

## Enhancing crop resilience through thiamine: implications for sustainable agriculture in drought-stressed radish

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### Abstract

During 21<sup>st</sup> century, abiotic stress has adversely affected the agriculture crop production around the globe. Keeping in view the food requirement under water shortage condition, a study was planned to investigate the effect of thiamine application on radish crop under drought stress conditions on plant. For study purpose, two varieties of locally available radish ('Early-Milo' and 'Laal-Pari') were grown with normal water application as well as thiamine (100 mg L<sup>-1</sup>) application while maintaining a stress condition (60% field capacity). Increasing water deficit stress linearly reduced plant growth, yield and biomass in both varieties by reducing water use efficiency, while significantly enhanced these attributes with thiamine application. Thiamine application under drought stress exerted significant impacts on physiological attributes in both varieties, including enhanced osmolytic attribute in drought conditions and improvements in superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), H<sub>2</sub>O<sub>2</sub>, and malondialdehyde (MDA) activities in plant leaves. Antioxidant and osmoprotectant upregulation positively linked to radish crop's drought tolerance. Moreover, PCA and heatmap analysis revealed a significant interdependence among various traits and interconnected in determining the crop's capacity to sustain growth under conditions of drought stress. In crux, thiamine

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application conclusively enhances radish growth, yield, biomass, physio-chemical and osmolytic attributes, ionic composition and enzymatic antioxidant potential. Therefore, it is recommended to consider the application of thiamine in commercial agriculture practices to mitigate the negative effects of drought stress on radish crop production.

**Keywords:** antioxidants potential; drought stress; foliar; growth; osmoprotectants; radish

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## Introduction

For the productivity and quality of crops the prevailing environmental conditions play a crucial role. Plants are exposed to various biotic and abiotic stresses, including drought, high temperature, heavy metals, pest, diseases, and salinity, which directly impact their metabolic processes (Morais *et al.*, 2018). The occurrence of extreme weather events like droughts and floods, attributed to global warming, further exacerbates the challenges faced by agricultural production (Srivastav *et al.*, 2021). Global food output is severely hampered by drought in particular, which happens when plant evapo-transpiration outpaces water availability (Ault, 2020; Seleiman *et al.*, 2021). Drought stress directly affects plant growth by disrupting water balance, leading to reduced cell division, expansion, and photosynthesis. It also indirectly induces secondary oxidative stress through the overproduction of reactive oxygen species (ROS) (Seleiman *et al.*, 2021). Maintaining an appropriate water balance is essential for turgor pressure formation, cell wall expansion, stomatal opening for CO<sub>2</sub> uptake during photosynthesis, and overall plant growth (Xiong and Nadal, 2020). Common symptoms of drought stress include leaf yellowing, leaf curling, stunted growth, leaf senescence, and permanent wilting. Drought stress at 50% field capacity (FC) significantly reduces important parameters such as ascorbic acid, total phenolic content, and biomass yield, despite the adaptive strategies that plants have developed to cope with water scarcity (Jabeen *et al.*, 2021). Plants have developed strategies to cope with drought stress, such as extensive root systems with deep, elongated capillary structures to absorb water from deeper soil layers (Ors and Suarez, 2017). Other adaptive mechanisms include solute accumulation through osmotic adjustment, activation of antioxidant defense systems, and dynamic regulation of stomatal closure during water scarcity (Seleiman *et al.*, 2021).

