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Research Article

Effects of Different Priming Treatments on Germination and Seedling Growth of Wheat under Drought Stress

Eda Günay[1] **Müge Teker Yıldız**[1] **Okan Acar**[2*]

[1]Graduate School of Natural and Applied Sciences, Biology Department, Çanakkale Onsekiz Mart University, Çanakkale, Turkey
[2]Faculty of Arts and Sciences, Biology Department, Çanakkale Onsekiz Mart University, Çanakkale, Turkey
*Corresponding author: oacar@comu.edu.tr

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Abstract

Wheat (*Triticum aestivum* L.) is a widely cultivated agricultural crop in dry areas. However, drought is one of the most limiting environmental stress factors for crop production in the world's agricultural areas. Seed priming is a physiological technique involving seed hydration and drying to improve metabolic processes before germination. The aim of this study is to determine the effect of four different priming treatments (hormo-priming (gibberellic acid, GA_3), redox-priming (hydrogen peroxide, H_2O_2), osmo-priming (polyethylene glycol, PEG) and thermo-priming (38^{o}C)) on germination percentage, shoot-root lengths, total chlorophyll content (SPAD), relative water content (RWC), specific leaf area (SLA) and H_2O_2 amounts in a local wheat variety (cv. Ekiz) under drought stress created with PEG-6000. Drought stress significantly decreased seed germination, shoot-root lengths, SLA, SPAD and RWC while increasing H_2O_2 content. Thermo-priming treatments fully improved the negative effects of drought on chlorophyll amount and germination compared to control plants. However, shoot-root lengths improved only in half level of control plants. While drought stress decreased seed germination by 14%, hormo-priming treatment ensured germination of all seeds. On the contrary of other priming treatments, hormo-priming and osmo-priming treatments increased RWC. Consequently, thermo-priming and hormo-priming treatments found most effective than the other priming treatments in drought stress resistance for cv. Ekiz.

Keywords: Drought tolerance, Osmotic stress, Seed priming, Wheat

Kuraklık Stresi Altında Farklı Priming Uygulamalarının Buğdayda Çimlenme ve Fide Büyümesi Üzerine Etkileri

Öz

Buğday (*Triticum aestivum* L.), kurak alanlarda yaygın olarak yetiştirilen bir tarım ürünüdür. Ancak kuraklık, dünya tarım alanlarında bitkisel üretim için en sınırlayıcı çevresel stres faktörlerinden biridir. Priming, çimlenmeden önce metabolik süreçleri iyileştirmek için tohum hidrasyonu ve kurutmayı içeren fizyolojik bir tekniktir. Bu çalışmanın amacı dört farklı priming uygulamasının (hormo-priming (gibberellik asit, GA_3), redoks priming (hidrojen peroksit, H_2O_2), ozmo-priming (polietilen glikol, PEG) ve termo-priming (38^{o}C)) PEG-6000 ile yaratılmış kuraklık stresi altındaki yerel bir ekmeklik buğday çeşidinin (cv. Ekiz) çimlenme yüzdesi, sürgün-kök uzunluğu, toplam klorofil içeriği (SPAD), bağıl su içeriği (BSİ), spesifik yaprak alanı (SYA) ve H_2O_2 miktarı üzerine etkilerini belirlemektir. Kuraklık stresi, H_2O_2 içeriğini arttırırken tohum çimlenmesini, sürgün-kök uzunluklarını, SYA, SPAD ve BSİ'yi önemli ölçüde azaltmıştır. Termo-priming uygulamaları, kontrol bitkilerine kıyasla kuraklığın klorofil miktarı ve çimlenme üzerindeki olumsuz etkilerini tamamen iyileştirmiştir. Bununla birlikte, sürgün-kök uzunlukları, kontrol bitkilerinin sadece yarısı düzeyinde iyileşmiştir. Kuraklık stresi tohum çimlenmesini %14 oranında azaltırken, hormo-priming uygulaması tüm tohumların çimlenmesini sağlamıştır. Diğer priming uygulamalarının aksine, hormo-priming ve ozmo-priming uygulamaları BSİ'yi arttırmıştır. Sonuç olarak, kuraklık stresine dayanıklılıkta cv. Ekiz için termo-priming ve ozmo-priming uygulamaları diğer priming uygulamalarından daha etkili olduğu bulunmuştur.

