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Drought stress represents a considerable risk to global agricultural yield, requiring novel strategies to alleviate its detrimental effects on agriculture. This research examines the impact of β-sitosterol and glycine betaine on the resilience of *Lupinus terms* (lupine) plants under drought circumstances by enhancing antioxidative defense mechanisms, increasing secondary metabolite synthesis, and facilitating osmolyte accumulation. Under drought stress, Lupinus plants showed reduced growth and elevated oxidative stress indicators malondialdehyde and H_2O_2. The combination of β-sitosterol and glycine betaine significantly enhanced plant development, demonstrated by improved photosynthesis and biomass of roots and shoots, with reduced oxidative stress. This improvement was ascribed to increased antioxidative enzymes, like catalase, superoxide dismutase, peroxidase, glutathione reductase, and ascorbate peroxidase, which jointly decreased reactive oxygen species levels and averted oxidative damage. Moreover, β-sitosterol and glycine betaine markedly increased the synthesis of osmolytes and secondary metabolites, allowing lupine plants to maintain cellular turgor and osmotic equilibrium under drought conditions, hence enhancing stress tolerance. In summary, our results provide significant insights into sustainable methods for improving crop resistance to drought, with potential agricultural applications to mitigate the detrimental impacts of climate change on crops.

Keywords: drought; enzymatic; Hormonal content; non-enzymatic antioxidants; ROS indicator

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INTRODUCTION

Globally, particularly in arid and semi-arid areas, water limitation is recognized as one of the most detrimental stresses affecting plant environments, decreasing plant development and growth (Al-Mokadem et al., 2023). The extended duration of water stress leads to considerable harm and, ultimately, the death of plant cells, primarily due to the overproduction of reactive oxygen species (ROS). This excess reactive oxygen species interferes with the scavenging capabilities of the antioxidant defense system components. Ongoing or repeated drought impacts nearly 45% of the global agricultural landscape (Hou et al., 2024).

ROS are produced in response to various environmental stresses, resulting in lipid peroxidation and the destruction of nucleic acids and proteins, disrupting plant metabolism (Hafez and Fouad, 2020, Alzamel, 2025). To mitigate the impacts of ROS, plants synthesize antioxidants, including several phytohormones, non-enzymatic and enzymatic components, and osmolytes, that manage the processes for sustaining optimal ROS levels (Choudhary et al., 2024).

The global reduction in water resources for agriculture is attributed to a rapidly increasing population, the substantial effects of global warming, climate change, and various human activities. Implementing multiple strategies, including using antioxidants and osmolytes, is essential to improve plant growth under stress forces. Notable examples include β-sitosterol and glycine betaine (Shafiq et al., 2021).

Over the past ten years, significant progress has been achieved in understanding the regulatory functions of phytosterols (including sterols and brassinosteroids) in influencing various traits crucial for agriculture, like photosynthesis, flowering time, and plant growth, in cultivated species. Notably, sterols and brassinosteroids have emerged as viable approaches to decrease the negative effects of numerous abiotic stresses, including heat, drought, and cold, enhancing crop productivity under current growth conditions (Wei and Li 2020). Phytosterols have recently emerged as a novel plant growth regulator, playing a significant role in various biological effects and gene expression. Their potential as a plant growth-promoting stimulant under stressful and normal conditions has garnered considerable interest (Du et al., 2022). Glycine betaine (GB) represents a prevalent quaternary ammonium compound utilized to alleviate and adjust to challenging environmental conditions. This compound is colorless, metabolically stable, water-soluble, and odorless, contributing to its effectiveness as an osmoprotectant. By oxidizing GB, chloroplasts stabilize their structure, and photosystem II retains its functionality under various stress conditions (Huang et al., 2020). Many researchers have observed that GB can safeguard plants against various stresses, like drought conditions, metal toxicity, and salinity (Oyebamiji et al., 2024). GB boosts several enzymatic and non-enzymatic antioxidants, which protect cells from oxidative stress. Furthermore, the external treatment of GB has demonstrated efficacy in mitigating the

toxic impacts of numerous abiotic stresses (Badawy et al., 2024).

Lupinus terms (lupine) is grown in several habitats. Lupine seeds have been farmed in Egypt since antiquity as one of the oldest grain legumes, with significant nutritional potential owing to their high oil content (10-15%) and protein content (35-45%). Egyptians and other nations have consumed its seeds as medicinal plants and snacks for millennia. Legumes are often regarded as fairly drought-resistant (Sharaf et al., 2009). Egypt is cultivating various crops, including Lupinus, on newly reclaimed soils despite drought affecting most of these soils. Enhancing the water stress tolerance of Lupinus is essential for augmenting global Lupinus production. Given this context, we predicted that the exogenous treatment of β-sitosterol and glycine betaine to field-grown Lupinus might enhance drought stress tolerance by mitigating oxidative damage via the up-regulation of tolerance mechanisms.

MATERIALS AND METHODS

Experimental procedures

During the winter of 2023, a Botanical farm at the Faculty of Science, Al-Azhar University Cairo, Egypt, cultivated healthy lupine seeds (*Lupinus termis* L.) (Giza 2). The seeds were acquired from the Egyptian Ministry of Agriculture and Land Reclamation (MALR) at Giza, Egypt. Identical-sized and colored seeds were selected for uniformity. The seeds were rinsed with distilled H_2O, sterilized in sodium hypochlorite (1%) for two minutes, and subsequently cleaned with distilled water. A room-temperature overnight dry was performed on the seeds. These were seeded in 3.0 m × 3.5 m plots, with rows spaced 70 cm apart and hills spaced 20–25 cm apart. For soil preparation and plant growth, MALR recommends supplying the entire dose of nitrogen (N), phosphorus (P), and potassium (K) fertilizer. The concentrations of calcium superphosphate, ammonium sulfate, and potassium sulfate were 359 kg ha^{-1}, 288 kg ha^{-1}, and 124 kg ha^{-1}, respectively, during the seed-bed preparation process. The soil's attributes are as follows: textured sandy loam, 27.56% sand, 25.29% silt, and 45.15% clay. To estimate the chemical and physical analyses of the soil collected at a depth of 30 cm before planting (Table 1). Twenty days after planting, the various irrigation levels were implemented. Foliar β-sitosterol or glycine betaine concentrations were administered 55 and 75 days after sowing. The following treatments were represented by the plots and divided into nine groups. Each treatment contained five replications:

Experimental scheme number	Description of an experimental scheme
T1	100 % water requirement (WR)
T2	100 % WR+ β-sitosterol 100 ppm
T3	100 % WR+ glycine betaine 100 ppm
T4	75 % WR.
T5	75 % WR+ β-sitosterol 100 ppm
T6	75 % WR+ glycine betaine 100 ppm
T7	50 % WR.
T8	50 % WR+ β-sitosterol 100 ppm
T9	50 % WR+ glycine betaine 100 ppm

Table 1. Chemical properties of soil

Character	
Soil characteristics	
Electrical conductivity (EC) mm hos/cm	1.21
pH	7.1
Total soluble salts (TSS, ppm)	772
Texture grade	sandy loamy
Soluble cations (meq L^{-1})	
Sodium (Na^+)	2.31
Calcium (Ca^{++})	2.32
Potassium (K^+)	0.51
Magnisum (Mg^{++}	1
Soluble anions (meq L^{-1})	
Carbon trioxide (CO_3^{--})	nd‡
Hydrogen carbonate (HCO_3^-)	1
Sulfate (SO_4^{--})	0.75
Chloride (Cl^-)	4.24
†Standard Error; ‡ not detected.	

