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3 RD INTERNATIONAL CONFERENCE ON APPLIED ENGINEERING AND NATURAL SCIENCES

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Türkiye dışından toplam 20 farklı ülkeden katılım sağlanmış olup, 735 adet bildirinin 371 tanesi yabancı katılımcı tarafından sunulmuştur.

Kongremize ilginiz için teşekkür ederiz.

Saygılarımızla,



Asst. Prof. Dr. Umut Özkaya

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Effects of Exogenous Glycine Betaine on the Reduction of Drought Stress Damage in Two Wheat Varieties with Different Drought Tolerance.

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Abstract – Drought stress, known to have a limiting effect on crop growth and yield, adversely affects wheat production worldwide due to global climate change. Osmolytes such as glycine-betaine (GB) play an osmoprotectant in the scavenging of reactive oxygen species formed under osmotic stress conditions such as drought stress and are naturally synthesized in some plants under stress conditions. It is known that GB has a vital role in the plant's abiotic stress resistance, including growth, photosynthesis, maintenance of membrane integrity, and oxidative defense. In this study, some physiological (shoot and root length, biomass, specific leaf area (SLA), total chlorophyll content (SPAD) and relative water content (RWC)) and biochemical (hydrogen peroxide (H_2O_2) amount and histochemical staining (H_2O_2)) changes caused by exogenous GB treatment in two wheat cultivars (drought-sensitive Selçuklu, drought-resistant Demir-2000) grown in Turkey with different drought tolerance were investigated. Accordingly, drought stress was initiated in 20-day-old wheat seedlings sprayed with 5 mM GB at 24 hours following the application and at the end of 7 days, leaf and root sampling were done.

Compared to Demir-2000, drought stress increased the amount of H_2O_2 and decreased other physiological parameters in Selçuklu. GB treatment reduced the drought-related damage in both genotypes. As a result, it was determined that drought damage in the susceptible genotype was improved at the resistant genotype level with GB treatment, except for the amount of H_2O_2.

Keywords – Drought Stress, Glycine-Betaine, Wheat, Growth, Histochemical Staining

1. INTRODUCTION

The world population is expected to exceed 9.8 billion in 2050 [6]. This situation highlights the need for a sustainable food source. On the other hand, global climate change limits potential yield losses. The drought triggered by the global climate change negatively affects the production of wheat (*Triticum aestivum* L.), which is a strategic agricultural product on a global scale. Moreover, the increase in sensitivity / decrease in resistance of existing varieties to environmental stresses indicates that the need for new varieties should be re-examined for suitable agricultural areas [15].

The term stress is expressed as adverse environmental conditions that negatively affect the growth and development of the plant. The stress tolerance of plants, the intensity and duration of the stress are directly related to the growth and development of that plant. Insufficient stress tolerance can lead to death. There are two types of stress in plants: biotic (infection of microorganisms (fungi, bacteria, and virus), attacks of harmful animals, etc.) and abiotic (water, temperature, radiation, chemicals, magnetic and electrical fields, etc.) factors [9,10].

In plants under drought stress, leaf temperature increases as a result of the closure of stomata with the decrease in the rate of water in the leaf. Therefore, membrane systems are damaged [7,5]. On the other hand, it damages photosynthetic

pigments, membrane lipids, proteins, and nucleic acids, causing overproduction of reactive oxygen species (ROS) produced at normal levels in the plant. This indicates oxidative stress due to drought [11]. Oxidative stress can lead to lipid peroxidation, oxidation of proteins, damage to nucleic acids, enzyme inhibition, programmed cell death (apoptosis) and ultimately cell death.

Osmolytes play a role as an osmo-protectant in the clearance of ROS formed by abiotic stress. They maintain turgor in the cytoplasm and protect cellular structures by facilitating the retention of sodium in the apoplast and vacuoles [17]. GB, which is among the well-known osmolytes such as proline, sugars, and polyols, is easily taken up by the leaf tissues when applied to the leaves of plants and provides drought tolerance resulting in increased yield in various plant species exposed to drought stress [19]. A few halophytes and several cereal plants, including *Poaceae*, synthesize and accumulate GB in response to environmental stresses.

