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Exogenous riboflavin-induced modulation in physio-biochemical attributes in drought-stressed canola (*Brassica napus* L.) plants

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Abstract

Drought stress adversely affects plants throughout their life cycle, from seed germination to full maturity, by impairing metabolic and physio-biochemical processes, ultimately reducing yield. Riboflavin, a water-soluble vitamin, is pivotal in photosynthesis and has been reported to promote growth and stress resistance in plants. To evaluate the efficacy of riboflavin in mitigating drought-induced adversaries, a trial was carried out on two *Brassica napus* L. (canola) cultivars, Super and TM, in two different water levels: 100% (well-watered) and 60% (drought-stressed) soil field capacity. Each treatment was replicated three times. Seeds were pre-soaked in a 100 µM riboflavin solution for 14 hours before sowing, and foliar application was applied after a 30-day drought period. Water deficit stress was imposed 15 days after germination and maintained for 30 days, and plants were collected after 15 days to determine morphological and physio-biochemical parameters. Drought stress significantly suppressed leaf area, and shoot and root biomass of both canola cultivars; it also markedly decreased the levels of chlorophyll (*a, b,* and total). In contrast, under drought conditions, there was a rise in relative membrane permeability, chlorophyll a/b ratio, levels of H_2O_2, MDA, glycine betaine, total soluble proteins, ascorbic acid, and the activities of various antioxidant enzymes. Exogenous application of riboflavin, both as pre-sowing and foliar treatment, mitigated the harmful effects of water deficit by enhancing plant biomass, chlorophyll *b*, and total chlorophyll contents. Additionally, riboflavin increased total phenolic content, relative water content (RWC), and the activities of peroxidase (POD) and catalase (CAT) enzymes. Between both canola cultivars, cv. Super exhibited greater drought tolerance than cv. TM. Overall, the beneficial effects of riboflavin on canola growth under drought stress appear to be associated with improved osmoprotectant accumulation and strengthened antioxidant defense mechanisms.

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Introduction

Human efforts to increase crop productivity are often hindered by abiotic stressors, with drought being one of the most significant and complex (Arooj et al., 2025). A slight reduction in water availability during the plant growth period can impede metabolic and physiological activities, thereby limiting crop production (Kapoor et al., 2020; Gavrilescu, 2021). Drought remains the main factor restricting agricultural output and continues to impair crop performance (Dietz et al., 2021; Yu et al., 2021). Plants respond to water deficit by activating mechanisms related to osmotic adjustment, hormonal regulation, morphological and physiological adaptations, and the expression of drought-tolerance genes. Depending on the timing and duration of drought during plant

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development, water shortage can cause multiple adverse effects. Stomatal closure restricts CO_2 entry into leaves, thereby reducing photosynthesis and gas exchange efficiency while minimizing water loss (Engineer et al., 2015).

Plants cope with water stress through avoidance, escape, and tolerance strategies, all of which contribute to drought resistance. Morphological adaptations such as thicker cuticles, wax deposition, and trichome or bark thickening help plants limit water loss (Hasanuzzaman et al., 2018). Reactive oxygen species (ROS), which can harm the cell membrane and impair photosynthesis, are also induced in response to water deficit. Key proteins and enzymes involved in metabolism may become denatured or inactive under such stresses (Mushtaq et al., 2021; Sachdev et al., 2021; Fathi et al., 2025). Plants produce antioxidants such as ascorbic acid, phenolics, as well as upregulate some key antioxidant enzymes to prevent oxidative damage (Akram et al., 2016; Aziz et al., 2018; Kohli et al., 2019; Ahmad et al., 2025). The improvement of water-stress-resistant crop varieties is therefore vital to minimizing yield losses under water-limited conditions, especially in light of increasing human populations and diminishing water resources (Khan et al., 2025).

Phytohormones are essential to control plant development and mediate reactions to environmental stressors like drought, which ultimately reduces crop production and creates a serious concern for global food availability (Ali et al., 2020). In the late 1870s, riboflavin (vitamin B_2) was first isolated as lactochrome, a yellow water-soluble pigment (Pinto and Zempleni, 2016). Recent studies have revealed that exogenous application of riboflavin enhances pathogen resistance in plants, e.g., under a 10% PEG6000 treatment, riboflavin was shown to improve drought tolerance by promoting antioxidant activity (Guhr et al., 2017). In plants, vitamin B_2 functions in stress priming, helping to establish baseline defense responses that enable a more effective reaction to subsequent stresses (Iftikhar and Perveen, 2024). As a water-soluble vitamin, riboflavin is involved in several metabolic processes, and its external application has been reported to enhance plant growth (Wu et al., 2024).