Biochemical studies

Photosynthetic Pigments

The 0.5 g of leaves were ground and centrifuged at 4500 rpm at 20 °C for 5 minutes in 80% acetone. The filtrate was examined at 470, 652, 665, and 750 nm wavelengths to quantify chlorophyll-a (Chla), chlorophyll-b (Chlb), and carotenoids (Lichtenthaler 1987).

Total soluble sugar contents

Total sugar concentrations were determined by Irigoyen et al., (1992) using anthrone and 80% sulfuric acid (H_2SO_4). An optical density of 620 nm was measured.

Determination of Total Soluble Protein

Following centrifugation for 10 minutes at 10,000 xg utilizing Folin-Ciocalteu reagent, the supernatant was extracted using cold phosphate buffer (pH 6.5; 0.05 M) and measured at 700 nm (Lowry et al., 1951).

Determination of Proline

Dried lupine leaves were tested for total proline (Bates et al., 1973). The compound was extracted using sulfosalicylic acid, equal to glacial acetic acid

and ninhydrin. Five milliliters of toluene were added after cooling and heating to 100 degrees Celsius. Spectrophotometers detected toluene layer absorption at 528 nm.

Determination of Phenols

Phenols were determined by Dihazi et al., (2003), who applied the Na_2CO_3 solution and Folin-Ciocalteu reagent (FCR). 6.5 ml of 50% methanol and 100 mg of dried lupine leaves were combined. After vortexing, the samples were allowed to stand at room temperature for 95 minutes before centrifuging at 15,000 xg for 5 minutes. The 765 nm absorbance was determined.

Determination of flavonoid

Absolute methanol was employed to soak 100 mg of dried leaf samples, which were then filtered to obtain the extract. After adding 5% $NaNO_2$ (0.3 ml), distilled water (4 ml), and 10% $AlCl_3$ (1 ml) to this filtrate, it was incubated for five minutes. Following incubation, distilled water (2.4 ml) and 4% NaOH. The Zhishen et al., (1999) approach measured absorbance at 510 nm.

Lipid peroxidation (MDA) content

Lipid peroxidation (MDA) was tested to assess oxidized lipids using Du and Bramlage (1992). Recent foliage was subjected to maceration with TCA and then centrifuged at 12,000 × g. Thiobarbituric acid was brought to the supernatant for 30 minutes in boiling water. Samples were measured at 532 nm for optical absorbance.

Determination of Relative Water Content

Leaf discs were measured for fresh weight (FW) and then soaked in distilled water for 4 hours at 25 ºC to assay the turgid weight (TW). The discs' dry weight (DW) was determined after 24 hours at 80 ºC. The equation from Barrs and Weatherley (1962) was to determine relative water content (RWC).

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

Reactive oxygen species (ROS): Hydrogen peroxide

For the extraction of leaf samples, 5% TCA was used and centrifuged for 15 minutes at 11,500 × g. After mixing phosphate buffer with KI, hydrogen peroxide (1 M) was determined at a wavelength of 390 nm. Jabs et al., (1996) determined superoxide anion (O_2) from a K-phosphate buffer. Incubating the extract with hydroxylamine hydrochloride began. Following 20 minutes of incubation, naphthyl and sulfanilamide were added, and samples were measured at 530 nm. Babbs et al., (1989) were used to compute the concentration of hydroxyl radicals (OH). The reaction mixture included KH_2PO_4 buffer, deoxyribose, $FeCl_3$, H_2O_2 (1 mM), 10^4 µM EDTA, and ascorbate (100 µM) in the last 1 mL. Samples were measured at 532 nm.

Determination Ascorbic acid (AsA)

The ascorbic acid (AsA) level in fresh leaf extracts of lupinus was determined using aqueous sulfosalicylic acid, following the methodology defined by Jagota and Dani (1982). The reaction mixture was composed of Na-molybdate at a concentration of 2%, H_2SO_4 at 0.15 N, and Na_2HPO_4 at 1.5 mM, along with the tissue extract. The absorbance was subsequently assessed at 660 nm.

Determination Glutathione

Determining leaf glutathione (GSH) content was conducted following the methodology outlined by (Owens and Belcher 1965). The leaf samples were homogenized in a 2% metaphosphoric acid solution and were centrifugated at 17,000 × g for 10 minutes. The absorbance was reported at 412 nm over one minute.

Determination Glycine Betaine

Fresh lupine leaves were milled in a toluene-water solution within a 20 mL test tube. Every tube underwent mechanical shaking for 24 hours. After filtration, the extract (0.5 mL) and 1 mL of HCl were mixed for 90 minutes in ice-cold water (Grattan and Grieve 1985). Samples were measured at 365 nm.

Antioxidant enzyme activities

Terminal buds and the first and second fully grown leaves from the plant apex were collected to test antioxidant enzymes such as catalase (CAT), peroxidase (POX), superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), and phenylalanine ammonia-lyase (PAL). Fresh plant tissue weighing 0.5 g was homogenized in 3 mL of TRIS buffer containing EDTA-Na (1 mM) and polyvinylpyrrolidone at 0–4°C. Homogenates underwent centrifugation at 10,000 × g for 20 minutes at 4 °C. The UV-160A spectrophotometer (Shimadzu, Japan) was measured at 25 °C. CAT was measured, and POX was measured by Vetter et al., (1958). Beauchamp and Fridovich (1971) measured SOD activity. The reduced absorbance at 265 nm was used to measure ascorbate peroxidase (APX) activity. GR activity was assayed by measuring NADPH oxidation at 340 nm for 1 minute (Jung et al., 1993). The enzyme reaction mixture Phenylalanine ammonia-lyase (PAL)

included 40 mM L-phenylalanine, 100 mM Tris-HCl, and a 1 ml enzyme aliquot at pH 8.8. After 30 minutes at 37 °C, 50 µL of 4 M HCl was added to stop the reaction. A UV spectrophotometer measured at 290 nm (Goldson et al., 2008).