Anahtar Kelimeler: Buğday, Kuraklık toleransı, Ozmotik stres, Priming

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important and strategic food for the world population. The increase in temperature and drought due to global warming negatively affects wheat production around the world. Drought stress, which is a major stress factor affecting the growth, development and productivity of plants (Singh et al., 2014), causes many metabolic, mechanical and oxidative changes in plants (Asseng et al., 2015). Seed germination is an important stage that affects plant growth and productivity and is directly affected by environmental factors. For this reason, pre-treatments in wheat are an effective strategy to ameliorate the harmful effects of drought stress. Especially, priming treatments are reported to be an easy and low-cost approach to increase the germination, growth and yield of field crops such as wheat (Gençtan, 2013).

Priming is defined as a physiological technique involving seed hydration and drying to improve metabolic processes prior to germination (Bradford, 1986). Water, inorganic salts, hormones, bacteria and organic substances are used as pre-sowing priming agents for improve on germination performance of seeds under stress conditions. Thus, by stimulating the plant antioxidant defense system in advance, it is possible to create a stress memory and increase stress tolerance (Sher et al., 2019). The effects of priming agents differ depending on their concentrations and the species of plant (Guo et al., 2022). Osmo-priming provides initiation of metabolic preparations for germination by controlling the water absorption in the seed under drought stress induced by polyethylene glycol (PEG) (Jisha et al., 2013; Abid et al., 2018).

Stress hormones are functional as signal molecules for plants at low concentrations and trigger plant defense against stress. Gibberellic acid (GA) breaking seed dormancy and signaling plant defense mechanisms against stress as a priming agent. The relationship between seed dormancy and germination is also balanced by the GA-ABA ratio, which is a key mechanism to combat abiotic stress conditions. As a signal molecule, hydrogen peroxide (H_2O_2) used for redox priming (Paparella et al., 2015; Cetinel et al., 2021). Moreover, it is a long-lived reactive oxygen species (ROS) and that can diffuse easily through membranes, reach targets far from production sites.

RWC is an important indicator of water status in plants and reflects the balance between water in leaf tissue and transpiration rate (Bradford, 1986). Low RWC levels result in chlorophyll degradation (Lugojan and Ciulca, 2011). Therefore, chlorophyll content is one of the important factors that should be examined to determine plant stress conditions (Nikolaeva et al., 2010; Tezcan et al., 2019). The decrease in cell growth of plants is the most sensitive response they have developed to drought stress. Leaf area affects the growth and development of the plant and also has an important role in light absorption. For this reason, it is accept an indicator for photosynthetic capacity and plant growth rate (Yalçın, 2018). SLA is the ratio of leaf biomass to leaf area and is a measure of plant growth versus environmental factors (Wilson et al., 1999).

The aim of this study is to determine the physiological effects (germination, shoot-root length, total chlorophyll content (SPAD), RWC, SLA and H_2O_2 accumulation) of four different priming treatments (hormo-priming (gibberellic acid, GA_3), redox priming (hydrogen peroxide, H_2O_2), osmo-priming (polyethylene glycol, PEG) and thermo-priming (38^{o}C)) under drought stress in a local wheat variety (cv. Ekiz).

Material and Method

In this study drought sensitive cv. Ekiz is variety of *Triticum aestivum* L. (Bahri Dagdas International Agricultural Research Institute) was used. Seeds were grown under controlled conditions (16/8 h light/dark photoperiod, 25±2°C and 60±5% humidity). The study was carried out according to the completely randomized block design with three replicates. Experiments are divided two groups for the effects of seed primings; control and 15% PEG-6000 (drought induced osmotic stress) (Michel and Kaufmann, 1973)(Table 1).

Seed Priming Treatments

Wheat seeds were sterilized by keeping them in 1% sodium hypochlorite for 5 min. Wheat seeds primed at different concentrations and times were grown in petri dishes under normal and drought stress conditions for 7 d. Primed and non-primed seeds were placed in petri dishes and wetted with distilled water for normal condition and PEG-6000 solutions for drought condition. Seeds of wheat were soaked for 12 h in H_2O_2 solution (50 µM) as redox priming, 12 and 24 h GA_3 solution (50

ppm) as hormo-priming, 12 h in PEG-6000 solution (10%) as osmo-priming and 30 and 60 min. at 38°C kept in the oven as thermo-priming treatment (Table 1).