Canola (*Brassica napus* L.) is predominantly cultivated as a winter crop in Asia and Europe, while spring varieties, including rapeseed cultivars, are better suited to the climates of northern Europe, Canada, and Australia (Markie et al., 2025). Among oilseed crops, canola ranks third in global production after palm and soybean (Givi and Haghighi, 2016). Canola oil is recognized for its high nutritional quality, containing approximately 61% oleic acid, 8.8% linoleic acid, 40-42% oil, and 25% protein. It contains less saturated fat than other vegetable oils, increasing its demand among health-conscious consumers (Bowen et al., 2019). In Pakistan, around 300,000 hectares are planted with mustard and rapeseed, contributing about 19.6% to the nation's edible oil production. Canola is also valuable as a rotation crop due to its resistance to soil-borne diseases and agricultural pests (Zheng et al., 2020). The canola plant parts used in food, medicine, and cosmetics include leaves, stems, roots, flowers, and seeds (Zhang et al., 2018). Canola requires higher nitrogen input compared to cereals such as barley and wheat (Lin et al., 2020; Porter et al., 2020). Based on these findings, we hypothesized that exogenous application of riboflavin through foliar spraying or seed priming could improve drought tolerance in *B. napus* plants. Thus, the major objective of the study was to determine the impacts of exogenously applied (presowing and foliar) riboflavin on growth and key physio-biochemical responses of canola plants under drought stress.

Materials and Methods

Experiment design

A pot trial was carried out from October to December 2023 in the Botanic Garden of Government College University, Faisalabad (Punjab, Pakistan), to assess the influence of various riboflavin concentrations on drought tolerance in canola. The experiment was arranged in a completely randomized design (CRD) with three factorial treatments and three replications, resulting in a total of 36 experimental units.

Plant material and soil preparation

The canola cultivars Super and TM were procured from the Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. Sandy loam soil was collected from Al-Rahim Nursery, Faisalabad, and 6 kg of this soil was filled into each of 36 plastic pots.

Seed priming, sowing, drought stress, and treatments

Seed priming was performed using a 100 µM riboflavin solution for 14 hours. Ten seeds were sown per pot, and after emergence, thinning was performed to ensure that each pot contained five uniformly sized plants. Soil water status was observed daily to ensure consistent water availability until stress treatments began. Fifteen days after germination, water stress treatments were imposed as water stress, control (100% field capacity), and drought stress (60% field capacity). Following a 30-day water deficiency treatment, riboflavin (100 µM) was sprayed on the leaves.

Sampling and data collection

Fifteen days after foliar treatment, two plants from each pot were sampled at the vegetative stage to record fresh and dry biomass of root and shoot. The remaining samples were stored at -20 °C for subsequent physiological and biochemical analyses. Data were recorded for various growth and physio-biochemical parameters.

Morphological attributes

The two plants uprooted from each replicate were rinsed thoroughly with distilled water and gently blotted to remove excess moisture. Morphological attributes, including fresh mass and lengths of roots and shoots, were recorded for each replicate. To obtain a constant dry weight, the plant samples were dried in an oven at 70 °C, after which their dry weights were determined using a digital weighing balance.

Chlorophyll contents

Chlorophyll content was determined following the procedure outlined by Arnon (1949). Fresh leaf (0.25 g) was homogenized and processed in 5 mL of 80% (v/v) acetone. A spectrophotometer was used to measure the absorbance of the resultant supernatant at 645 and 663 nm following proper centrifugation. Then, chlorophyll *a*, *b,* and total chlorophyll concentrations were determined spectrophotometrically.

Relative water contents (RWC)

The method outlined by Jones and Turner (1978) was used to measure the relative water contents of the leaves. Fresh tissues were initially used to measure fresh weight. They were then immersed in water for three hours, and afterwards their turgid weight was recorded. For dry weight measurements, the leaves were placed in an oven at 70 °C and dried for three days.

Relative membrane permeability (RMP)

The protocol of Yang et al. (1996) was used to measure relative membrane permeability with slight modifications. Fresh leaf material (500 mg each) was cut into small pieces and placed in 10 mL of distilled water. Following a 2-hour incubation at room temperature, the solution's initial electrical conductivity (EC_0) was recorded. The samples were kept overnight at 4 °C, after which electrical conductivity was re-measured to obtain EC_1 values. Subsequently, the samples were autoclaved at 121 °C for 20 minutes, and the resulting final electrical conductivity (EC_2) was determined.

Proline content

The Bates et al. (1973) method was employed to estimate proline content. After homogenizing 0.5 g of leaf tissues in 3% sulfosalicylic acid, the mixture was passed through a filter paper. The filtrate was treated with glacial acetic acid and acidified ninhydrin reagent. The mixture was placed in a water bath at 100 °C for 1 h. Once cooled, 2 mL toluene was added, and the liquid was thoroughly mixed. Two distinct layers were formed; the toluene layer containing the chromophore was gently removed. The absorbance at 520 nm was determined with a spectrophotometer.