Estimation of Hormone Content

The phytohormones (indole acetic acid (IAA), gibberellic acid (GA_3), and abscisic acid (ABA)) were measured by Knegt and Bruinsma (1973). Using 80% cold MeOH at 4 ºC, a 5-gram sample was extracted overnight in darkness. The residue was dissolved in phosphate buffer (0.1 M) at -18 °C for 24 h. The extract was centrifuged at 17000 ×g at 4 °C. Repeated partition extract against diethyl ether removed the organic phase. Diethyl ether partitioned and ejected the aqueous phase twice when 5 N HCl reached pH 2.5. Next, MeOH–acetic acid (2%) mobile phase and 1.0/min flow rate separated endogenous plant hormones (IAA, GA_3, ABA) in the C18 sep-pack cartridge reversed-phase HPLC.

Estimation of Mineral Ion Contents

Phosphorus (P), potassium (K^+), and nitrogen (N) were assessed using dried powdered tissues. P was determined, K^+ was evaluated with a flame photometer, and N was estimated using a micro-Kjeldahl apparatus (Sen Tran et al., 1988).

Statistical analysis

All measured parameters were tested for significance using the analysis of variance (SPSS 27). Before doing the variance analysis, all data were validated for variance homogeneity. A comprehensive data analysis was performed, and notable differences between groups were assessed at $P \leq 0.05$ using Fisher's multiple range test.

RESULTS

Morphological characteristics

Results in Figure 1 demonstrate that drought stress at a water requirement (75 %, 50% WR) significantly reduced the growth parameters of Lupinus plants (shoot length (29.37%, 54.65%), root length (31.38%, 45.14%), the number of leaves plant (40.86%, 73.12%), shoot fresh (34.60%, 59.80%), and dry weight (45.75%, 77.83%) plant, and root fresh (54.32%, 79.86%), and dry weight (43.32%, 72.73) plant, compared to the control. Nevertheless, exogenously applied β-sitosterol and glycine betaine notably raised all growth parameters (glycine betaine recorded better enhancements) compared to the control plants. The foliar treatment of β-sitosterol and glycine betaine to drought-stressed plants significantly influenced the growth parameters of Lupinus, with comparable results to plants cultivated under full irrigation without treatment. The exogenous treatment of β-sitosterol or glycine betaine alleviated the detrimental impacts of water deficit stress on Lupinus growth, as proven by the significant enhancement in β-sitosterol and glycine-treated Lupinus plants subjected to water deficit compared to that irrigation 75 %, 50% WR.

Chlorophyll contents

Results in Figure 2 demonstrate a decrease in the content of chl a, b, carotenoids, and total pigments in Lupinus leaves at different water irrigation compared to control plants (100% WR). Conversely, drought duress resulted in a significant increase in carotenoid content. Compared to stressed plants, the content of chl a, b, carotenoids, and total pigments increased significantly when treated with β-sitosterol or glycine betaine. The most significant rise was noticed in plants applied with glycine betaine, followed by β-sitosterol.

Osmolytes content

Osmolytes content (Total soluble sugar, protein, proline, phenols, and flavonoids in shoots of Lupinus plants rose under different water irrigation (75 %, 50% WR) as compared with untreated plants (100 % WR) (Figure 3). Compared to the control plant, applied with β-sitosterol or glycine betaine significantly affected sugar, protein, proline, phenols, and flavonoid content, irrigated at 100% WR. Moreover, sugar, protein, proline, phenols, and flavonoid content were significantly raised in plants treated with β-sitosterol or glycine betaine compared with moderate and severe drought stress.

ROS indicator, MDA, and RWC

Data in Figure 4 revealed that MDA, H_2O_2, O_2, and OH content significantly increased in plants under different water irrigation compared with non-stressed. The most pronounced decreases were observed in the glycine betaine treatments, followed by plants treated with β-sitosterol. In addition, the RWC in the leaves of Lupinus plants significantly decreased under different water irrigation methods when compared with non-stressed plants. Furthermore, applying β-sitosterol and glycine betaine to foliar spray resulted in significant increases in the relative water content in the stressed and non-stressed lead plants compared to control and stressed plants.

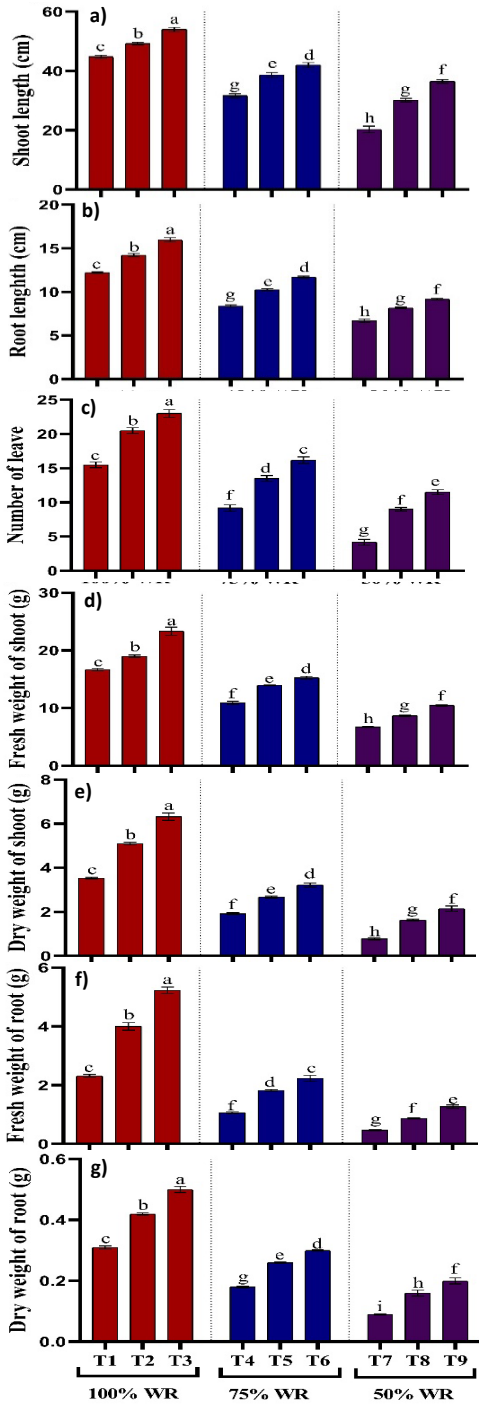

Figure 1. Effect of β-sitosterol and glycine betaine on morphological characters a) shoot length, b) root length, c) the number of leaves, d) fresh weight of shoot, e) dry weight of shoot, f) fresh weight of root, and g) dry weight of root of *Lupinus termis* L. plant under drought stress. Each value is a mean of 3 replicates ± standard error of means. Different lower-case letters are significantly different by post-hoc-Tukey's. T1: 100% WR, T2: 100% WR+ β-sitosterol 100 ppm , T3: 100% WR+ glycine betaine 100 ppm ,T4: 75% WR, T5: 75% WR+ β-sitosterol 100 ppm , T6: 75% WR+ glycine betaine 100 ppm , T7: 50% WR. T8: 50% WR+ β-sitosterol 100 ppm, T9: 50% WR+ glycine betaine 100 ppm.