Thiamin, also known as vitamin B1, impart important contribution in mitigating drought stress and is a vital component of the vitamin-B group. It acts as a coenzyme in the decarboxylation of  $\alpha$ -keto acids, such as pyruvic acid and keto-glutamic acid, which are involved in the metabolism of fats and carbohydrates (Goyer, 2010; Sadak *et al.*, 2022). Thiamine application has been shown to improve the growth, development, and chemical composition of maize plants under environmental stresses (Kaya *et al.*, 2015). It plays a significant role in plant responses to water stress, as exogenous application of thiamine can enhance the antioxidant defense mechanism through the involvement of the transketolase enzyme in the glycolytic cycle (Sadak *et al.*, 2022). Thiamin also promotes the growth of microscopic plants in underwater environments and enhances plant growth overall. Organic fertilizers have been found to increase the concentration of vitamin B1 in plants, and plant roots are capable of absorbing it (Mozafar, 1994). Moreover, thiamine activates plant defense mechanisms, reducing the incidence of pathogen and nematode infections, as well as aphid settling and reproduction (Hamada *et al.*, 2018). It also affects nodule development in plant roots, promoting nodulation and eliminating growth defects and chlorosis (Nagae *et al.*, 2016). Thiamine is involved in the response to DNA damage and is essential for actively dividing meristematic stem cells and organ initial cells. It is also considered to fulfill hormone requirements in higher plants (Martinis *et al.*, 2016). Thiamine treatment triggers the transient expression of pathogenesis-related genes in plants and upregulates protein kinase C activity (Ahn *et al.*, 2005).

Radish (*Raphanus sativus* L., Brassicaceae) is a widely cultivated root vegetable known for its exceptional nutritional and phytochemical properties (Nishio, 2017). The edible thickened hypocotyl, commonly referred to as “globular roots”, of radish is abundant in essential macro- and micronutrients, flavonoids, polyphenols, terpenes, fats, and glucosinolates, which contribute to its characteristic pungent flavor (Gamba *et al.*, 2021). Like many other crops, radish cultivation is significantly influenced by water scarcity (Stagnari *et al.*, 2018; Henschel *et al.*, 2022). However, radish exhibits remarkable adaptability and flexibility in response to drought conditions by modulating its growth and defense mechanisms. This makes it a viable option for food production in regions and periods with limited water availability (Stagnari *et al.*, 2018). Furthermore, the short growth cycle and the presence of globular roots make radish an excellent model for studying biomass production and allocation patterns under drought stress. So, the present trial was hypothesized that foliar applied thiamine ameliorates the adverse impacts of drought stress in radish. This study intends to, 1) assess the growth-toxic effects of drought stress, biochemical, tissues health and quality attributes of radish and 2) assess the effect of foliar applied thiamine for the mitigation of drought stress in radish.

## Materials and Methods

A study was conducted in the Old Botanical Garden at the University of Agriculture Faisalabad to investigate the impact of exogenously applied thiamine on radish (*Raphanus sativus* L.) plants under drought conditions. The experiment took place during the summer of 2022. Two radish varieties, namely Early Milo and Laal Pari, were chosen, along with two levels of drought stress (0% and 60% Field Capacity). Two concentrations of thiamine (0 mg L<sup>-1</sup> and 100 mg L<sup>-1</sup>) were applied externally. The experimental setup followed a completely randomized design (CRD) with four replicates. Each pot, filled with 8 kg of soil, contained eight seeds of each radish variety. During the vegetative and reproductive stages, six seedlings of equal length were retained for further analysis.

### *Growth, yield and biomass attributes*

The calculated growth parameter included crop root and shoot length, fresh crop root and shoot weight, dried root and shoot weight and radish crop yield estimation. From each replication, three plants were taken, and their roots and shoots were divided in order to calculate and record the growth variables. Fresh weight of root and shoot was recorded immediately after harvesting using weight balance to avoid any moisture loss. The root and shoot length of the radish plant was measured using a graduated scale. For estimation of the dry weight of the roots and shoots, they were dried in oven at 70 °C for 48 hr. as reported by Ghafar *et al.* (2019) and Tufail *et al.* (2023). To estimate the crop yield area of 1 m<sup>2</sup> was selected in random and crop yield was estimated by multiplying the crop yield with area of one hectare.