Glycine betaine (GB)

Glycine betaine contents in the leaf tissues were determined following the method of Grieve and Grattan (1983). Fresh tissues (0.5 g each) were ground with 0.5% toluene and centrifuged. To a clean test tube, 1 mL of sulfuric acid, 1 mL of the filtrate, 0.5 mL of the plant extract, and 0.2 mL of KI were added and mixed thoroughly. Two separate layers formed as a result of cooling the mixture in an ice bath. After careful collection of the lower layer, its absorbance was determined at 365 nm with a spectrophotometer.

Malondialdehyde (MDA)

The MDA contents were determined using a slightly modified procedure of Cakmak and Horst (1991). Fresh leaf tissue (25 g each) was homogenized in 5 mL of 0.1% (w/v) trichloroacetic acid (TCA). Following a 15-minute centrifugation at 10,000 × *g*, 1 mL of the resulting supernatant was mixed with 4 mL of TBA solution (0.5% w/v) prepared in 20% TCA. The solution was rapidly chilled on ice following a 30-minute incubation period at 95 °C in a water bath. Absorbance of the supernatant was recorded at 532 nm, and nonspecific turbidity was adjusted by subtracting the absorbance at 600 nm. The concentration of MDA was quantified based on its extinction coefficient.

Ascorbic acid (AsA*)*

The method outlined by Mukherjee and Choudhuri (1983) was used to determine the ascorbic acid content. A fresh tissue (0.25 g each) sample was mixed with 6% TCA, and the mixture. Next, 1 mL of 10% thiourea and 2 mL of dinitrophenyl hydrazine were mixed with 2 mL of the leaf extract. The solution was incubated at 100 °C for 15 minutes and subsequently cooled to room temperature. Afterwards, the cooled mixture was mixed with 5 mL sulfuric acid (80%). The measurement of absorbance was carried out at 530 nm.

Hydrogen peroxide (H_2O_2)

According to Velikova et al. (2000), 0.5 g of fresh leaf tissue was homogenized in 5 mL of 1% TBA. The filtrate (1 mL) was then combined with 5 mL of phosphate buffer and 1 mL of 1 *M* KI. A spectrophotometer was used to assess the mixture's optical density at 390 nm.

Total phenolics

Total phenolics were estimated following the procedure outlined by Julkenen-Titto (1985). After homogenizing fresh tissue samples (0.5 g each) in 80% acetone, they were subjected to centrifugation for 10 minutes. The extract was mixed with 5 mL of 20% Na_2CO_3 and 1 mL of Folin-Ciocalteu phenol reagent. Absorbance was measured at 750 nm.

Total soluble proteins

The Bradford's (1976) protocol was used to measure total soluble proteins. Fresh tissue samples (0.5 g each) were homogenized using 5 mL of phosphate buffer. The Bradford reagent was used to measure the absorption of soluble proteins. To prepare the solution, 100 mg of Coomassie Brilliant Blue G-250 Plus was dissolved in 100 mL of 85% phosphoric acid and 50 mL of 95% ethanol. Subsequently, the absorbance was recorded at 595 nm.

Activity of the peroxidase (POD) enzyme

As per Chance and Maehly (1955), a reaction mixture was prepared by mixing 0.1 mL of leaf extract with 1.8 mL of phosphate buffer and 0.1 mL of 5% guaiacol in a cuvette. The absorbance was measured at 470 nm.

Activity of the superoxide dismutase (SOD) enzyme

The Giannopolitis and Ries (1977) method was employed to assess superoxide dismutase activity. A mixture was prepared by adding 50 µL of leaf extract, 250 µL of Triton-X and potassium phosphate buffer, and 100 µL of methionine. Using a spectrophotometer, the light absorption was measured for 180 seconds at 470 nm.

Activity of the catalase (CAT) enzyme

The catalase activity was measured following the method of Chance and Maehly (1955). To prepare the reaction mixture, 0.1 mL of the leaf extract was mixed with 1 mL of H_2O_2 and 1.9 mL of potassium phosphate buffer. Using a spectrophotometer, the light absorption was measured at 240 nm.

Statistical analysis

The complete dataset was subjected to analysis of variance (ANOVA). ANOVA for each parameter was conducted using the COSTAT software (CoHort Software, Berkeley, USA). The least significant difference (LSD) test at 5% probability was used to compare treatment means, as described by Snedecor and Cochran (1980).

Results

Plant growth was assessed by measuring the dry and fresh weights of roots and shoots of the two cultivars of canola (Super and TM) under water stress [(60% field capacity (F.C.)]. Drought stress markedly suppressed ($p \leq 0.001$) all these growth attributes. The plant growth regulator (PGR), riboflavin, applied externally through seed priming or foliar spray, proved effective in enhancing the growth parameters of both canola cultivars. Riboflavin treatment notably enhanced shoot and root lengths, especially during drought stress (**Figure 1**).