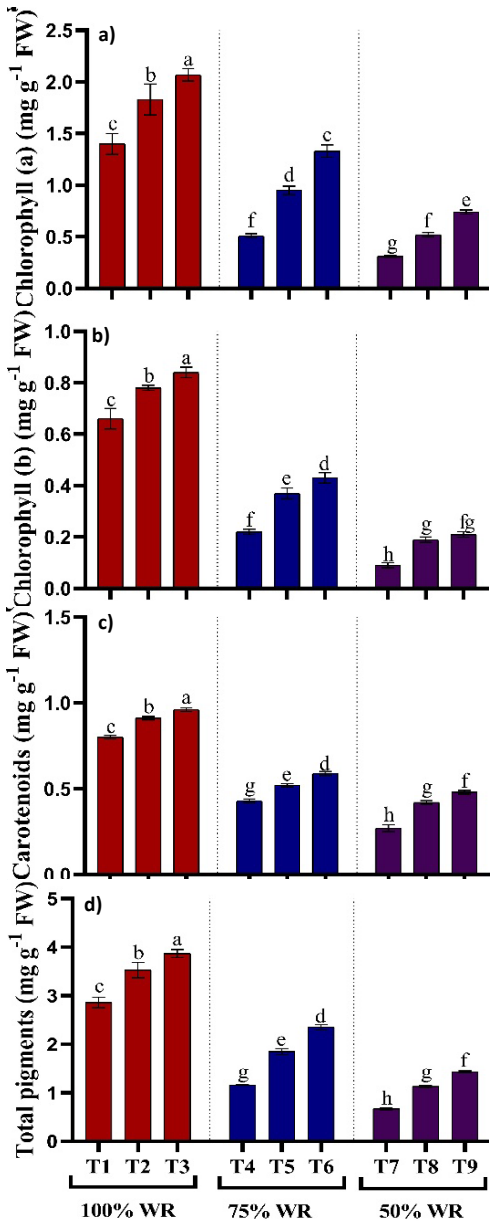

Figure 2. Effect of β-sitosterol and glycine betaine on Chlorophyll contents a) chlorophyll a, b) chlorophyll b, c) carotenoids, d) total pigment of *Lupinus termis* L. plant under drought stress. Each value is a mean of 3 replicates ± standard error of means. Different lower-case letters are significantly different by post-hoc-Tukey's. T1: 100% WR, T2: 100% WR+ β-sitosterol 100 ppm , T3: 100% WR+ glycine betaine 100 ppm ,T4: 75% WR, T5: 75% WR+ β-sitosterol 100 ppm , T6: 75% WR+ glycine betaine 100 ppm , T7: 50% WR. T8: 50% WR+ β-sitosterol 100 ppm, T9: 50% WR+ glycine betaine 100 ppm.

Determination of Stress Response Factors

The drought markedly increased ascorbic acid, glutathione, and glycine betaine (Figure 5). Nonetheless, applying β-sitosterol and glycine betaine to drought-stressed plants significantly raises glutathione, ascorbic acid, and glycine betaine levels compared to the stressed plants.

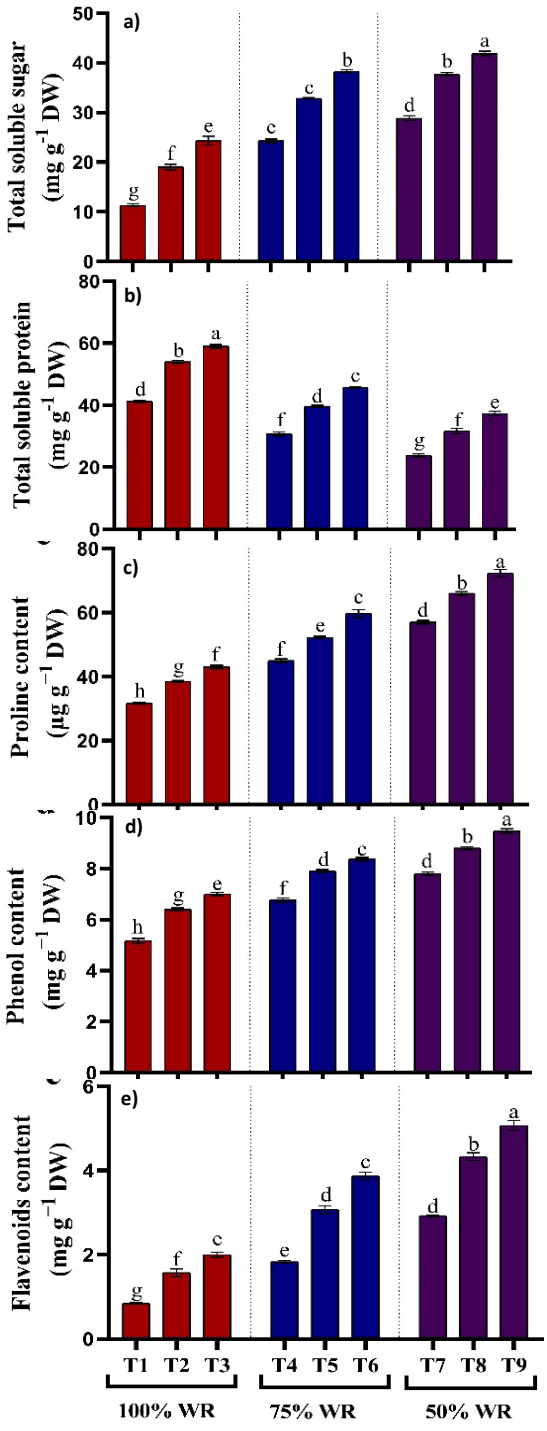

Figure 3. Effect of β-sitosterol and glycine betaine on a) Total soluble sugar, b) Total soluble protein, c) proline, d) phenols, and e) flavonoids of *Lupinus termis* L. plant under drought stress. Each value is a mean of 3 replicates ± standard error of means. Different lower-case letters are significantly different by post-hoc-Tukey's. T1: 100% WR, T2: 100% WR+ β-sitosterol 100 ppm , T3: 100% WR+ glycine betaine 100 ppm ,T4: 75% WR, T5: 75% WR+ β-sitosterol 100 ppm , T6: 75% WR+ glycine betaine 100 ppm , T7: 50% WR. T8: 50% WR+ β-sitosterol 100 ppm, T9: 50% WR+ glycine betaine 100 ppm.