In this study, we focused on the determination of some physiological (shoot and root length, biomass, specific leaf area (SLA), total chlorophyll content (SPAD) and relative water content (RWC)) and biochemical (hydrogen peroxide (H_2O_2) amount and histochemical staining (H_2O_2)) changes caused by exogenous GB treatment in two wheat cultivars with different drought tolerance grown in Turkey.

2. MATERIALS AND METHOD

2.1. Plant materials and growing conditions

Triticum aestivum L. is known as a bread wheat species belonging to the *Poaceae* family. In this study, seeds of drought-sensitive Selçuk and drought-resistant Demir-2000 cultivars were obtained from Bahri Dağdaş International Agricultural Research Institute and Ankara Field Crops Central Research Institute, respectively. Surface sterilization of seeds was carried out with a 5% sodium hypochlorite solution. The seeds germinated in moist blotting papers were transferred to pots with washed perlite. Plants were grown in the cabin in the plant growing room, at 24±2 °C and a photoperiod of 16/8 h day/night
cycle, irrigated with 100% Hoagland nutrient solution [18]. The 20-day seedlings are divided into 4 groups, including a control group with no treatment (K), a drought group (D), a group with glycine-betaine treatment (GB) and a group with a water scarcity applied after GB (GB+D) (Figure 1). The 20-day-old wheat seedlings have 5 mM GB applied by spraying them into the leaves. Drought stress followed by 24. the water scarcity was created and started at the end of 7 days and the leaf and root sampling was done.

Some physiological (shoot and root length, biomass, specific leaf area (SLA), total chlorophyll content (SPAD) and relative water content (RWC)) and biochemical (hydrogen peroxide (H_2O_2) amount and histochemical staining (H_2O_2)) changes were investigated in samples taken from plants.



Figure 1: Drought stress and the effects of GB treatment on wheat seedlings. (C: Control D: Drought GB: Glycine-Betaine GB+D: Glycine-Betaine +Drought).

2.2. Plant measurements and analysis methods

2.2.1. Shoot and root length

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 plant seedlings with the help of a ruler.

2.2.2. *Determination of Biomass*

The weight of the plant seedlings was determined by weighing a total of 9 seedlings with 3 replicates from each group (g).

2.2.3. Determination of Specific Leaf Area (SLA)

Leaf area was calculated in Image J program by photographing the leaves of plant seedlings. After that, the samples were dried in an oven at 70°C for 24 hours and weighed on a precision balance. SLA was calculated by applying the weighing results to the following formula [21].

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

2.2.4. Determination of Relative Water Content (RWC)

Mature leaves of plant seedlings (first leaf under flag leaf) were used for wet weight measurement. The turgor weights were recorded after the plants, whose wet weights were taken, were placed between the drying papers, and kept in a tub containing pure water for 4 hours. After this process, the leaves were dried in an oven at 70 ºC for 24 hours and their dry weight was determined. Relative water contents were calculated by applying these values to the formula [16].

RWC=(FW-DW) / (TW-DW) X 100
RWC: Relative water content FW: Fresh weight
TW: Turgor weight DW: Dry weight

2.2.5. Total Chlorophyll Amount

Total chlorophyll contents of plants were determined from leaves of plants with SPAD (Minolta SPAD-502, Osaka, Japan) [13].

2.2.6. Amount of Hydrogen Peroxide (H_2O_2)

0.1 g of plant sample was homogenized with a mixture of 3 ml of H_2SO_4 (Sulfuric Acid) and cold acetone homogenization buffer and centrifuged. Centrifuged samples were read in a blank at 550-800 nm against water by preparing a reading buffer containing H_2SO_4, purified water, ferrous ammonium sulfate, xenol orange, sorbitol, and ethanol (e-FOX) [4].

2.2.7. Determination of H_2O_2 Accumulation by Histochemical Staining

The leaves were incubated in a solution containing 1 mg/ml of 3',3'-diaminobenzidine (DAB) at 25°C for 12 hours. Incubated leaves were decolorized by immersion in boiling ethanol (90%) for 15 min to visualize reddish brown spots of H_2O_2. Then stained leaves were photographed against a contrasting background for proper visual [8].

