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(Research Article)

Effect of glycine betaine and silicon on drought-stressed durum wheat (*Triticum durum* Desf.) plants

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Abstract

Water deficit is the major factor limiting durum wheat (*Triticum durum* Desf.) production and productivity. In this context, a first experiment was conducted to study the effect of seed priming by glycine betaine (GB) on the germination of durum wheat under water deficit stress induced by polyethylene glycol (PEG 6000). Five treatments were applied (T0: Control (0 g/l PEG, 0 mM GB); T1: 0 PEG, 50 mM GB; T2: 0 PEG, 100 mM; T3: 125 g/l PEG, 0 mM GB;T4: 125 g/l PEG, 50 mM GB;T5: 125 g/l PEG, 100 mM GB. A second experiment has focused on the evaluation of the effect of silicon (Si) foliar application on the physiological response of durum wheat to water stress in the field under semi-arid conditions. Two treatments were applied (whithout Si (Si-) and with Si (Si+)). Main results showed that durum wheat seed priming by GB improved germination and seedling growth under water deficit conditions. The highest values of germination percentage (75.83%), vigor index (434.45) and shoot length (4.45 cm) were obtained using 50 mM of GB. The foliar application of silicon improvedphysiological performance of different studied varieties. Highest values of relative water content (57.31%), leaf area (31.56 cm^2) were recorded with the application of Si. Results showed that seed priming with glycine betaine and the foliar application of silicon could be among new approaches to alleviate adverse effects of water deficit stress.

Keywords: Durum wheat; Water deficit stress; Glycine betaine; Silicon

1. Introduction

Within an agricultural context, drought is defined as an extended period of insufficient precipitation that adversely affects crop growth and yield. Due to global warming, drought is expected to become more frequent and severe in the near future [1]. This environmental stress impacts plants throughout their developmental cycle [2]. Seed germination is particularly sensitive to drought conditions [3]. Early exposure to water stress significantly affects root formation, leading to reduced grain reserves and delayed or even arrested germination [4]. In wheat, water deficit reduces germination rate, vigor, coleoptile length, and both root and shoot length [5].Water deficit stress is the primary abiotic factor limiting durum wheat (*Triticum durum* Desf.) production [6]. It inhibits cell division, there by restricting leaf growth [7]. Additionally, it reduces leaf size and surface area, leading to curling and, in severe cases, complete wilting

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[8].Relative water content is a widely used indicator of a plant's water balance [9]. Under drought conditions, a decline in relative water content leads to a significant reduction in total biomass production [10].

To defend against drought stress, cereal crops employ various strategies at both cellular and molecular levels. They modify their metabolic pathways and synthesize specific osmolytes to enhance tolerance to abiotic stresses [11]. Glycine betaine (GB) has been identified as an effective compound for protecting plants against multiple abiotic stresses, including water stress [12]. GB is a low-molecular-weight compound characterized by high solubility and low viscosity. Due to these properties, it is considered one of the most effective osmoprotectants for enhancing water stress tolerance [13]. GB seed priming has been shown to improve abiotic stress tolerance in various species, particularly during germination and early seedling growth [14]. In durum wheat, seed priming with GB enhances germination and seedling growth under water stress conditions [15]. The improved tolerance induced by GB seed priming may be attributed to increased antioxidant activity, reduced accumulation of reactive oxygen species (ROS), lower lipid peroxidation, decreased electrolyte leakage, and enhanced membrane stability.

Additionally, silicon (Si) is a multifunctional element [16] and a beneficial nutrient, similar to nitrogen, phosphorus, and potassium. It plays a crucial role in the physiological activity of plants exposed to water stress [17]. Silicon is the second most abundant element in the Earth's crust (27.7%), following oxygen [18]. However, it is primarily found in the inert form of silicon dioxide (SiO_2), which is not readily accessible to plants. Plants absorb Si in the form of uncharged monosilicic acid [$Si(OH)_4$], which remains soluble in soil solutions with a pH below 9 [19]. Metabolic changes in plant cells following silicon (Si) treatment suggest that it may act as an activator of plant defense responses [20]. Si can be used as a mineral nutrient to enhance plant resistance to various stress conditions [21].The application of Si has been shown to improve germination rate, plant growth, biomass production, photosynthesis, and stomatal conductance in wheat under water deficit conditions [22,23]. Moreover, its use has been associated with reduced antioxidant enzyme activity and lipid peroxidation [24]. Si also enhances fresh weight, dry weight, germination energy, seedling length, and vigor index [16]. Furthermore, it strengthens plant resistance to different stress levels and improves the overall mechanical stability of both stressed and unstressed plants [25].