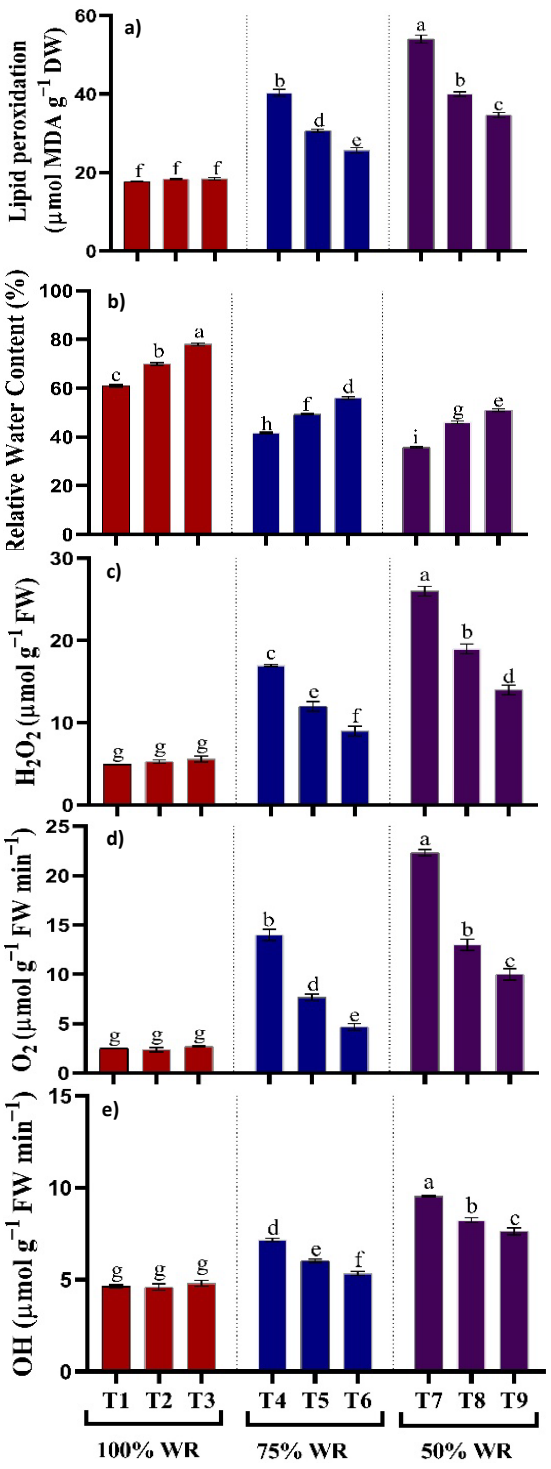

Figure 4. Effect of β-sitosterol and glycine betaine on a) lipid peroxidation, b) relative water content, c) H_2O_2, d) O_2, and e) OH of *Lupinus termis* L. plant under drought stress. Each value is a mean of 3 replicates ± standard error of means. Different lower-case letters are significantly different by post-hoc-Tukey's. T1: 100% WR, T2: 100% WR+ β-sitosterol 100 ppm , T3: 100% WR+ glycine betaine 100 ppm ,T4: 75% WR, T5: 75% WR+ β-sitosterol 100 ppm , T6: 75% WR+ glycine betaine 100 ppm , T7: 50% WR. T8: 50% WR+ β-sitosterol 100 ppm, T9: 50% WR+ glycine betaine 100 ppm.

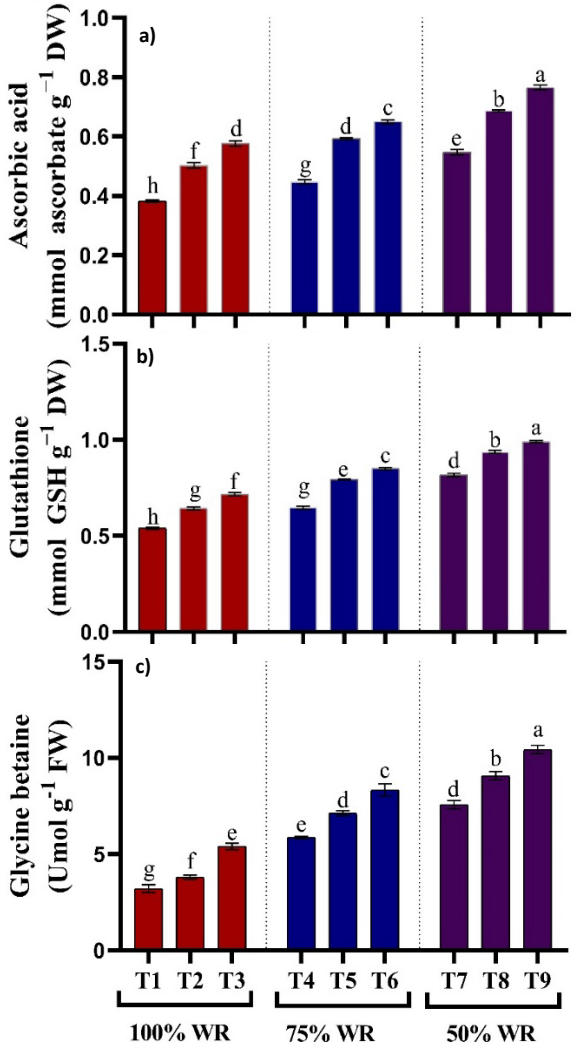

Figure 5. Effect of β-sitosterol and glycine betaine on a) ascorbic acid, b) glutathione, c) glycine betaine, of *Lupinus termis* L. plant under drought stress. Each value is a mean of 3 replicates ± standard error of means. Different lower-case letters are significantly different by post-hoc-Tukey's. T1: 100% WR, T2: 100% WR+ β-sitosterol 100 ppm , T3: 100% WR+ glycine betaine 100 ppm ,T4: 75% WR, T5: 75% WR+ β-sitosterol 100 ppm , T6: 75% WR+ glycine betaine 100 ppm , T7: 50% WR. T8: 50% WR+ β-sitosterol 100 ppm, T9: 50% WR+ glycine betaine 100 ppm.