Table 1. Seed priming treatments and growth condition

Growth Condition	**Treatment**		
Normal	Distilled water		
Drought stress	15% PEG-6000		
Methods of Seed Priming	**Agents of Seed Priming**	**Duration**	**Group**
Non-primed seeds	None	-	C
		-	D
Hormo-priming	50 ppm Giberellic acid (GA_3)	12 h	G1
		24 h	G2
Osmo-priming	10% Polyethylne glycol (PEG-6000)	12 h	P
Redox priming	50 µM Hydrogen peroxide (H_2O_2)	12 h	H
Thermo-priming	Incubation at 38°C	30 min	T3
		60 min	T6

Wheat seeds primed at different concentrations and times were grown in petri dishes under normal and drought stress conditions for 7 d. Primed and non-primed seeds were placed in petri dishes and wetted with distilled water for normal condition and PEG-6000 solutions for drought condition. (C: Control (non-primed seeds), G1 and G2: Seeds primed gibberellic acid for 12 and 24 h, H: Seeds primed hydrogen peroxide for 12 h, P: Seeds primed PEG-6000 for 12 h, T3 and T6: Seeds primed 38°C for 30 and 60 min, D: Drought (non-primed seeds)).

Physiological and Biochemical Analyses

Germination Percentage (%)

Germination percentage is an estimate of germination percentage was determined by calculating the ratio of germinated wheat seeds to the total number of seeds (Formula 1).

Germination Percent (%) = (*number of germinated seeds*/ *number of experimental seeds*)x100 (1)

Root and Shoot Length

The lengths of the root and shoot parts of the plants were measured with a ruler.

Total Chlorophyll Content (SPAD)

Total chlorophyll amounts of leaf samples were measured with chlorophyll meter (Minolta, SPAD-502) (Peryea and Kammereck, 1997).

Relative Water Content (RWC)

To determine RWC, 9 leaves from each group were weighed immediately (FW) after harvesting the plant. Leaves were then placed in distilled water for 4 h and then turgid weight (TW) was measured. Then the leaves were dried in oven at 70°C for 24 h to obtain their dry weight (DW). RWC was calculated by the following formula 2 (Smart and Bingham, 1974).

$$\%RWC = (FW - DW) / (TW - DW) \times 100 \quad (2)$$

Spesific Leaf Area (SLA)

SLA was calculated using the leaf photos of wheat seedlings in the Image J program. Then the samples are dried in an oven at 70°C for 24 h and weighed on a precision scale. SLA is calculated by the formula 3 (Wilson et al., 1999).

$$SLA = Area\ (cm^2) / Dry\ weight\ (mg^{-1}) \quad (3)$$

Hydrogen peroxide (H_2O_2) Content

Leaf H_2O_2 content was determined by adopting the method of Cheeseman (2006). Plant samples were homogenized with a homogenization buffer consisting of sulfuric acid (H_2SO_4) and cold acetone. After ferrous ammonium sulfate/xylenol orange (e-FOX) was used to determine the H_2O_2 contents of the extracts. It was calculated by absorbance at 550-800 nm in a spectrophotometer (µg ml^{-1}).

Histochemical Localization of H_2O_2 Using DAB

Leaves were immersed in a solution containing 1 mg ml^{-1} 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 (Kumar et al., 2014).

Statistical Analysis

The data were made with Tukey test using one-way analysis of variance (ANOVA). SPSS (Statistical Package for the Social Sciences, version 21.0) program was used for statistical analysis. Significance levels were shown in graphs. Those comparisons with $P \leq 0.05$ were taken as significantly different.

Results and Discussion

Germination Percentage (%)

Drought (D) caused by osmotic stress decreased the germination percentage of wheat seedlings by 14% compared to the control (C) and increased it by 16% in the G1 group compared to the D group. On the other hand, priming treatments in G2, T3 and T6 groups increased germination by 20% compared to D and all seeds were germinated (Figure 1).