Therefore, this study aimed to evaluate the effectiveness of glycine betaine seed priming and silicon foliar application on durum wheat germination and plant growth under water deficit conditions.

2. Materials and Methods

2.1. Experiment 1: Effect of glycine betaine seed priming on durum wheat seed germination under water deficit conditions

The plant material used consists of seven durum wheat varieties: Maâli, Om rabiaa, Ben Bechir, Karim, Salim, Dhahbi and Inrat 100,

2.1.1. Preparation of the polyethylene glycol (PEG) solutions

The PEG solution, with an osmotic potential of -3.02 bar, was prepared by dissolving 125 g of PEG 6000 (molecular weight: 6000 g mol^{-1}, purity>99.0%; Sigma-Aldrich, St. Louis, USA) in one liter of distilled water. Polyethylene glycol (PEG) is a water-soluble, non-ionic polymer that is impermeable to plant cells [26]. It is commonly used to simulate water deficit conditions by reducing water availability to plants [27]. For the hydropriming treatment, seeds of the tested varieties were soaked in distilled water for 12 hours [28]. For osmopriming, seeds of each variety were soaked in a glycine betaine solution at three concentrations: 0, 50, and 100 mM, corresponding to 0, 3.752 g/L, and 7.506 g/L, respectively. Priming was conducted in the dark for 12 hours, followed by three rinses. The seeds were then dried on filter paper for 48 hours until they regained their initial weight [15].

2.1.2. Germination

The disinfected and treated seeds were germinated at a rate of 10 seeds per Petri dishes and placed in an incubator under controlled conditions (humidity: 50%; average temperature: 22 ± 2°C, day and night). The experimental design was completely randomized, with three replicates for each variety and treatment.

Every two days, 5 mL of either the PEG solution or distilled water was added to the Petri dishes according to the following treatments:

- **T0:** Control (0 g/L PEG, 0 mM GB)

- **T1:** 0 g/L PEG, 50 mM GB
- **T2:** 0 g/L PEG, 100 mM GB
- **T3:** 125 g/L PEG, 0 mM GB
- **T4:** 125 g/L PEG, 50 mM GB
- **T5:** 125 g/L PEG, 100 mM GB

2.1.3. Parameters measured

Four parameters were measured:

-Germination percentage PG = (total number of germinated seeds / total number of observed seeds)*100

- Vigor index (VI)

VI = % germination* average seedling length

-Shoot length (SL, cm):

-Root length (RL, cm): measured from the base of shoot to the tip of the root

2.2. Trial 2: Effect of foliar application of silicon on the performance of durum wheat grown under semi-arid conditions

2.2.1. Experimental Site Description and Experimental Design

A field experiment was conducted during the 2022–2023 growing season using a randomized split-plot design with three replicates at the INRAT (National Institute of Agronomic Research) station in Kef, a semi-arid region of Tunisia. The following durum wheat varieties were used: Maâli, Karim, Salim, Dhahbi, and Inrat 100. Foliar silicon (Si) treatments, using sodium metasilicate ($Na_2SiO_3·9H_2O$), were applied at the early tillering and anthesis stages at a concentration of 100 mg/L. Two treatments were tested: without silicon (Si-) and with silicon (Si+). Average monthly rainfall and mean temperatures during the growing season are presented in Table 1.

Table 1 Average monthly rainfall and mean temperatures during 2022-2023 growing season

	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	Mai
Mean Temperature	26.6	21.3	16.25	9.8	10.05	10.6	12.4	15.2	22
Rainfall (mm)	3.6	25.3	10.8	44	52	27	32	30.6	8.8

2.2.2. Crop management

The plots were manually sown on November 22, 2022, at a rate of 25 seeds per 2.5 m. Nitrogen fertilization (33.5% ammonium nitrate) was applied at a rate of 100 kg/ha during the tillering and stem elongation stages.