2.2.8. Statistical analysis

Statistical analysis of the data was calculated using the one-way analysis of variance (ANOVA) method in IBM SPSS Statistics 21 program.

3. RESULTS

3.1. Shoot and root length

Drought stress reduced shoot and root length of the Selçuklu cultivar by 12% and 5%, respectively, compared to control. With GB+D treatment, these reductions were determined as 7% and 3%, respectively.

Similarly, in Demir 2000, drought stress reduced shoot and root length by 12% and 17%, respectively, compared to the control. With GB+D treatment, these reductions were determined as 8% and 15%, respectively.

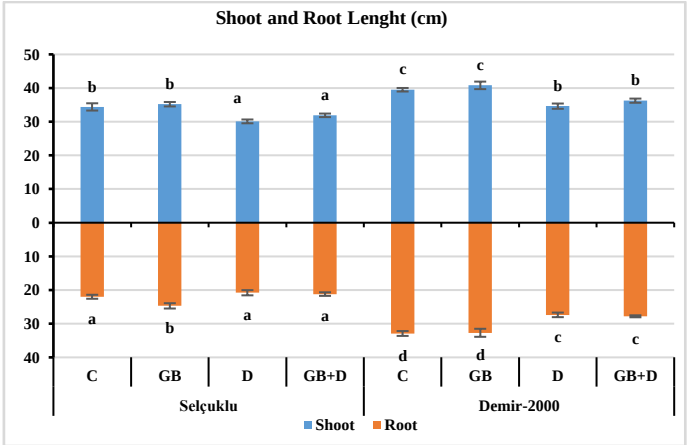


Figure 2: Effects of drought stress and GB treatment on shoot and root length. (C: Control D: Drought GB: Glycine-Betaine GB+D: Glycine-Betaine +Drought).

3.2. *Biomass*

Compared to the biomass control in Selçuklu cultivar, it decreased by 41% with drought stress and decreased by 23% with GB+D treatment.

Drought stress biomass of Demir-2000 cultivar decreased by 27% compared to control, while it decreased by 21% with GB+D treatment.

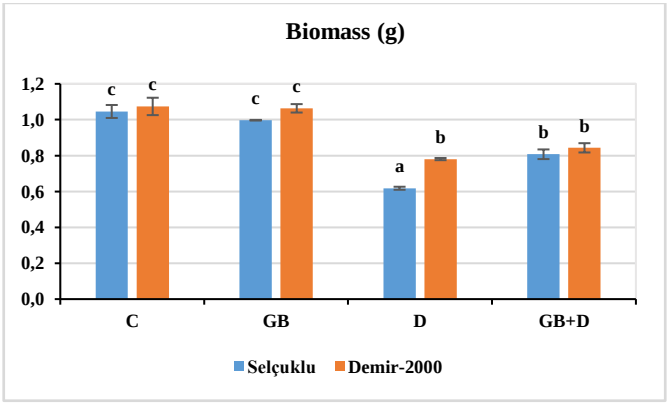


Figure 3: Effects of drought stress and GB treatment on biomass. (C: Control D: Drought GB: Glycine-Betaine GB+D: Glycine-Betaine +Drought).

3.3. Specific Leaf Area (SLA)

Drought stress decreased leaf area of Selçuklu and Demir-2000 cultivars by 14% and 8%, respectively, compared to the control. GB treatment reduced drought damage in Selçuklu and Demir-2000 cultivars, and SLA was determined as 5% and 1%, respectively, compared to control.

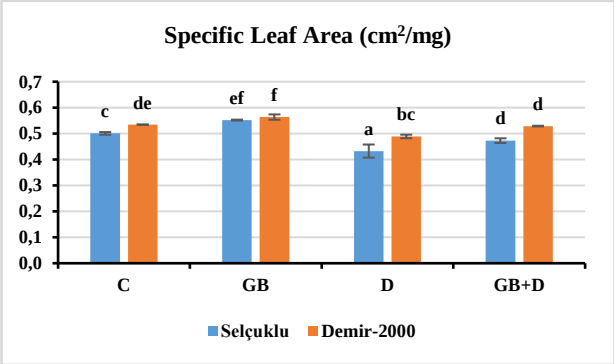


Figure 4: Effects of drought stress and GB treatment on specific leaf area. (C: Control D: Drought GB: Glycine-Betaine GB+D: Glycine-Betaine +Drought).