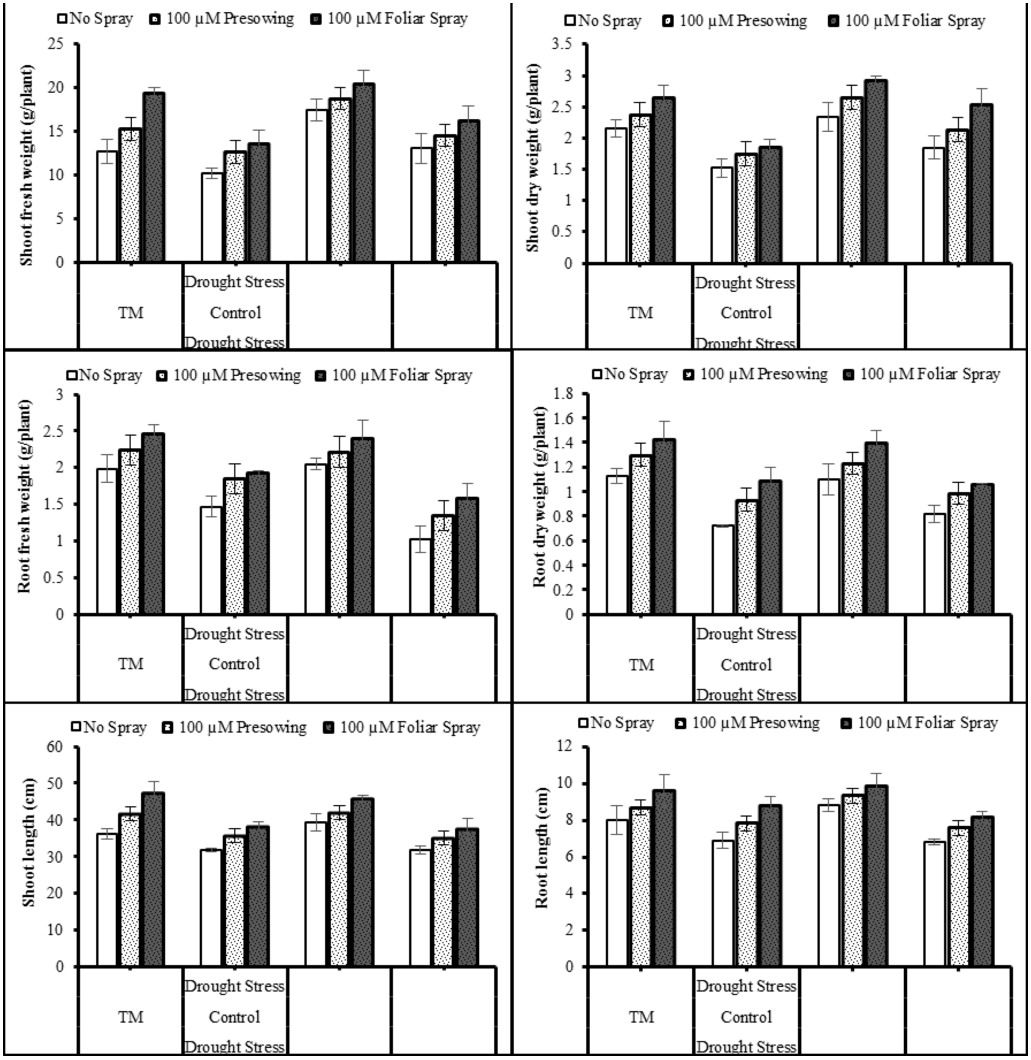

Figure 1: Shoot and root fresh and dry weights, and shoot and root lengths of drought-stressed (60% field capacity) and non-stressed (100% field capacity) plants of two canola (*Brassica napus* L.) cultivars (Super and TM) treated with riboflavin as seed priming or foliar spray (Mean ± S.E.).

Relative water contents of both canola cultivars decreased, while relative membrane permeability rose noticeably ($p \leq 0.001$) during water deficit conditions. However, foliar-applied riboflavin improved the relative water content ($p \leq 0.01$), while decreasing RMP in each canola cultivar under drought-stress regimes.

When water was scarce, the amount of chlorophyll *a, b, a/b,* and total chlorophyll contents of both canola cultivars markedly decreased ($p \leq 0.001$). Exogenously applied riboflavin, through both seed priming and foliar spray, did not show a significant effect on chlorophyll contents under both control and water stress conditions. Under various water regimes, the responses of the two canola cultivars in terms of chlorophyll contents were nearly the same (**Figure 2**).