For weed control, the herbicide Puma Evolution Zoom (containing 65.9% Dicamba and 4.1% Triasulfuron) was applied at a dosage of 1 L/ha.

2.2.3. Parameters measured

- Leaf area (LA)

At anthesis, from three plants, leaves were cut at random to measure leaf area.

LA = leaf length × leaf width × 0.78

- Relative water content (RWC)

Relative water content was estimated according to Barrs [29] as follows: two leaves were immediately weighed (FW). Then, the same samples were oven-dried at 70 ºC for 48 h, and the dry weight (DW) was recorded. RWC% was determined using the formula:

$$RWC\ \% = [(FW - DW)/FW] \times 100$$

- Leaf membrane stability index (MSI)

Membrane stability index was determined indirectly by measuring electrical conductivity (EC) following the protocol of Kocheva et al. [30]. Glass vials containing 1 gram of plant material and 10 mL of double-distilled water were shaken for 24 hours at 10°C. The initial electrical conductivity (EC_1) was measured using a conductivity meter after bringing the samples to 25°C. Next, the samples were autoclaved at 0.1 MPa for 10 minutes, cooled to 25°C, and the final electrical conductivity (EC_2) was recorded. Leaf MSI was then calculated according to Blum & Ebercon [31] using the following formula:

$$MSI\ \% = [1 - (EC1 / EC2)] \times 100$$

where, EC1 and EC2 refer to readings before and after autoclaving, respectively.

2.2.4. Statistical analysis

All data were subjected to variance (ANOVA) to test treatment effects and means were separated with Duncan's test at P = 0.05, P < 0.01 and P <0 .001.

3. Results and discussion

3.1. Effect of seed priming with glycine betaine on durum wheat germination under water deficit conditions

Variance analysis revealed a highly significant effect (p<0.001) of the applied treatments and the studied varieties on all measured parameters (table 2).

Table 2 Variance analysis (mean square and F-test) for germination percentage (GP), vigor index (VI), shoot length (SL) and root length (RL) of durum wheat tested varieties under different treatments

Source of variation	ddl	GP (%)	VI	CL (cm)	SL (cm)	RL (cm)
Treatments	5	882.222***	1533283.215***	19.577***	75.293***	72.633***
Varieties	3	4648.148***	1434495.300***	1.602***	39.381***	222.901***
Treatments × Varieties	15	148.148*	225201,404***	0.391*	8.206***	36.447***

ddl: degree of freedom; ns: not significant; *: significant at p<0.05; ** significant at p<0.01; ***significant at p<0.001

3.1.1. Germination percentage

Obtained results showed that the lowest germination percentage (65%) was recorded under water deficit conditions. Similar results were obtained by Mujtaba et al. [32] in six wheat genotypes. However, hydropriming and osmopriming by 50 mM glycine betaine (GB) showed the highest GP (89.16% and 75.83%) under unstressed and stressed conditions (Figure 1 A). Hussain et al. [33] showed that seed osmopriming with GB for 12 h improved seed germination in rice. In addition, Mahmood et al. [34] revealed that seed priming using 50 mM GB increased the drought tolerance of five wheat cultivars.

