are significantly different at P <0.05).*

Under normal conditions, CAT activity increased by 12% and 7% with drought stress (D) and T30, respectively, and decreased by 2-3% with T60, DT30 and DT60. However, CAT activity increased by 7% with heat stress (H). CAT activities significantly reduced by 20% in all HD treatments, except for 10% increase in HT60 compared to heat stress (Figure 10).

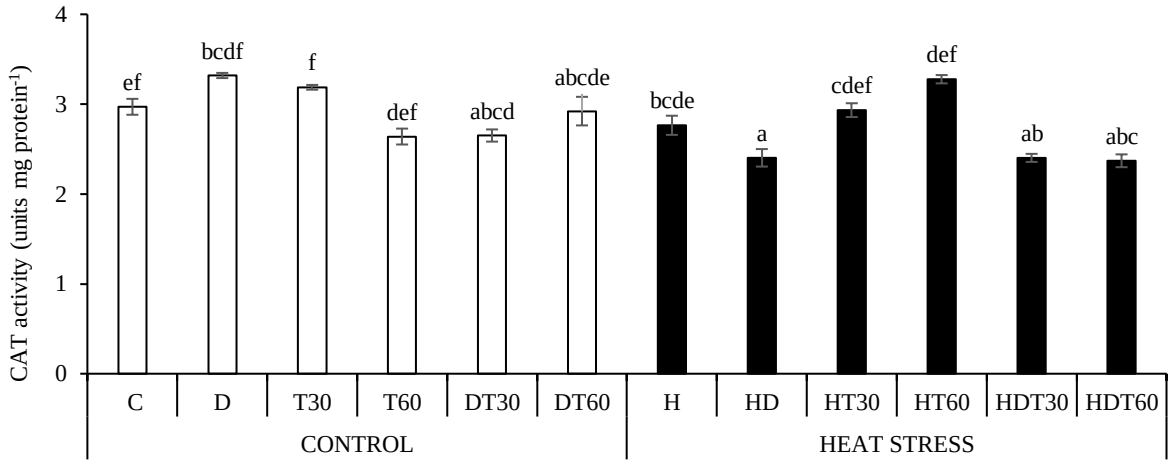


Figure 10. *The effects of thermo-priming on CAT activity in 7d old wheat seedlings under drought and heat stress. (C: Control, D: Drought, T30: Thermo-priming 30 min, T60: Thermo-priming 60 min, DT30: Drought + Thermo-priming 30 min, DT60: Drought + Thermo-priming 60 min; H: Heat stress (42°C for 3 h), HD: Drought and Heat stress, HT30: Thermo-priming 30 min. + Heat stress, HT60: Thermo-priming 60 min. + Heat stress, HDT30: Thermo-priming 30 min. + Drought + Heat stress, HDT60: Thermo-priming 60 min. + Drought + Heat stress) (Means values followed by different letters are significantly different at P <0.05).*

Hydrogen peroxide content in the tissues was determined by the histochemical staining method used for the direct detection of H_2O_2 in plant leaf tissue (Figure 11 (b)). Accordingly, the H_2O_2 content increased by 5% and 9% with D and DT60, respectively, compared to the control, and decreased by 7% and 3%, respectively, with T30 and DT30. Under heat stress conditions, the H_2O_2 content increased by 4% with HD and decreased by 12% with HDT60 compared to H (Figure 11 (a)).