Under situations of water deficit, the accumulation of proline and glycine betaine (GB) levels improved considerably ($p \leq 0.001$) in both canola cultivars. Furthermore, riboflavin applied externally, either through seed priming or foliar spray, led to improvement in GB and proline accumulation in both cultivars under water deficit stress. Water stress led to a considerable rise ($p \leq 0.05$) in malondialdehyde and hydrogen peroxide levels in both canola cultivars.

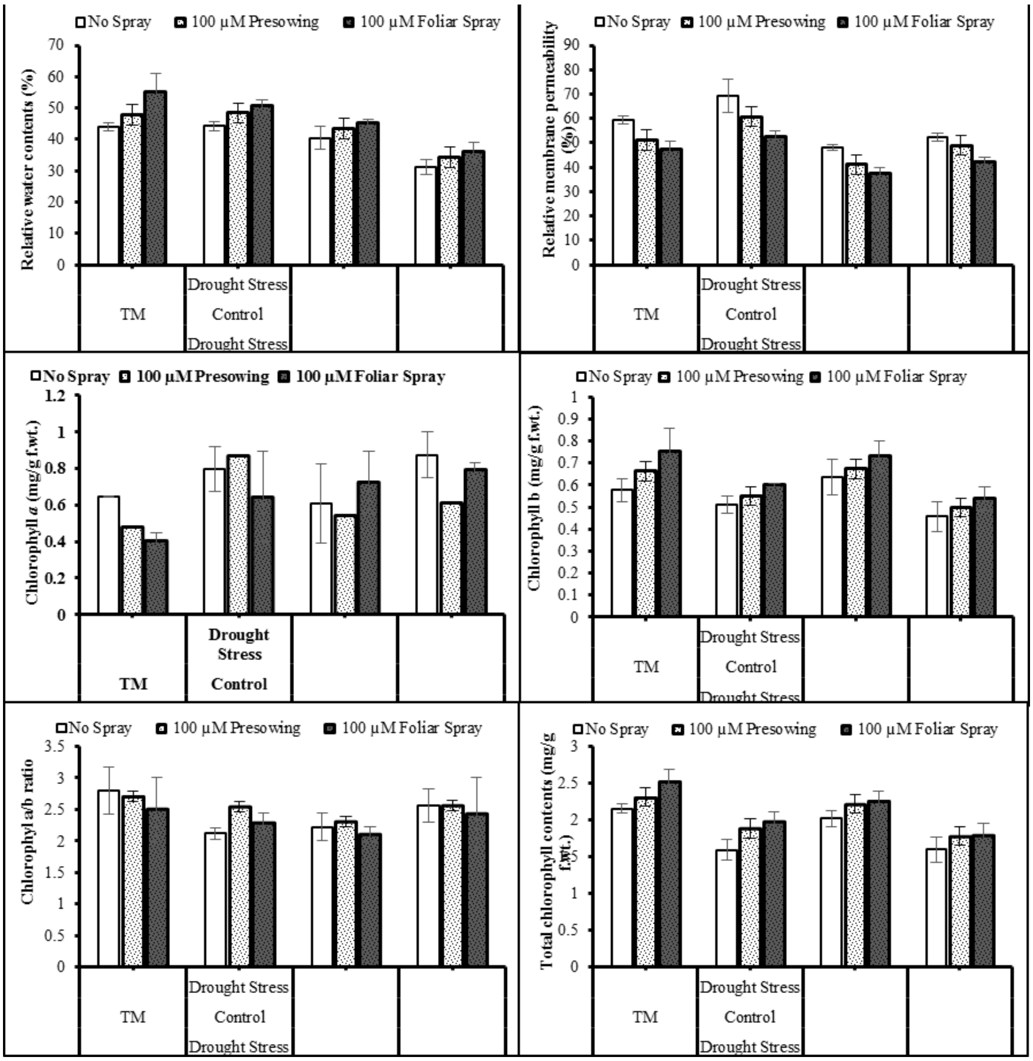

Figure 2: Relative water contents, relative membrane permeability (RMP), chlorophyll a, chlorophyll b, chlorophyll a/b, and total chlorophyll of drought-stressed (60% field capacity) and non-stressed (100% field capacity) plants of two canola (*Brassica napus* L.) cultivars (Super and TM) treated with riboflavin as seed priming or foliar spray (Mean ± S.E.).

However, hydrogen peroxide levels were non-significantly impacted by the exogenous administration of riboflavin through foliar spray or seed priming in plants grown in either control or water shortage situations. Both canola cultivars showed a statistically significant increase ($p \leq 0.001$) in total phenolic and ascorbic acid levels during the water scarcity. Exogenous application of riboflavin, through seed priming or foliar spray, enhanced total phenolics significantly ($p \leq 0.05$) across both water regimes. Foliar application was more effective than pre-sowing treatment. However, both cultivars showed a non-significant difference in their response under varying irrigation regimes and exogenous application of riboflavin (**Figure 3**).