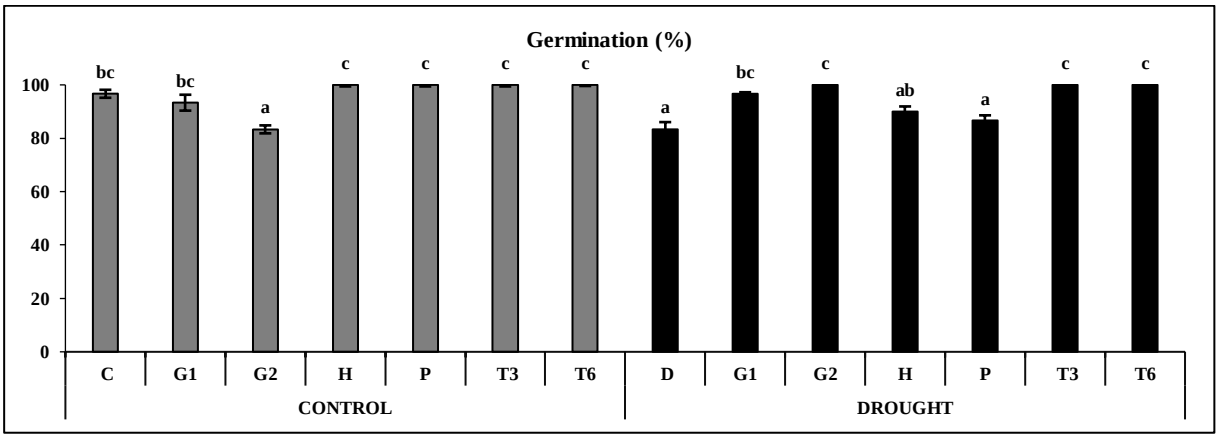

Figure 1. The effects of different primings on germination percentage of *T. aestivum* L. cv. Ekiz (C: Control (non-primed seeds), G1 and G2: Seeds primed GA_3 for 12 and 24 h, H: Seeds primed H_2O_2 for 12 h, P: Seeds primed PEG-6000 for 12 h, T3 and T6: Seeds primed 38^0C for 30 and 60 min, D: Drought (non-primed seeds)) (Means values followed by different letters are significantly different at $P < 0.05$).

Shoot and Root Length

Under normal conditions, shoot length increased by 13% only in the G1 group and decreased by 25% with thermo-priming treatments. In drought conditions, shoot length decreased by 58% compared to the control. On the contrary, it increased by 31% in G1 and G2 groups. Our findings showed that root lengths were significantly reduced under normal conditions in the G2 group with hormone priming and in the T3 groups with thermo-priming compared to the control. In contrast, root length decreased dramatically with drought stress by 37%. Moreover, root length of G1, G2 and T3 seedlings increased by 37%, 36% and 21%, respectively (Figure 2. A, B).