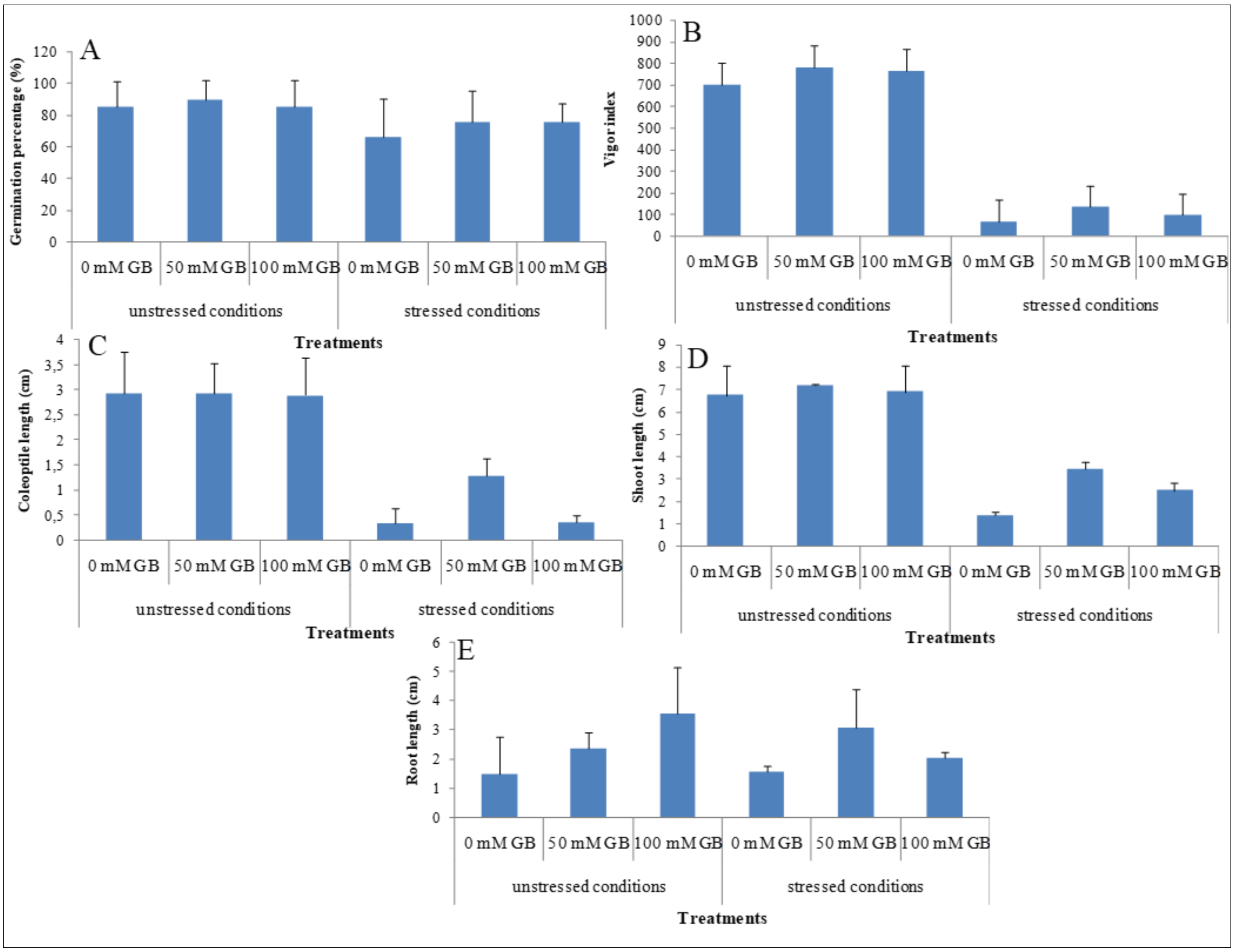

Figure 1 Effect of water deficiency and seed priming with glycine betaine treatments on germination percentage (A), vigor index (B), coleoptile length (C), shoot length (D) and root length (E) of durum wheat varieties under different treatments

3.1.2. Vigor index

Figure 1 B showed that water deficit reduced vigor index of durum wheat seedlings. Under water deficit conditions, osmopriming with GB improved it essentially by using a concentration of 50 mM. The results obtained by Ahmed et al. [15], revealed that the most significant increase in VI was recorded with the application of GB in wheat.

3.1.3. Shoot length

Abiotic stresses restrained the growth of different plants [35]. In the present study, water deficiency has a negative effect on the seedling shoot length (Figure 1 D). This may be the result of reduced protoplasm extension and dehydration, which are linked to limited cell expansion and prevention of mitosis [36]. Under both treatments (with and without water deficit) the application of 50 Mm GB recorded highest values 4.45 and 7.17 cm of SL respectively.

3.1.4. Coleoptile length

The coleoptile is linked with the emergence capacity, and it is essential for plant vigor and successful emergence, which leads to increase grain yield [37]. According to Figure 1 C water stress reduced coleoptile length (CL). Sassi et al. [4] explained that the effect of water deficit on the decrease in CL depends on the applied stress severity. However, osmopriming with 50 mM GB improved this trait in different durum wheat varieties.