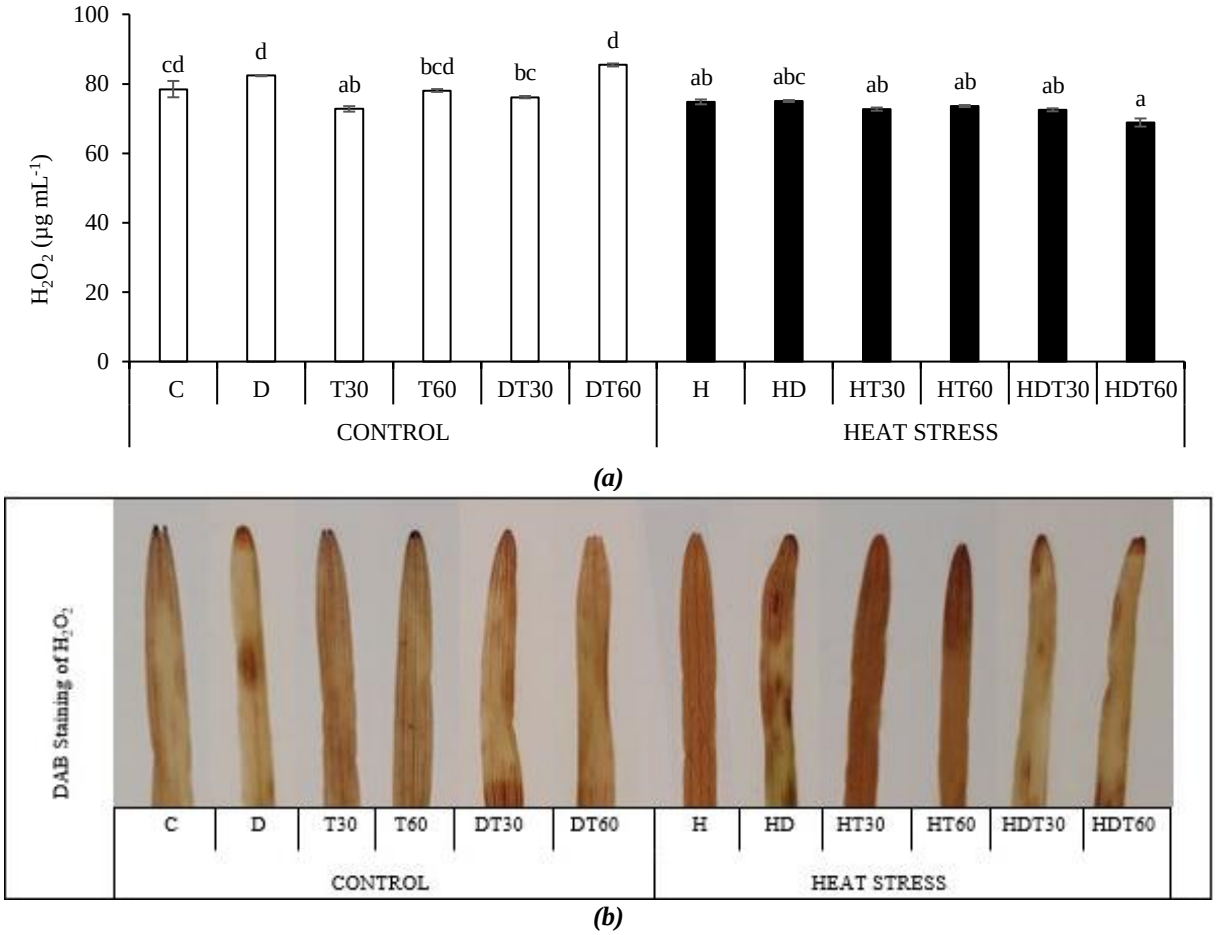


Figure 11. *The effects of thermo-priming on hydrogen peroxide content (H_2O_2) (µg ml^{-1}) (A) and histochemical staining (B) in 7d old wheat seedlings under drought and heat stress. (C: Control, D: Drought, T30: Thermo-priming 30 min, T60: Thermo-priming 60 min, DT30: Drought + Thermo-priming 30 min, DT60: Drought + Thermo-priming 60 min; H: Heat stress (42°C for 3 h), HD: Drought and Heat stress, HT30: Thermo-priming 30 min. + Heat stress, HT60: Thermo-priming 60 min. + Heat stress, HDT30: Thermo-priming 30 min. + Drought + Heat stress, HDT60: Thermo-priming 60 min. + Drought + Heat stress) (Means values followed by different letters are significantly different at P <0.05).*

IV. DISCUSSION

Drought is a major abiotic factor limiting plant growth. Therefore, drought stress reduces growth and yield in wheat [36], [37]. Similarly in our study, drought stress decreased root and shoot length, RWC, SLA, biomass and total chlorophyll amount in Ekiz variety. On the other hand, heat stress significantly reduces productivity in wheat during the grain filling [38]. In addition, it was reported that high temperature reduces biomass in winter wheat and photosystem II is damaged due to insufficient antioxidant defense [20]. Heat stress treatment did not change root and shoot lengths, SLA, biomass, germination rate and GR activity of Ekiz variety. However, it decreased the total amount of chlorophyll, RWC, H_2O_2 content, APX activity and CAT activity.

There are reports that priming treatments affect growth parameters positively in wheat [21], [22]. Some studies have shown that thermo-priming treatments promote seed germination and seedling emergence [17], [18]. Our results showed that thermo-priming limited the reduction in root length with drought treatment but did not change the decrease in shoot length. On the other hand, heat treatment did not change root and shoot lengths significantly, while shoot lengths decreased dramatically with drought.

Accordingly, it was determined that the growth in double stress treatment decreased with the effect of drought stress and the thermo-priming treatment did not affect this.

Although the positive effect of priming treatments on seed germination has been [17], [18], our research results showed that all treatments, including thermo-priming, did not have a significant effect for seed germination of Ekiz variety. It has been reported that biomass decreases in wheat seedlings under drought stress [39], while GR24 priming treatment increases biomass [40]. However, it was determined that the decrease of SLA and biomass caused by drought and heat stresses did not affect with thermo-priming treatments. Additionally, the decreases in RWC, total chlorophyll content and the protein amount were increase with thermo-priming effect. Increases in protein content and antioxidant enzyme activities with sodium nitroprusside (SNP) priming treatment in wheat support our research results [41]. Interestingly, APX activities increased significantly, especially with 60 min thermo-priming treatments. However, GR activities were not significantly altered by drought and heat stress. However, it was determined that the CAT activities, which increased with D and decreased with H, decreased in thermo-priming treatment. On the other hands similar thermo-priming study in wheat which prevents damage to grain yield, increases chlorophyll content and antioxidant enzyme activities supports our results [37]. While H_2O_2 content increased with drought stress under normal conditions, it decreased with heat stress. A statistical decrease was determined only in HDT60 under temperature stress conditions and histochemical staining results support it. [20] reported that thermo-tolerance in wheat seedlings exposed to high temperature stress by heat priming in wheat is largely a result of enhanced antioxidant conditions at the intracellular level. In this context, the decrease in H_2O_2 amount in our results can be related to the increased APX activity with the effect of thermo-priming and suggesting that the improvement in growth parameters is related to it.

V. CONCLUSION

When strategically important cereals such as wheat are adversely affected by drought stress, biomass and yield decrease [35]. It is accepted that priming treatments are promising in solving the growth and yield problem that will be caused by increasing global warming in wheat production. In our study, it was determined that short-term thermo-priming treatment improved the biochemical parameters of Ekiz variety. Therefore, there is a need for a detailed investigation of the effect of thermo-priming on growth and development in wheat with longer-term treatments. As a result, it can be said that short-term thermo-priming treatment increases the antioxidant capacity of wheat and reduces the damage of double stress.

VI. REFERENCES

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