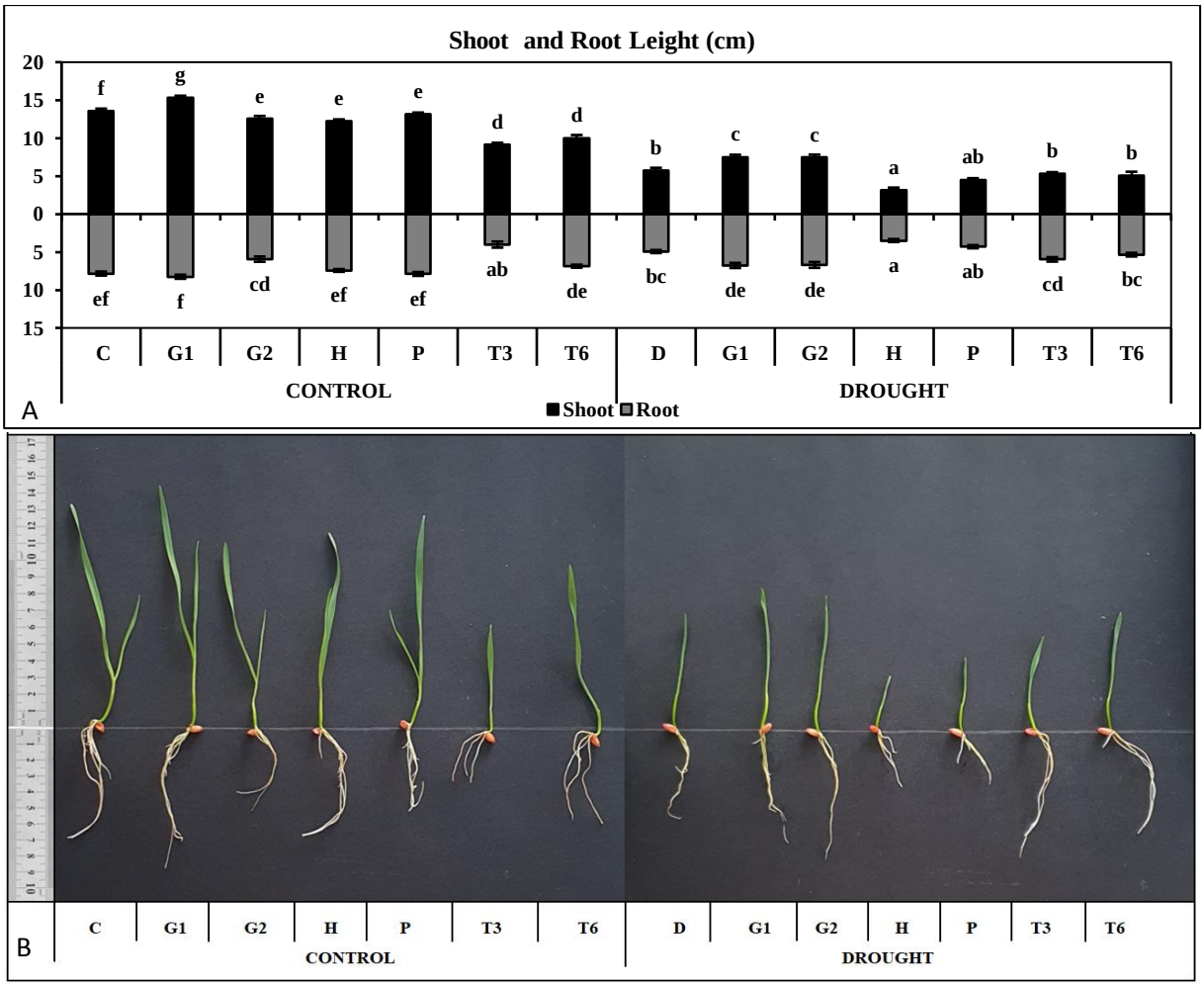

Figure 2. The effects of different primings on shoot and root length of *T. aestivum* L. cv. Ekiz (A). Effects of seed primings on seedling growth of Ekiz wheat variety (B). (C: Control (non-primed seeds), G1 and G2: Seeds primed GA_3 for 12 and 24 h., H: Seeds primed H_2O_2 for 12 h, P: Seeds primed PEG-6000 for 12 h, T3 and T6: Seeds primed 38^0C for 30 and 60 min, D: Drought (non-primed seeds)) (Means values followed by different letters are significantly different at P <0.05).

Total Chlorophyll Content (SPAD)

Under normal conditions, there is no significant change in total chlorophyll content in priming treatments compared to control. However, drought was decreased chlorophyll content by 44%. Additionally, chlorophyll content was increased by 65%, 71%, 74%, 44%, 102% and 95% in all primed groups under drought condition compared to the D, respectively (Figure 3).

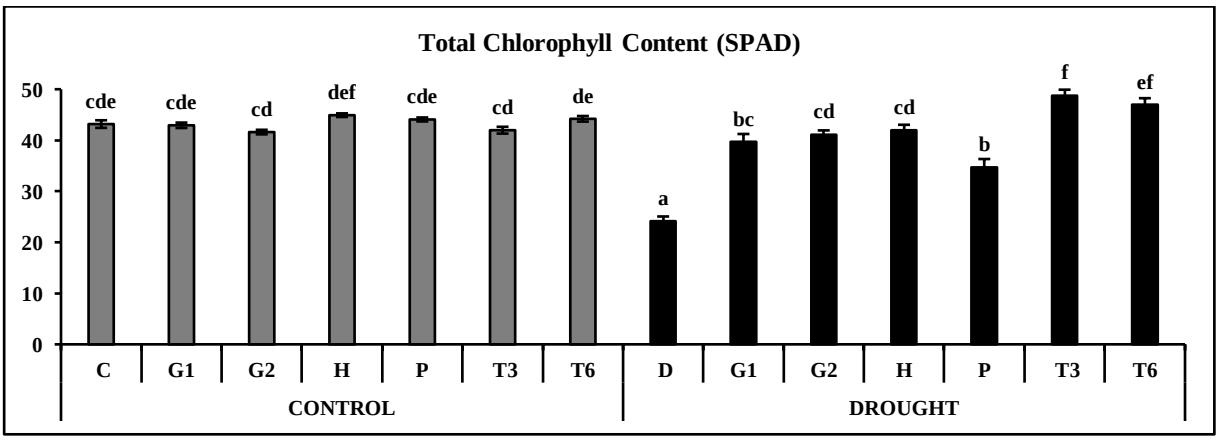

Figure 3. The effects of different primings on total chlorophyll content of *T. aestivum* L. cv. Ekiz (C: Control