The activities of enzymatic antioxidants like superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were considerably ($p \leq 0.001$) enhanced in drought-stressed canola plants. The activities of POD, CAT, and SOD in both canola cultivars under different water regimes were not considerably impacted by the application of riboflavin, either as seed priming or foliar spray. Likewise, both cultivars, when exposed to water-deficient conditions, exhibited a similar response in terms of the activities of the key enzymes (**Figure 4**).

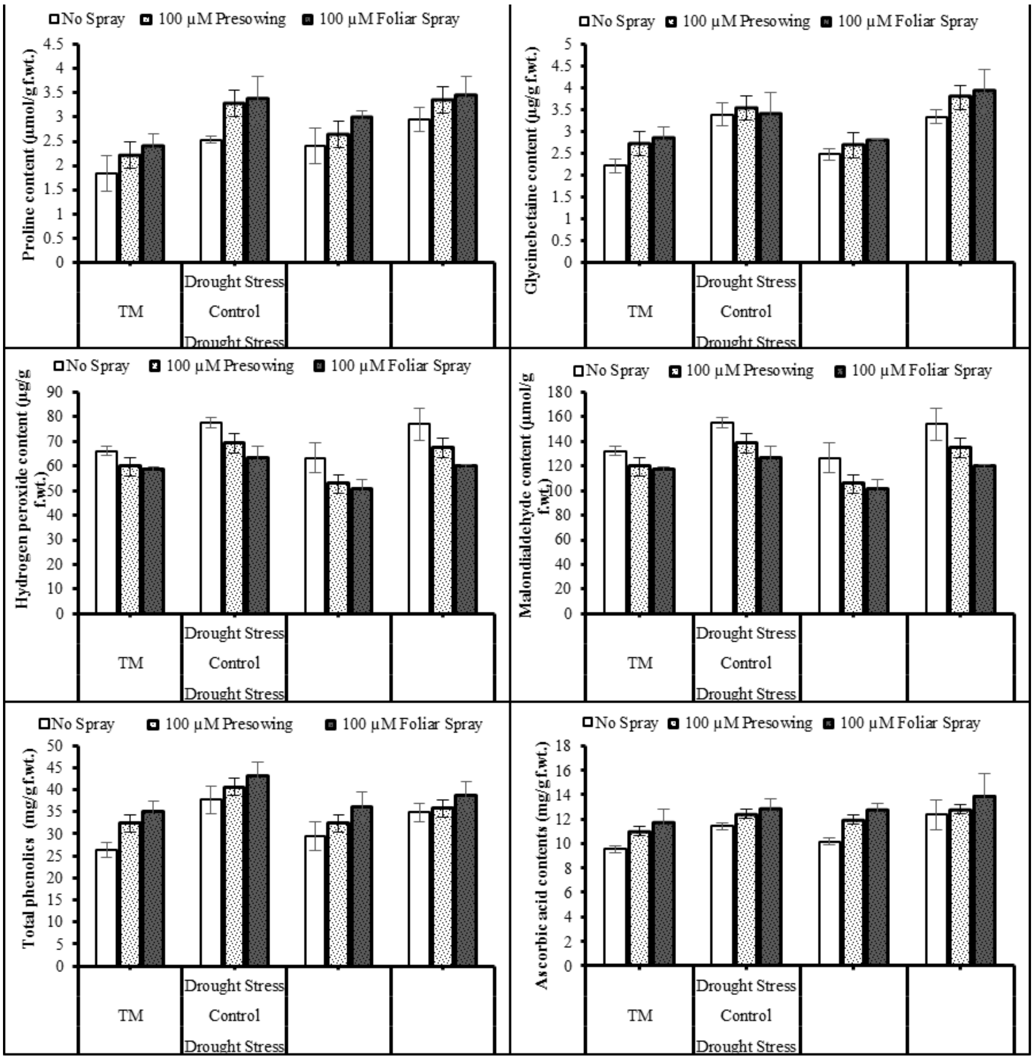

Figure 3: Proline, glycine betaine, hydrogen peroxide, malondialdehyde, total phenolics, and ascorbic acid of drought-stressed (60% field capacity) and non-stressed (100% field capacity) plants of two canola (*Brassica napus* L.) cultivars (Super and TM) treated with riboflavin as seed priming or foliar spray (Mean ± S.E.).