3.4. Relative Water Content (RWC)

Drought stress reduced RWC by 11% in Selçuklu variety and 5% in Demir-2000 variety compared to control.

GB and GB+D treatments increased RWC by 5% and 2%, respectively, in Selçuklu cultivar compared to the control. Accordingly, GB treatment preserved RWC better in Demir-2000 variety than Selçuklu variety.

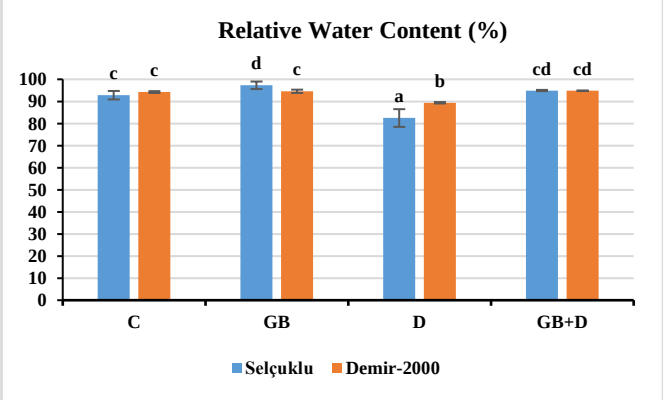


Figure 5: Effects of drought stress and GB treatment on relative water content. (C: Control D: Drought GB: Glycine-Betaine GB+D: Glycine-Betaine +Drought).

3.5. Total Chlorophyll Amount

While the total amount of chlorophyll in Selçuklu cultivar decreased by 46% with drought stress compared to control, it increased by 7% with GB+D treatment. It was determined that the amount of chlorophyll decreased by 17% with drought compared to the control in Demir-2000 cultivar and decreased by 3% as a result of GB+D treatment.

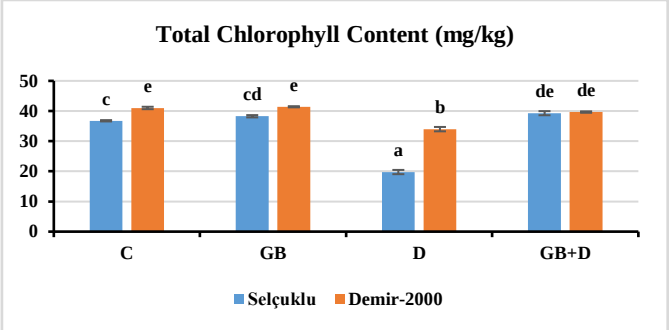


Figure 6: Effects of drought stress and GB treatment on total chlorophyll content. (C: Control D: Drought GB: Glycine-Betaine GB+D: Glycine-Betaine +Drought).

3.6. H_2O_2 Amount and Histochemical Staining

Drought stress increased the H_2O_2 content of Selçuklu and Demir-2000 cultivars by 14% and 6%, respectively, compared to the control. GB treatment increased the H_2O_2 content by 6% and 1%, respectively, compared to the control. Histochemical staining supports these data (Figs 7, 8 and 9).

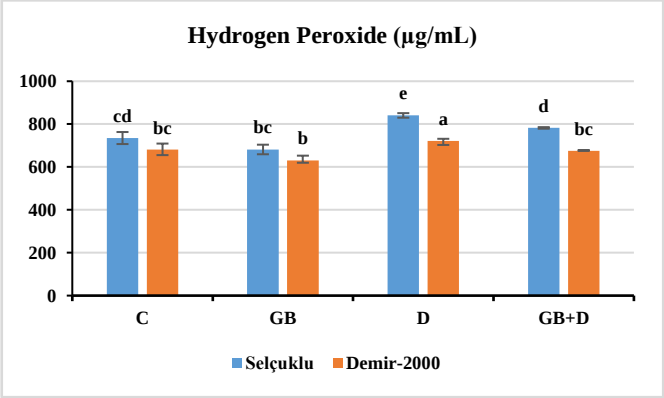


Figure 7: Effects of drought stress and GB treatment on H_2O_2 amount. (C: Control D: Drought GB: Glycine-Betaine GB+D: Glycine-Betaine +Drought).