3.1.5. Root length

Water deficit significantly reduces root length (RL)(Figure 1 E). Sassi et al. [4] found that water stress reduced RLin six durum wheat varieties. Nevertheless, osmopriming with glycine betaine increased this trait. The highest length (3.08 cm), under water deficit conditions, was obtained using 50 mM GB. Ahmed et al. [15] showed that durum wheat seed priming with GB stimulated root elongation.

3.2. Effect of foliar application of silicon on the response of durum wheat to water stress

The aim of this trial was to study the effect of foliar treatment with silicon on stimulating the tolerance of durum wheat varieties, grown in the field, to water stress in a semi-arid climate. Analysis of variance revealed a significant effect ($p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$) of treatments and varieties on all the measured parameters (Table 3).

Table 3 Variance analysis of (mean square and F test) of physiological parameters: relative water content (RWC, %), leaf area (LA, cm^2) and membrane stability index (SMI, %) of durum wheat varieties under different treatments

Source of variation	ddl	TRE (%)	SF (cm^2)	MSI (%)
Treatments	1	67.480*	42.392**	1485.727***
Varieties	4	236.626**	113.916***	288.958***
Treatments × Varieties	4	397.296***	130.447***	161.229***

ddl: degree of freedom; ns: not significant; *: significant at p<0.05; ** significant at p<0.01; ***significant at p<0.001