Enzymatic antioxidants

The antioxidant enzymes (POX, SOD, CAT, GR, APX, PAL) were significantly elevated in the shoots of Lupinus plants subjected to different water irrigation. Furthermore, all treatments assist the plant in mitigating different water irrigation by enhancing the accumulation of antioxidant enzymes compared to control plants (100% WR) (Figure 6). The glycine betaine treatment substantially enhanced antioxidant enzymes relative to unstressed and stressed plants.

Hormonal contents

Drought stress decreased the IAA and GA_3 contents compared to control plants (100% WR) (Figure 7). Exposure to moderate and severe drought significantly increases the ABA contents compared to control plants. In addition, β-sitosterol or glycine betaine stimulated the activities of the hormonal contents (IAA, GA_3, and ABA). Lupinus plants increased with the foliar treatment of glycine betaine exhibited the highest IAA (55.56%, 61.54%) and GA_3 (30.40%, 32.29%) while significantly increasing the ABA (46.67%, 35.71%) contents in comparison to plants grown with moderate and severe drought stress, respectively.

Mineral contents

Compared to the non-drought-stress plants, drought stress decreased the Lupinus seed's N, P, and K amounts (Figure 8). Nevertheless, compared to plant irrigation with control plants (100% WR), applying β-sitosterol or glycine betaine considerably increased Lupinus Plants' N, P, and K contents (Figure 8). Compared to plants grown under different water irrigation, the exogenous application of glycine betaine showed the most effective treatment effect.

DISCUSSION

Plants in semi-arid and arid areas experience drought or water deficit stress when the water supply to roots is restricted owing to elevated transpiration rates and increased temperatures. Our findings indicated that moderate to severe drought stress reduced lupinus development. Due to drought stress, comparable outcomes have been shown in the decreased development of lupinus, faba bean, and soybean plants (Zhou et al., 2024). The dysregulation of elongating cells, which arises from the disruption of water transport from the xylem to these cells, decreased levels of growth-promoting hormones, and compromised processes of cell elongation, mitosis, and expansion during cell division, may be the cause of the drought-induced reduction in plant growth. (Riaz et al., 2023).

Global climate change induces drought stress episodes. Consequently, new management will be necessary to address this issue using antioxidant chemicals. The application of β-sitosterol or glycine betaine to lupinus plants resulted in enhanced growth. Numerous studies indicate that foliar application of β-sitosterol in crops like wheat might mitigate severe drought stress effects on plant development by improving the antioxidant system

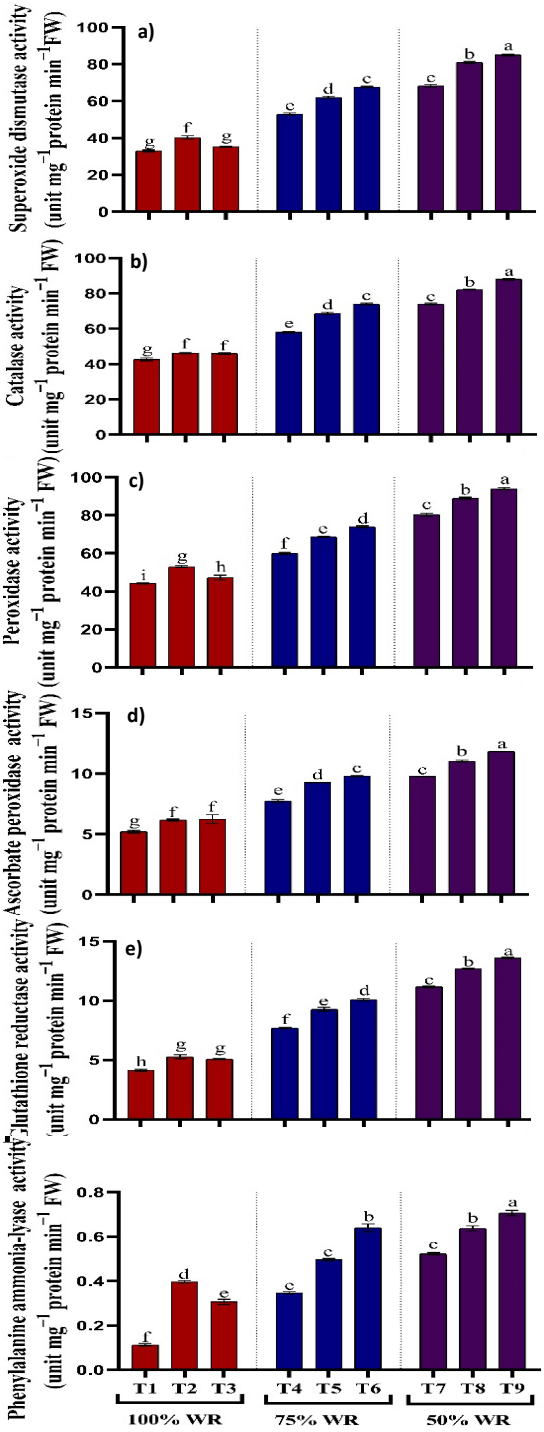

Figure 6. Effect of β-sitosterol and glycine betaine on a) Superoxide dismutase, b) catalase, c) peroxidase, d) ascorbate peroxidase, e) glutathione peroxidase and f) Phenylalanine ammonia-lyase of *Lupinus termis* L. plant under drought stress. Each value is a mean of 3 replicates ± standard error of means. Different lower-case letters are significantly different by post-hoc-Tukey's. T1: 100% WR, T2: 100% WR+ β-sitosterol 100 ppm , T3: 100% WR+ glycine betaine 100 ppm ,T4: 75% WR, T5: 75% WR+ β-sitosterol 100 ppm , T6: 75% WR+ glycine betaine 100 ppm , T7: 50% WR. T8: 50% WR+ β-sitosterol 100 ppm, T9: 50% WR+ glycine betaine 100 ppm.