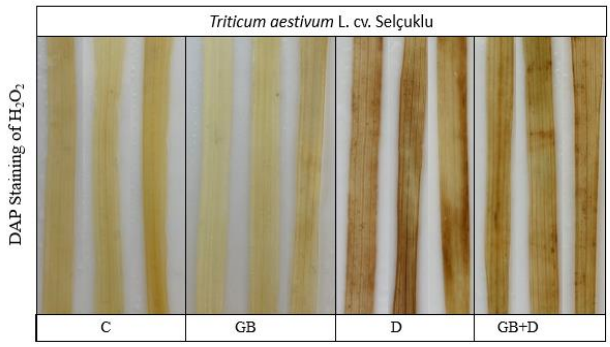


Figure 8: The effects of drought stress and GB treatment on the amount of hydrogen peroxide in Selçuklu cultivar by histochemical staining. (C: Control D: Drought GB: Glycine-Betaine GB+D: Glycine-Betaine +Drought).

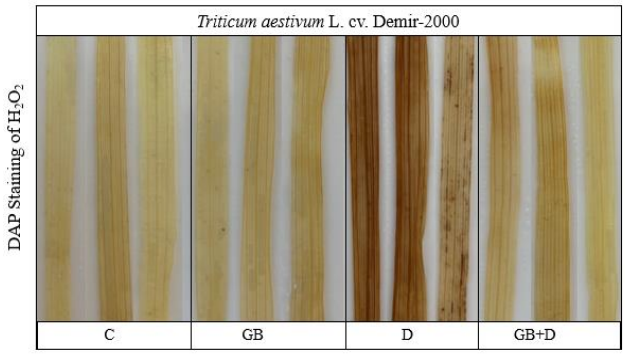


Figure 9: The effects of drought stress and GB treatment on the amount of hydrogen peroxide in Demir-2000 cultivar by histochemical staining. (C: Control D: Drought GB: Glycine-Betaine GB+D: Glycine-Betaine +Drought).

4. DISCUSSION

As an osmo-protectant, foliar exogenous GB treatment to plants that cannot synthesize GB or can only synthesize small amounts of GB can alleviate the effects of abiotic stress [2]. Drought stress is a limiting abiotic stress factor for wheat production and food security (Raza et al., 2014). On the other hand, exogenous GB treatment improves plant biomass and yield in different crops exposed to water scarcity [12,3]. In addition, it was reported that the GB treatment of wheat seedlings under drought stress compensates for the reduction in root length in cultivars with different drought tolerance, while maintaining shoot length only in drought-resistant cultivars [1]. Moreover, it was shown that exogenous GB significantly improved spike length, grain number per spike, grain yield, plant water potential, osmotic potential, and turgor potential in two wheat cultivars under drought stress [14].

In addition, it was determined that GB treatment protected the drought-resistant variety better from drought-induced chlorosis compared to the susceptible variety in wheat [1]. However, it has been reported that exogenous betaine treatment in barley under drought stress improved the drought-related decreases in plant height, root length and root dry weight, chlorophyll contents and photosynthesis parameters [20].

Drought stress reduces chlorophyll content, biomass, root and shoot length, yield, water potential, osmotic potential and turgor potential in wheat and barley. Our research results showed that the amount of H_2O_2 increased under drought stress, while other physiological parameters decreased in the drought-sensitive Selçuk compared to the drought-resistant Demir-2000. However, GB treatment reduced drought-related damage in both cultivars. As a result, exogenous GB treatment was found more effective for reducing drought stress damage in root and shoot lengths, biomass, SLA, RWC, chlorophyll content and H_2O_2 amount for susceptible variety compared to resistant variety.

5. CONCLUSION

In our study, the responses of two wheat cultivars with different drought tolerance to exogenous GB treatment under drought stress were different. Accordingly, it has been shown that negative effects such as growth restricted by drought stress, decreased amount of chlorophyll and increased oxidant concentration can be reduced by exogenous GB. Moreover, it was determined that drought susceptible Selçuk variety was affected much better from exogenous GB treatment compared to resistant Demir-2000 variety.

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