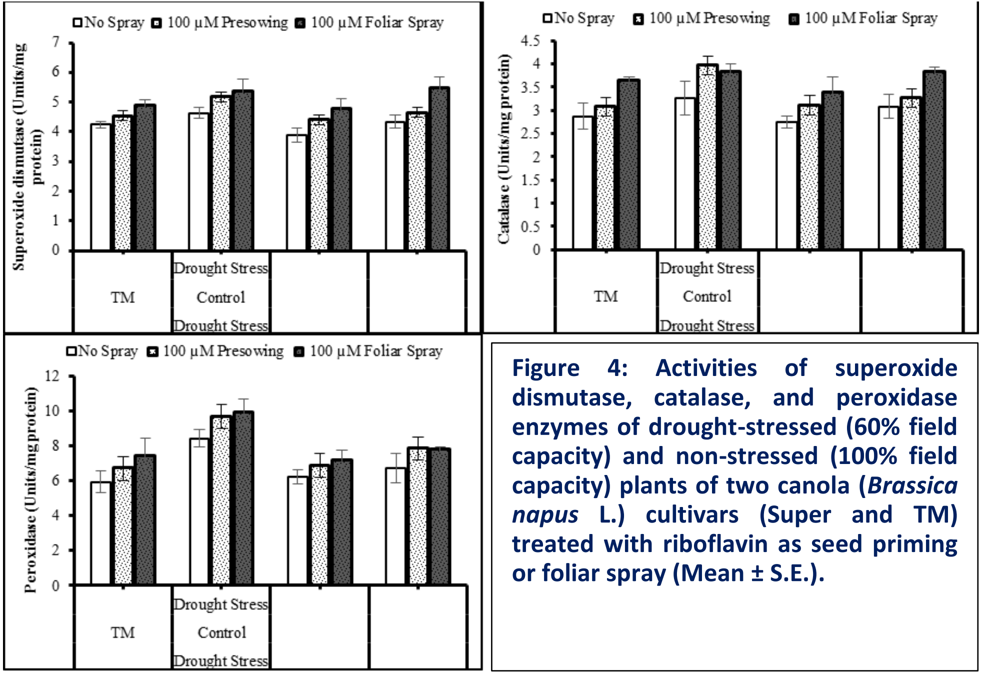

Figure 4: Activities of superoxide dismutase, catalase, and peroxidase enzymes of drought-stressed (60% field capacity) and non-stressed (100% field capacity) plants of two canola (*Brassica napus* L.) cultivars (Super and TM) treated with riboflavin as seed priming or foliar spray (Mean ± S.E.).

Discussion

Drought stress is a critical ecological constraint that adversely impacts cell expansion, plant metabolism, and overall productivity (Seleiman et al., 2021; Mohan et al., 2025). Under conditions of water scarcity, plants generally exhibit slow developmental rates and suppressed growth. Overproduction of reactive oxygen species (ROS) can result in oxidative damage or free radical disruption of essential cellular membranes, which can cause significant metabolic impairment and damage to the photosynthetic systems, eventually restricting plant yield (Sharma and Zheng, 2019). Even mild water stress can significantly impair the growth of economically important crops, leading to noticeable reductions in their productivity (Arvin et al., 2012; Mohan et al., 2025).

It has been observed that water shortage substantially reduced the growth attributes of the two canola cultivars. Similar findings have been documented in several earlier published studies, where water deficit stress induced significant alterations in various physiological and biochemical processes, ultimately affecting growth traits in different crops such as *Zea mays* (Jabeen et al., 2008; Kim and Lee, 2023), *Brassica napus* (Tesfamariam et al., 2010; Morsi et al., 2023), *Helianthus annuus* (Hossain et al., 2010; Ashraf and Siddiqi, 2024), and *Oryza sativa* (Mostajeran and Rahimi-Eichi, 2009; Afra et al., 2025). However, root and shoot biomass of canola cultivars were significantly improved by treating them exogenously with riboflavin supplemented either by pre-sowing or foliar spray. Likewise, working with fragrant rice, Wu et al. (2024) have reported enhanced growth and yield in this crop due to foliar-applied riboflavin. Similarly, long ago, Deng et al. (2014) also observed a significant improvement in drought tolerance of tobacco plants with exogenously applied riboflavin. All these reports, including the current study, show that riboflavin supplementation can effectively promote growth in plants exposed to drought stress.

Drought stress-induced reduction in chlorophyll pigments is a well-known response of plants (Ashraf and Harris, 2013; Latif et al., 2016; Karami et al., 2025). For example, similar results were observed in the present research, wherein chlorophyll contents of both canola cultivars (Super and TM) showed considerable reduction on exposure to water deficiency. Moreover, when both cultivars of canola were exposed to pre-sowing or foliar treatments of riboflavin (100 µM), under both control and water stress conditions, their chlorophyll pigments were found to be improved. Likewise, Deng et al. (2014) observed considerable improvement in chlorophyll content in drought-stressed tobacco plants fed with riboflavin. Recently, Lan et al. (2025) have observed that riboflavin can delay the degradation of chlorophyll content in postharvest okras by modulating key hormonal pathways.