### *Physiological and osmolytic attributes*

Physiological attributes included net CO<sub>2</sub> assimilation (A), transpiration rate (E), stomatal conductance, carotenoid (CAR), chlorophyll a (Chl a), chlorophyll b (Chl b), chlorophyll a/b ratios (Chla/b), Total Chlorophyll (T. Chl), water use efficiency (WUE), ratio of intercellular to ambient CO<sub>2</sub> partial pressures (C<sub>i</sub>/C<sub>a</sub>), Osmolyte and total soluble protein (TSP). Transpiration rate and stomatal conductance was calculates using standard procedure given by Percy *et al.* (2000) while standard method described by Rodriguez-Concepcion *et al.* (2018) was adopted for carotenoid (CAR). 1.0 g leaf sample from harvested plants was selected taking care of any damage and leaves were cut in 0.50 cm sections. Overnight, at 4 °C extraction process was performed using 80% acetone solution for CAR calculations. Using formulae 1, 2, 3 and 4 the Chl<sub>a</sub>, Chl<sub>b</sub>, Chl<sub>t</sub>, and CAR concentrations were calculated after absorbance estimating of the supernatant using Hitachi, U-2800 spectrophotometer at 645, 652, 663, and 480 nm.

$$\text{Chl}_a \left( \frac{\text{mg}}{\text{g FW}} \right) = [(12.7 \times 663 \text{ OD}) - (2.69 \times 645 \text{ OD})] \times \frac{V}{1000} \times W \quad (1)$$

$$\text{Chl}_b \left( \frac{\text{mg}}{\text{g FW}} \right) = [(22.9 \times 645 \text{ OD}) - (4.68 \times 663 \text{ OD})] \times \frac{V}{1000} \times W \quad (2)$$

$$\text{Chl}_t \left( \frac{\text{mg}}{\text{g FW}} \right) = [(20.2 \times 645 \text{ OD}) + (8.02 \times 663 \text{ OD})] \times \frac{V}{1000} \times W \quad (3)$$

$$\text{CAR} \left( \frac{\mu\text{g}}{\text{g FW}} \right) = \frac{A_{\text{car}}}{E_{\text{max}}^{100}} \quad (4)$$

$$A_{\text{car}} = (480 \times \text{OD}) + 0.114 (663 \times \text{OD}) - 0.638 (645 \times \text{OD}) \quad (5)$$

Here  
and

$$E_{\text{max}}^{100} (\text{cm}) = 2500 \quad (6)$$

Whereas:

V, OD and W presents sample volume, designates optical density and sample weight respectively.

Ci/Ca was calculated using standard procedure described by Tan *et al.* (2017). The calculation of water use efficiency took into account the period from 24 to 42 days after sowing. It involved determining the ratio of the total increase in dry weight to the overall amount of water transpired by the crop. Transpiration was calculated by subtracting the fresh weight increase and evaporation from the water supply. The estimation of evaporation was based on the weight loss of trays without plants at the beginning of the experiment (Marcelis and Van Hooijdonk, 1999). A fresh leaf sample weighing 0.50 g was collected and processed using a buffer solution with a pH of 7.2. The buffer contained protease inhibitors at a concentration of 1 M and was combined with a saline phosphate buffer. The saline buffer was prepared by dissolving 1.37 mM NaCl, 2 mM KH<sub>2</sub>PO<sub>4</sub>, 2.7 mM KCl, and 10 mM Na<sub>2</sub>HPO<sub>4</sub> in 1.0 L of deionized water. To adjust the pH of the buffer, HCl was added to the solution. The resulting buffer solution was then autoclaved and subjected to centrifugation for 5 minutes to separate the supernatant. Proline contents were determined using established protocols discussed by Maehly (2006). Soluble sugar contents and soluble protein contents were analyzed using standard method outlined by Giannakoula *et al.* (2008) and Bradford (1976), respectively.

#### *Antioxidant attributes*

Supernatant obtained from 1.0 g of radish leaves was used for enzyme activity determination using 50.0 mM phosphate buffer. Centrifugation was performed at approximately  $(15 \times 10^3) \times g$  for 10 minutes as standard methodology outlined by Velikova *et al.* (2000) for peroxidase activity (POD) measurements. Catalase activity (CAT) and superoxide-dismutase activity (SOD) was measured according to the standard protocols reported by Aebi (1984) and Beauchamp and Fridovich (1971) respectively. The relative water content (RWC) measurement was carried out using the method described by Turner and Kramer (1980) and the calculation was performed using the equation 7.

$$\text{RWC} = \left[ \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \right] \times 100 \quad (7)$$

Here, FW, TW and DW are fresh weight, turgid weight and dry weight respectively.