(non-primed seeds), G1 and G2: Seeds primed GA_3 for 12 and 24 h., H: Seeds primed H_2O_2 for 12 h, P: Seeds primed PEG-6000 for 12 h, T3 and T6: Seeds primed 38^0C for 30 and 60 min, D: Drought (non-primed seeds)) (Means values followed by different letters are significantly different at P <0.05).

Relative Water Content (RWC)

Our results showed that RWC was not change significantly in the treatment groups under normal conditions. However, it decreased by 9% in D under drought conditions. On the contrary, RWC levels were increased in all priming treatments compared to D. According to our results, RWC increased in G1, G2 and P groups compared to D (Figure 4).

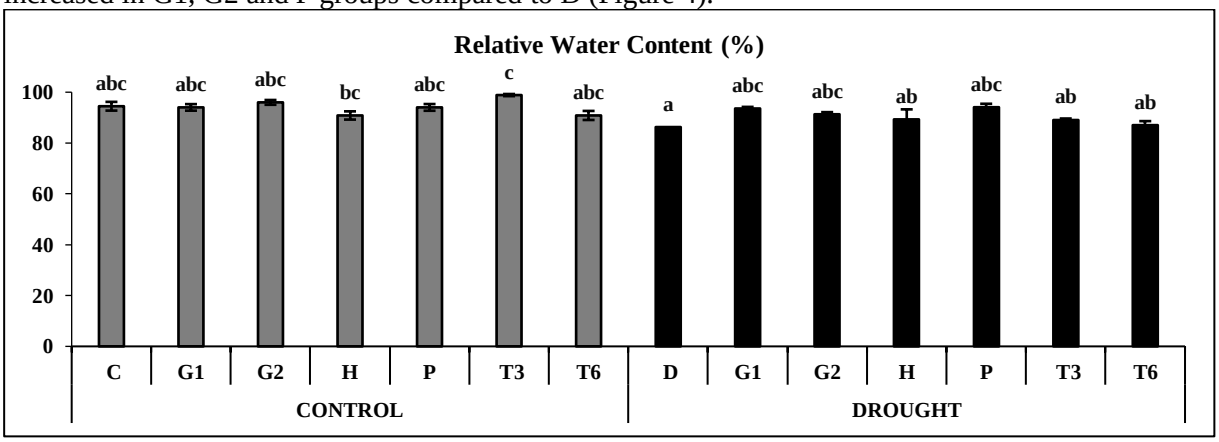

Figure 4. The effects of different primings on total chlorophyll content of *T. aestivum* L. cv. Ekiz (C: Control (non-primed seeds), G1 and G2: Seeds primed GA_3 for 12 and 24 h., H: Seeds primed H_2O_2 for 12 h, P: Seeds primed PEG-6000 for 12 h, T3 and T6: Seeds primed 38^0C for 30 and 60 min, D: Drought (non-primed seeds)) (Means values followed by different letters are significantly different at P <0.05).

Specific Leaf Area (SLA)

Under normal conditions, the SLAs of the priming groups except G1 were not changed compared to the control. On the other hand, drought treatment reduced SLA by 76% compared to control. However, it was determined that all priming treatments increased SLA approximately 2.5 times compared to D (Figure 5).