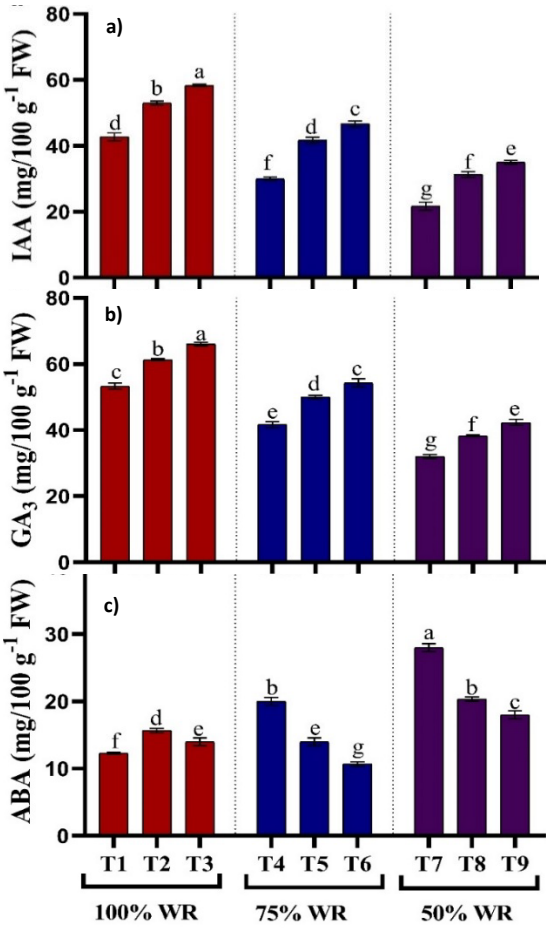

Figure 7. Effect of β-sitosterol and glycine betaine on a) indole acetic acid (IAA), b) Gibberellic acid (GA_3), c) Abscisic acid (ABA) of *Lupinus termis* L. plant under drought stress. Each value is a mean of 3 replicates ± standard error of means. Different lower-case letters are significantly different by post-hoc-Tukey's. T1: 100% WR, T2: 100% WR+ β-sitosterol 100 ppm , T3: 100% WR+ glycine betaine 100 ppm ,T4: 75% WR, T5: 75% WR+ β-sitosterol 100 ppm , T6: 75% WR+ glycine betaine 100 ppm , T7: 50% WR. T8: 50% WR+ β-sitosterol 100 ppm, T9: 50% WR+ glycine betaine 100 ppm.

and osmoprotectant metabolism (Elkeilsh et al., 2019). The exogenous treatment of glycine betaine mitigates drought stress by promoting plant growth by activating antioxidant mechanisms, reducing oxidative damage, and enhancing the expediting proline accumulation and synthesis of compatible solutes, thereby improving photosynthesis and yield characteristics (Haque et al., 2024).

Exposure of lupinus plants to different irrigation of drought stress-induced oxidative stress, decreasing chlorophyll content. This may result from pigment photooxidation and chlorophyll degradation. Comparable outcomes are documented in *Vicia faba* and common bean plants. Plants react to drought conditions by shutting stomata to minimize water

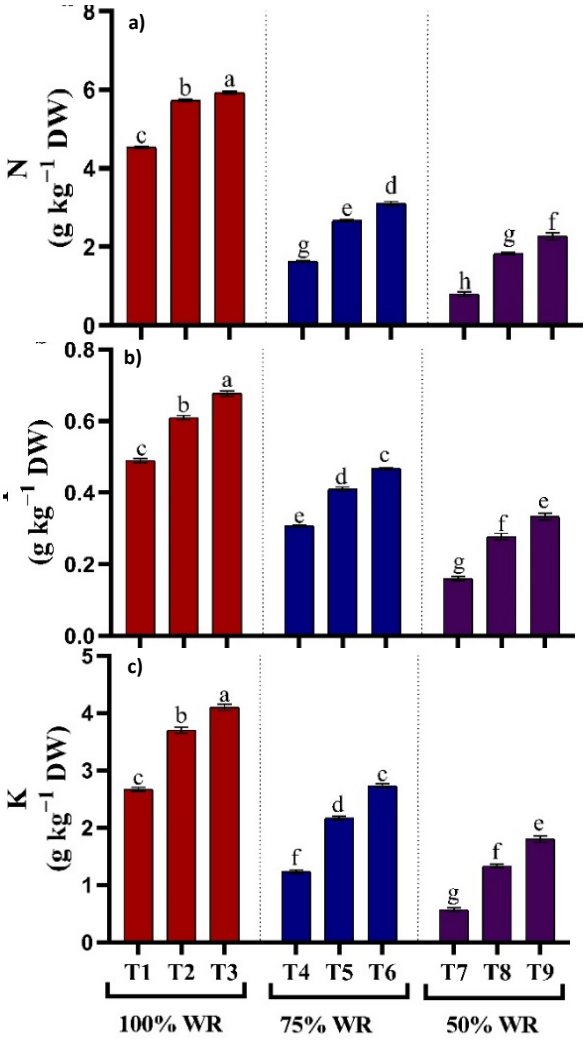

Figure 8. Effect of β-sitosterol and glycine betaine on a) nitrogen (N), b) phosphorus (P), and c) potassium (K) of *Lupinus termis* L. plant under drought stress. Each value is a mean of 3 replicates ± standard error of means. Different lower-case letters are significantly different by post-hoc-Tukey's. T1: 100% WR, T2: 100% WR+ β-sitosterol 100 ppm , T3: 100% WR+ glycine betaine 100 ppm ,T4: 75% WR, T5: 75% WR+ β-sitosterol 100 ppm , T6: 75% WR+ glycine betaine 100 ppm , T7: 50% WR. T8: 50% WR+ β-sitosterol 100 ppm, T9: 50% WR+ glycine betaine 100 ppm.

loss, decreasing carbon transport, and diminishing ATP synthase and Rubisco activity (Haghpanah et al., 2024). Lupinus plants' photosynthetic pigments increased after antioxidant treatment. Antioxidants may increase pigment synthesis by reducing chlorophyllase activity and increasing chlorophyll biosynthetic gene expression. β-sitosterol is significant in photosynthesis and proline synthesis. β-sitosterol can neutralize ROS induced by water stress and may reduce lipid peroxidation, thereby preserving chlorophyll content and promoting plant growth (Elkeilsh et al., 2019).