To measure malondialdehyde (MDA) levels, a fresh shoot sample weighing 0.1 g was ground and mixed with 5 mL of 0.1% (w/v) trichloroacetic acid (TCA). The mixture underwent centrifugation (Allegra 4R) at  $10,000 \times g$  for 15 minutes, and the resulting supernatant was collected. A solution comprising 20% (w/v) TCA and 0.5% (w/v) thiobarbituric acid (TBA) in a 1:1 ratio was prepared, and 2.5 mL of the supernatant was combined with this mixture. The solution was then heated in an oven at 95 °C for 30 minutes and subsequently cooled using an ice bath. Absorbance readings at 532 nm and 600 nm were recorded using a UV/VIS spectrophotometer (UV752D). MDA concentration was determined using Beer-Lambert's equation (Jambunathan, 2010). For hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) content measurement, a fresh shoot sample weighing 0.20 g was ground, and 5.0 mL of 0.10% TCA was added. The mixture was then subjected to centrifugation at  $10,000 \times g$  for 15 minutes. The resulting supernatant was combined with a mixture of 10 mM potassium phosphate (KP) buffer (pH = 7) and 1.0 M potassium iodide (KI) in a 1.0:1.0:2.0 ratio. The absorbance of the solution was measured at 390 nm as reported by Sergiev *et al.* (1997).

### *Ionic attributes*

For the determination of Na<sup>+</sup> and K<sup>+</sup> ion levels, the plant samples underwent a digestion process. Specifically, dried and ground plant material (1 g) was digested with concentrated nitric acid (20 mL) in digestion tubes. Subsequently, the digested leaf samples from different radish genotypes were analyzed using a Flame photometer (Jenway PFP-7, UK) to measure the concentrations of Na<sup>+</sup> and K<sup>+</sup>. To ensure accurate quantification, a standard curve was created using a series of graded standards (ranging from 10 to 100 mg L<sup>-1</sup>) of Na<sup>+</sup> and K<sup>+</sup> (Ayyub *et al.*, 2016; El-Beltagi *et al.*, 2022).