An increase in hydrogen peroxide (H_2O_2) levels in plants under water stress has been well documented (Luna et al., 2005; Jubany-Marí et al., 2009). Furthermore, increased malondialdehyde (MDA) content under water-deficit conditions was linked to higher H_2O_2 levels, suggesting increased oxidative damage and lipid peroxidation (Akram et al., 2017). In the current study, hydrogen peroxide contents of both canola cultivars were increased significantly, but riboflavin applied aa seed priming and foliar spray exhibited a non-significant effect under drought stress conditions. In contrast, Jiadkong et al. (2024) reported that riboflavin application decreased both H_2O_2 and MDA contents in rice seedlings under saline stress.

Generally, MDA accumulation increases in plants exposed to different types of stress (Morales and Munné-Bosch, 2019). For example, MDA contents increased significantly in *Ctenantha setosa* (Terzi and Kadioglu, 2006), tomato (Zeinali et al., 2014), and wheat (Hameed and Iqbal, 2014) when these plants were exposed to water stress. Likewise, in the present study, MDA levels in *B. napus* increased predominantly on exposure to drought stress. However, no change was observed in this attribute of canola plants by applying riboflavin exogenously.

In the current research, the proline concentration was noticeably enhanced in drought-stressed canola plants. Furthermore, riboflavin applied as a pre-sowing or foliar treatment enhanced proline contents of both canola cultivars. A positive effect of riboflavin on proline content was also observed in maize subjected to salt stress (Tuna et al., 2013). Under water-limited conditions, no improvement was observed in the proline content of quinoa plants; however, glycinebetaine content increased under water stress (Aziz et al., 2018). The detrimental effects of free radicals can be mitigated by elevated ascorbic acid content, which functions as a potent antioxidant (Farouk, 2011; Ye et al., 2012; Akram et al., 2017). In the current investigation, the accumulation of ascorbic acid was not affected by drought stress or the application of riboflavin. However, in contrast, in wheat, Seĉenii et al. (2009) noted that the ascorbic acid contents were positively promoted when the wheat plants were exposed to drought stress. Phenolic and other non-enzymatic antioxidants function as secondary metabolites that stop lipid peroxidation, DNA denaturation, and protein structural alterations, while also mitigating the oxidative damage (Quan et al., 2016; Xu et al., 2025). The data of this study reported that the concentration of total phenolics in *B. napus* was enhanced when exposed to drought stress. When canola plants were exposed to pre-sowing or foliar treatment of riboflavin, the amount of phenolics was found to have significantly increased. Although not under drought stress but under cold stress, Castro-Cegri et al. (2024) observed a substantial increase in phenolic content in postharvest zucchini fruit. In the recent research, SOD and CAT activities in the two canola cultivars remained non-significant, while the activity of the peroxidase enzyme improved considerably under drought conditions. However, when canola cultivars were exposed to pre-sowing or foliar treatments of riboflavin, the superoxide dismutase activity showed no significant change, but the catalase and peroxidase enzyme activities were enhanced under water stress conditions. Consistent with the findings of the current study, Deng et al. (2014) reported that the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) were significantly improved in tobacco plants fed with low or moderate levels of riboflavin.

Overall, drought stress adversely affected all growth attributes, chlorophyll *a*, *b,* and total chlorophyll pigments, relative water contents, and total soluble proteins in the canola plants. However, under stress conditions, organic osmolytes, antioxidant enzymes, relative membrane permeability, and non-enzymatic antioxidants were elevated. The growth, as well as chlorophyll pigments, total phenolics, the activities of catalase, peroxidase, and proline contents, were enhanced by the exogenous administration of riboflavin as a pre-sowing or foliar treatment. So, exogenous application of riboflavin was found to be efficient in increasing drought tolerance of the canola plants in terms of growth and the oxidative defense system.

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Contribution of authors

Conceptualization and planning of research: MI, MS, NAA. Conduction of research: MI. Data collection: MI. visualization and interpretation: MI, MS, NAA. Statistical analysis: MI, NAA. Preparation of initial draft: MI, MS, NAA. Review of initial draft: MI, NAA. Revisions and corrections: MI, MS, NAA. Proofreading and approval of the final version: All authors.

Permissions and ethical compliance

This study does not involve human/animal subjects, and thus no ethical approval is required.

Handling of bio-hazardous materials

The authors certify that all experimental materials were handled with great care during collection and experimental procedures. After completion of the study, all materials were properly discarded to minimize/eliminate any types of bio-contamination(s).

Supplementary material

No supplementary material is included with this manuscript.

Conflict of interest

The authors declare no conflict of interest.

Availability of primary data and materials

As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher/Journal management. These are available with the corresponding author (s) and/or with other author(s) as declared by the corresponding author (s) of this manuscript.

Authors' consent

All authors have critically read this manuscript and agreed to publish in IJAaEB.

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