3.2.1. Relative water content

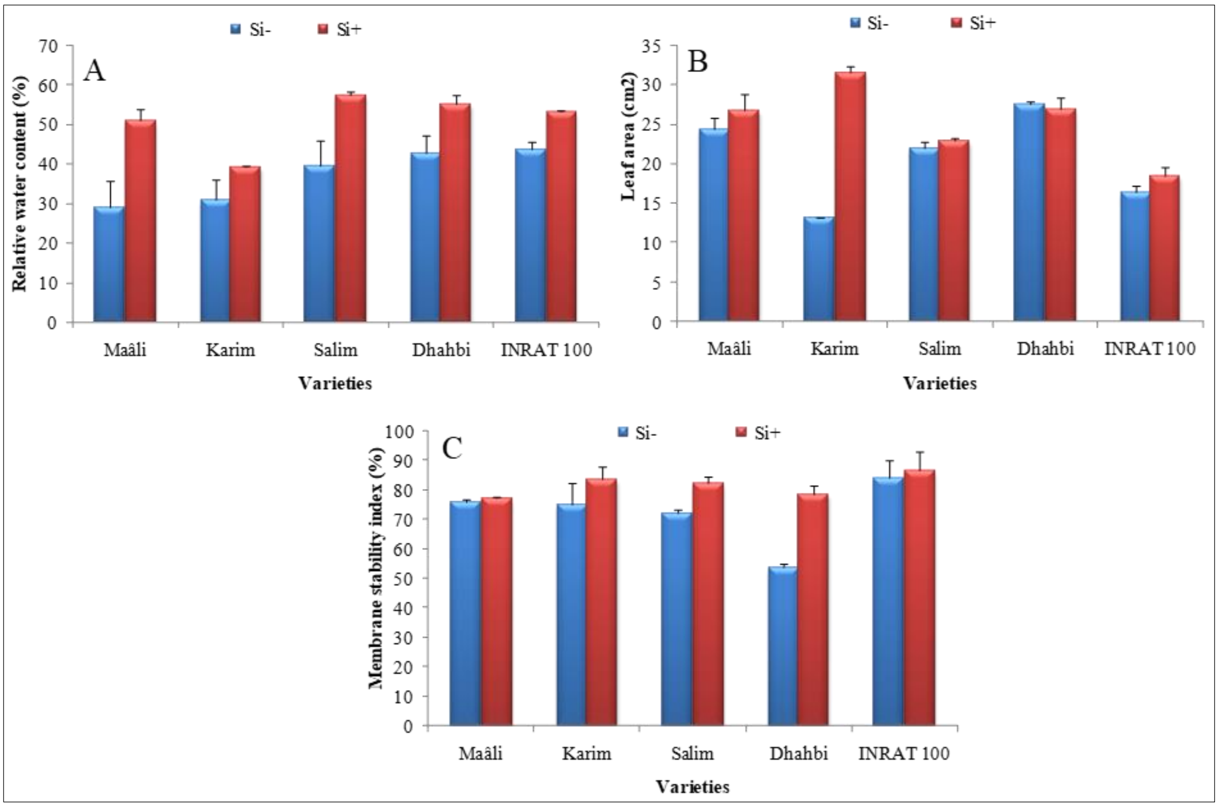

Figure 2 Effect silicon foliar application on relative water content (A), leaf area (B) and membrane stability index (C) of different durum wheat varieties grown under rainfed conditions

According to figure 2 A, the lowest relative water content (RWC) (29.08%) was recorded in Maâli variety under rainfed conditions. This variety had the highest percentage increase (74.96%) in RWC when Si was applied. RWC decreased considerably in plants subjected to water stress conditions [38]. There are two adaptation mechanisms which are responsible for maintaining a high RWC value under stress, either by maintaining high tissue elasticity or by reducing osmotic pressure [39]. Furthermore, the results showed that foliar treatment with Si improved this parameter in all studied varieties. Gong and Chen [40] showed that in wheat leaves treated with Si, the relative water content and water potential were maintained compared with untreated leaves, indicating that Si can also be used to improve the water status of wheat under drought conditions in the field. The results obtained by Behboudi et al. [41] revealed that water stress significantly reduced RWC, whereas, the application of Si significantly increased it. Si can improve the accumulation and the storage of water in leaves, increase the amount of water in the plant body, reduce transpiration and improve water absorption capacity [42].

3.2.2. Leaf area

The lowest leaf area (LA) value was recorded under the rainfed regime without Si application in Karim variety (13.12%) (figure 1 B). The water deficit stress reduced LA, which could be explained by the reduction in photosynthesis following a lack of water availability at the plant root level. In this respect, Alem et al. [43] revealed that the reduction in photosynthesis was essentially due to the reduction in leaf area. Drought stress leads to a significant reduction in total biomass production, by reducing the number of leaf-bearing organs [44]. In addition, water deficit leads to a reduction in the leaf area, leaf life and photosynthetic capacity, as well as a reduction in the plant's turgor pressure, causing a loss of water from the cell contents. This loss of turgidity can in turn have harmful physiological effects on the plant [45].

Obtained results showed that the addition of Si significantly improved LA. The highest values (31.56 cm^2 ; 28.89 cm^2 and 26.71 cm^2) were obtained in the presence of Si in Karim, Dhahbi and Maâli varieties respectively. Si foliar application promoted positive effects on plant organism, mostly by acting in the optimization of physiological and biochemical processes, which influenced growth indicators [46]. Si improved in leaf anatomy, which assured good nutrients absorption and assimilates translocation to the cells which certainly resulted in vigorous growth [47].

3.2.3. Leaf membrane stability index

Drought stress caused a decrease in membrane stability index (MSI) (Figure 2 C). Water stress has a typically significant effect on the alteration of the cellular membrane, which resulted in a dysfunction. Moreover, in plants the preservation of membrane integrity and stability under stress is a main component of stress tolerance [48]. However, obtained results revealed that silicon improved MSI in all durum wheat varieties. Under both treatments (Si- and Si+), the INRAT 100 variety had the highest values. These results are similar to those obtained by Maghsoudi et al. [17] for wheat cultivars. According to Ma and Yamaji [49], silicon is deposited in the stems and leaves, where it helps to increase the strength and solidity of the cell walls and reduce transpiration from the cuticles. It also increases light interception by helping to keep the leaves upright.

4. Conclusion

The present study demonstrated that drought stress negatively affected durum wheat germination, seedling growth, and physiological attributes. However, glycine betaine seed priming improved germination percentage, vigor index, and shoot and root length under induced water deficit conditions. Additionally, foliar application of silicon enhanced relative water content, leaf area, and membrane stability index in the studied durum wheat varieties under drought stress.

Further research is needed to explore the molecular mechanisms by which GB and Si regulate drought tolerance in wheat.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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