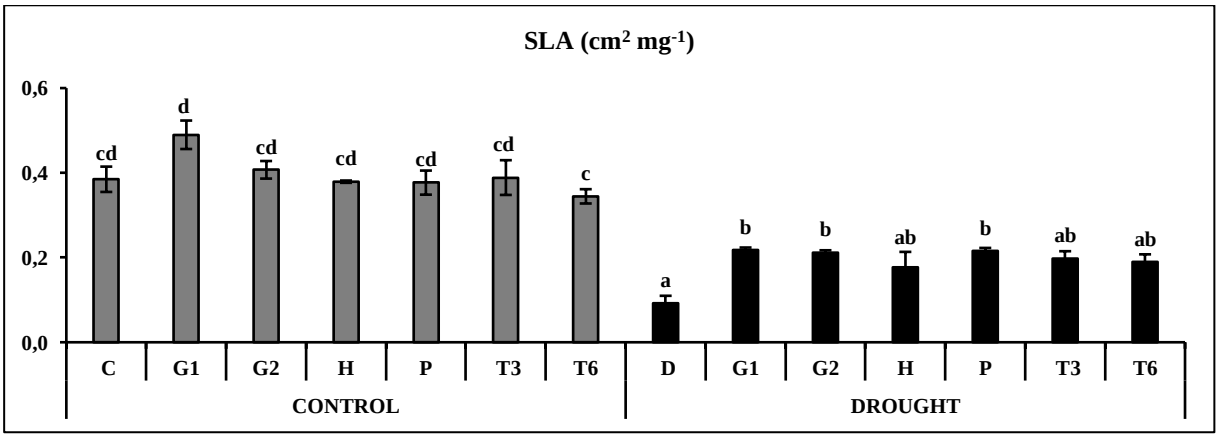

Figure 5. The effects of different primings on specific leaf area of *T. aestivum* L. cv. Ekiz (C: Control (non-primed seeds), G1 and G2: Seeds primed GA_3 for 12 and 24 h., H: Seeds primed H_2O_2 for 12 h, P: Seeds primed PEG-6000 for 12 h, T3 and T6: Seeds primed 38^0C for 30 and 60 min, D: Drought (non-primed seeds)) (Means values followed by different letters are significantly different at P <0.05).

Hydrogen Peroxide (H_2O_2) Content

All priming treatments in the control group significantly reduced the H_2O_2 content compared to the control. Drought treatment increased the amount of H_2O_2 by 41%. H_2O_2 amounts in the G1, G2, H, P, T3, and T6 groups were decreased by 7%, 9%, 25%, 15%, 3%, and 7%, compared to the D group respectively (Figure 6).

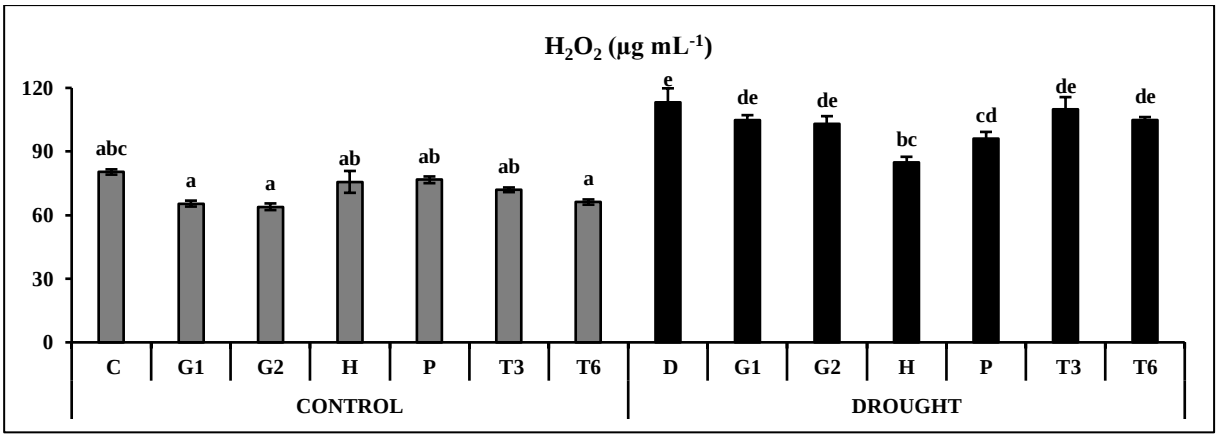

Figure 6. The effects of different primings on hydrogen peroxide content of *T. aestivum* L. cv. Ekiz (C: Control (non-primed seeds), G1 and G2: Seeds primed GA_3 for 12 and 24 h, H: Seeds primed H_2O_2 for 12 h, P: Seeds primed PEG-6000 for 12 h, T3 and T6: Seeds primed 38^0C for 30 and 60 min, D: Drought (non-primed seeds)) (Means values followed by different letters are significantly different at P <0.05).