In addition to protecting photosynthetic activity, exogenous glycine betaine interacts with enzymes to maintain protein structure and activity. In Lupinus plants, total soluble sugar, proline, phenol, and flavonoid concentrations rise under different irrigation of drought conditions. Antioxidant treatment mitigates the negative impact of drought stress. Proteins are essential molecules for all cellular functions. Drought leads to decreased protein synthesis, likely attributable to reduced polysomal complexes in tissues with diminished water content, reducing plant growth and crop yield (Sallam et al., 2019). ROS generation induced amino acid oxidation and could disrupt protein structure under drought stress. Due to a reduction in photosynthesis during drought stress, soluble protein levels have declined. Consequently, drought conditions lead to a decrease in photosynthesis, which in turn reduces or halts protein synthesis (Jarin et al., 2024). Proteolysis resulted in a gradual reduction in protein content during periods of water deficiency in plants, with the released amino acids utilized for osmotic adjustment. This indicates a potentially slow recovery of this parameter, likely due to the reliance of proteins on the synthesis of other nitrogen compounds. The findings align with those of Nalina et al., (2021). In plants, exogenous glycine betaine protects proteins by adhering to protein surfaces, thereby preventing ROS-induced damage to native proteins and membranes (Haque et al., 2024). The exogenous treatment of GB enhances endogenous proline levels, thereby reducing cell osmotic potential and facilitating water uptake, ultimately promoting plant growth. Proline mitigates drought-induced oxidative stress by lowering H_2O_2 concentrations and enhancing the antioxidant defense mechanisms (Urmi et al., 2023).

Under different irrigation conditions of drought conditions, ASA and GSH levels increased with the treatment of β-sitosterol and GB. Furthermore, under drought stress, applying glycine betaine elevated the content of ASA and GSH in pea plants (Osman 2015). This may serve as a preventive mechanism, as elevated GSH levels have been demonstrated to inhibit free radicals and effectively decrease oxidative damage. GSH is essential for regenerating the water-soluble antioxidant AsA via the ASC-GSH cycle, which may enhance AsA levels during drought stress (Altaf et al., 2022).

This research found that drought stress exposure in Lupinus led to increased hydrogen peroxide production, adversely affecting membrane structural integrity and resulting in lipid peroxidation. Ramzan et

al., (2023) demonstrated that drought treatment caused membrane damage in mustard cultivars, leading to changes in membrane function. Song et al., (2024) showed that treatment with glycine betaine decreased ROS production, leading to a notable reduction in lipid peroxidation within the membrane. This study confirmed the role of protection. Glycine betaine enhances the membrane structures of lupinus under drought and non-drought conditions. Numerous studies have established that soil drought induces membrane damage by developing lipid peroxidation and ROS. The antioxidant enzymes (PAL, GR, CAT, POX, SOD, and APX) significantly increased in the Lupinus plants subjected to different irrigation of drought stress. To mitigate oxidative damage and resistance to drought, plants employ both enzymatic defenses (GR, CAT, SOD, and PAL) and non-enzymatic antioxidant defenses (Altaf et al., 2022).

Treatment with glycine betaine resulted in synergistic effects and enhanced enzymatic antioxidants in Lupinus plants. Patade et al., (2014) similarly reported increased APX activity in sugarcane by applying 20 mM glycine betaine. Additionally, the application of glycine betaine to foliage wheat plants enhanced the activities of CAT, SOD, and POX. Enhanced activity of CAT, SOD, and POX can effectively neutralize the detrimental effects of ROS (Hasanuzzaman et al., 2020). SOD is the primary enzyme responsible for scavenging ROS, converting superoxide radicals (O_2) into molecular oxygen (O_2) and water (H_2O). APX and CAT modified the commutation of H_2O_2, a potent and harmful oxidizing agent, to O_2 and H_2O. Shemi et al., (2021) demonstrated that glycine betaine treatment resulted in significant variations in APX activity in maize plants subjected to drought stress. The application of exogenous β-sitosterol and glycine betaine increased GR activity, suggesting its potential role in the recycling and enhancement of endogenous GSH levels (Haque et al., 2024). Exogenous glycine betaine increased GR activity in the lupinus plants. Thus, GSH protects enzymes and structural protein groups containing sulfhydryl from oxidation and stabilizes the structure of biological macromolecules. Enhanced GSH levels promote the AsA-GSH cycle, raising the amount of AsA and improving the activity of APX, PAL, and GR. These enzymes remove ROS due to their glycine betaine effects (Altaf et al., 2022). Several studies indicate that drought- and heat-resistant plants have a higher GSH content due to increased GSH synthesis and/or degradation. Exogenous glycine betaine has demonstrated greater efficacy than AsA in enhancing the activities of GR, APX, and SOD alongside elevated GSH and AsA contents. Exogenous glycine betaine stimulates the AsA-GSH cycle (Hasanuzzaman et al., 2020).

Under drought stress, endogenous phytohormones like IAA and GA_3 decreased, whereas ABA levels increased with higher drought concentrations. Liu et al., (2024) reported analogous findings, noting a rise in ABA in response to drought, while IAA exhibited a reduction. The increase in ABA levels inhibits soybean growth and plant development during drought stress and triggers stomatal closure, which affects photosynthesis. ABA is crucial for synthesizing and aggregating osmoprotectants, including proline and dehydrins, in response to dehydration caused by drought stress and generating reactive oxygen species (Riyazuddin et al., 2022). Applying glycine betaine to Lupinus plants under drought stress resulted in the highest levels of IAA and GA_3.

In contrast, ABA levels were minimized compared to control plants subjected to stress. This was followed by treatment with β-sitosterol. Glycine betaine increased the expression of ABA and IAA in wheat seedlings subjected to drought stress. Aldesuquy (2014) demonstrated that drought stress significantly decreased GA_3 and IAA levels in wheat plants. Applied with glycine betaine increased IAA and GA_3 content compared to drought-stressed plants . The correlation between drought stress and nutrient deficiencies is well established. The interaction between drought levels and treatment significantly enhanced K, N, and P foliar uptake. The administration of glycine betaine enhanced micronutrient absorption in drought conditions. Potassium is a critical plant macronutrient, essential for stomatal function, cell expansion, enzyme activity, osmoregulation, and neutralizing non-diffusible negatively charged ions (Sofy et al., 2022). Phosphorus (P) concentrations decrease rapidly under drought stress due to the precipitation of phosphate ions with Ca^+ ions, rendering them inaccessible to plants. P is an essential nutrient characterized by its mobility and potential to become obstructed in the phloem due to drought stress. Manganese and potassium interact synergistically. Manganese activates dehydrogenase and decarboxylase, essential for nitrogen metabolism, photosynthesis, and nitrogen absorption (Al-Mokadem et al., 2022).

CONCLUSION

Drought stress negatively impacts chlorophyll production, protein levels, and the development of Lupinus species. The adverse effects of drought

conditions on plants with antioxidants have been markedly diminished by the upregulation of an antioxidant protection system, enabling the swift neutralization of ROS and thus averting oxidative damage to membranes and proteins. The delivery of glycine betaine led to the upregulation of antioxidant enzymes and proline production in stressed plants, potentially protecting cellular membranes from damage induced by excessive ROS created under drought stress. The greatest notable enhancements were seen in plants subjected to glycine betaine treatment.

DECLARATIONS

Ethics approval and consent to participate

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