Histochemical Detection of H_2O_2 Accumulation

High levels of H_2O_2 can cause toxicity to cell membranes and damage plant cells. Among the stained samples, the highest staining was observed in the leaf tissue of group D. This shows high H_2O_2 accumulation in the leaf samples. The least staining was determined in the G1 group under drought stress (Figure 7).

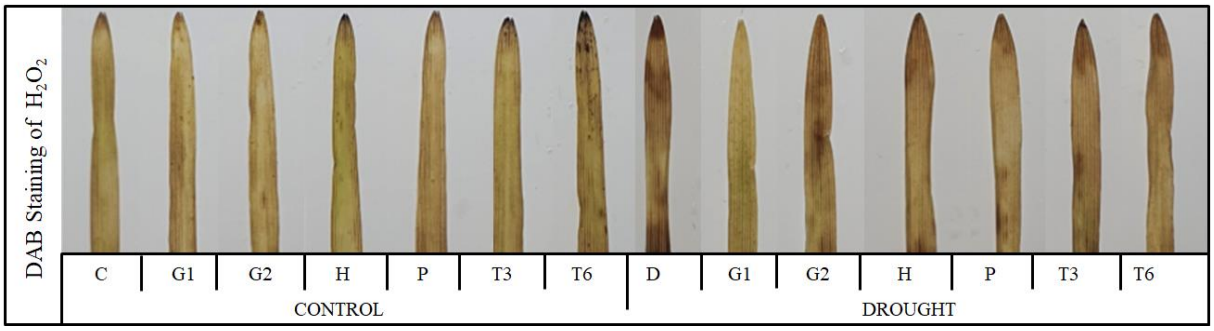

Figure 7. Detection of H_2O_2 using DAB staining in leaves of *T. aestivum* L. cv. Ekiz. The brown areas reflect H_2O_2 accumulation. (C: Control (non-primed seeds), G1 and G2: Seeds primed GA_3 for 12 and 24 h, H: Seeds primed H_2O_2 for 12 h, P: Seeds primed PEG-6000 for 12 h, T3 and T6: Seeds primed 38^0C for 30 and 60 min, D: Drought (non-primed seeds)).

Discussion

Drought limits plant growth and development, creates a serious pressure on agricultural production with the effect of increasing global warming. Although research continues to develop drought-resistant varieties in order to increase agricultural production (Kumar et al., 2019; Liaqat et al., 2020), priming treatments offer important opportunities for the development of drought resistance of existing varieties due to their ease and low cost.

In this study, the effects of four different priming treatments on germination and growth of Ekiz variety seeds under drought stress were studied comparatively. Drought reduces the water absorption of plants and inhibits the alpha-amylase enzyme activity, required for germination (Nawaz et al., 2013). It has been reported that drought stress in wheat reduces root and shoot length (Balkan, 2012; Acar et al., 2020), chlorophyll content (Aghanejad et al., 2015; Elmas and Acar 2021) and RWC (Siddique et al., 2000). According to our results drought treatment decreased seed germination, root-shoot length, chlorophyll amount, RWC, SLA and increased H_2O_2 amount in Ekiz variety. On the other hand, hormo-priming and thermo-priming treatments increased seed germination, root-shoot length, chlorophyll amount, RWC, SLA, and decreased H_2O_2 amount. However, 10% PEG-6000 and 50 µM H_2O_2 treatments only improved chlorophyll and SLA and decreased the amount of H_2O_2. Similarly, it has been shown that 50 ppm GA_3 treatment reduces the negative effects of drought in early seedling stage wheat seedlings (Ghobadi et al., 2012). In addition, it has been reported that

priming the wheat seeds 50 µM H_2O_2 reduces the stress effects (Demirbaş et al., 2018; Elmas and Acar 2021). Interestingly, it was reported that temperature induced thermo-priming remembered with wheat for thermo-tolerance (Zhang et al., 2016) and high antioxidant capacity against high temperature stress compared to non-primed plants (Maroufi et al., 2011). Likewise, increased antioxidant activity found effectively against negative effects of heat stress in wheat (Wang et al., 2014).

Conclusion

Consequently, our findings show that hormo-priming treatments better improve on seed germination potential and seedling growth than other priming treatments on germination and growth of Ekiz variety under drought stress.

Authors' Contributions

The authors declare that they have contributed equally to the article.

Conflicts of Interest Statement

The authors declare that they have no conflict of interest.

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