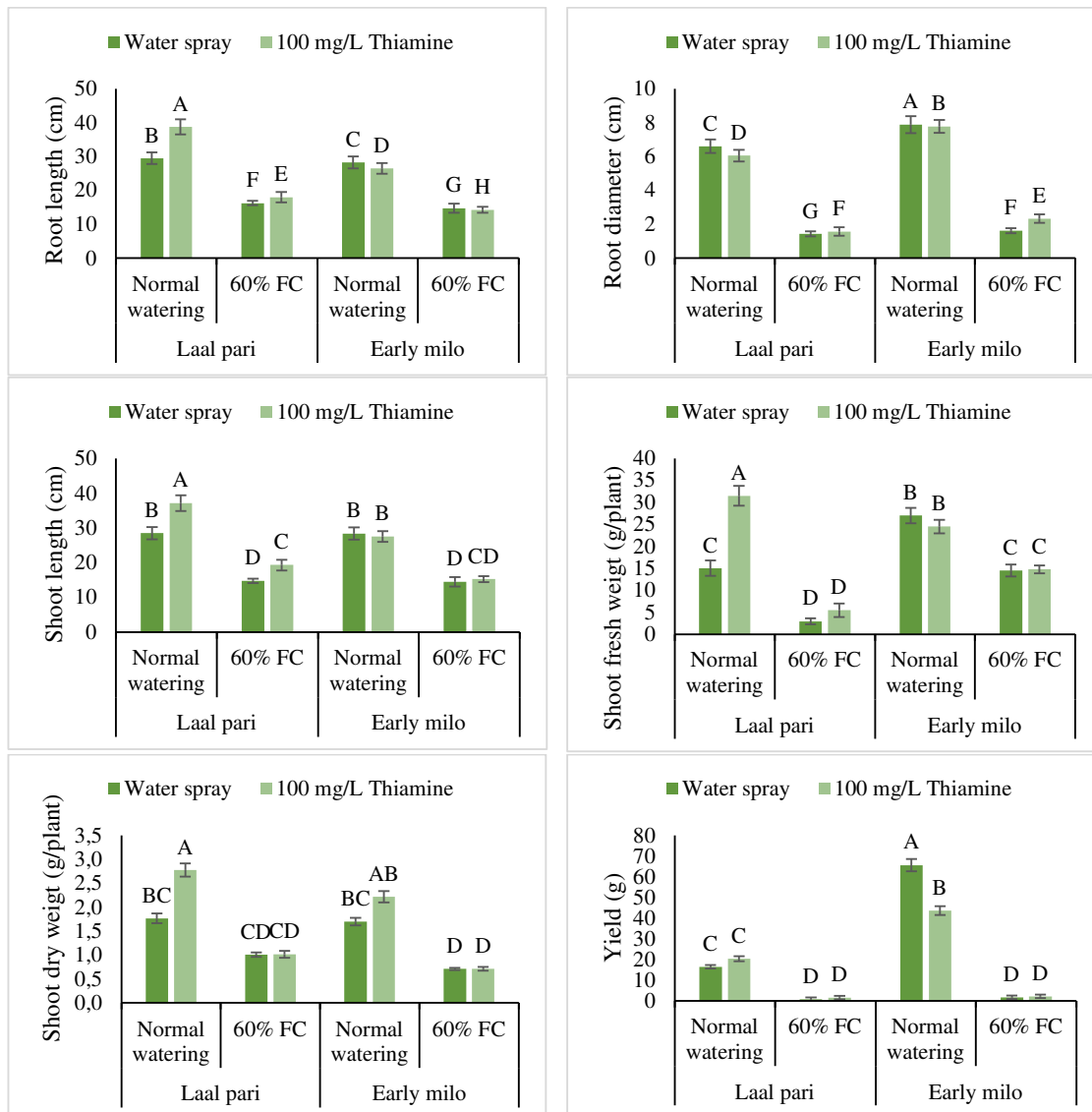
### *Statistical analysis*

The collected data underwent statistical analysis using the Fisher's Analysis of Variance (ANOVA) technique. To compare means, the Least Significant Difference (LSD) test at a 5% probability level was employed (Steel *et al.*, 1997). Principal Component Analysis (PCA) and heatmap analyses were conducted using the R statistical software. All statistical computations were performed using Statistix software, version 8.1. Graphical representations were created using Microsoft Excel (2016 version) for this study.

## **Results**

### *Growth, yield and biomass attributes*

Drought stress and thiamine affected the growth, yield and biomass attributes significantly in the tested varieties. In 'Laal Pari', root length was higher (38.75 cm) where normal water along with thiamine was applied followed by normal water spray (29.5 cm), whereas, root length was assessed lower (16.25 cm, 18 cm) in case of drought and thiamine application, respectively. Similar but decreasing trend in root length was observed in 'Early Milo'. Drought stress (60% FC), in 'Laal Pari', reduced the root diameter (1.43 cm) and 1.57 cm root diameter was observed in case of drought stress along with thiamine was applied as compared to normal water spray where root diameter was higher. 'Early Milo' exhibited the similar but increasing trend in root diameter in all applied treatments. Shoot length, in 'Laal Pari', was higher (37.12 cm) in case of normal watering along with thiamine application followed by normal water spray (28.5 cm), whereas, lower root length (14.75 cm, 19.31 cm) was observed where drought and thiamine were applied, respectively. Similar but decreasing trend in shoot length was observed in 'Early Milo'. In 'Laal Pari', shoot fresh weight was higher (31.47 g/plant) where normal watering and thiamine was applied as compared to normal water spray (15.02 g/plant), whereas, reduced shoot fresh weight (2.95 g/plant, 5.47 g/plant) was observed drought and thiamine treated conditions, respectively. In 'Early Milo', shoot fresh weight was high (27 g/plant, 24.5 g/plant) in case of normal water and thiamine application, respectively, whereas, drought as well as thiamine application exhibited 14.5 g/plant and 14.75 g/plant shoot fresh weight, respectively. In 'Laal Pari', higher shoot dry weight (2.77 g/plant) was observed where normal watering and thiamine was applied as compared to normal water spray (1.76 g/plant), whereas, drought as well as thiamine showed shoot dry weight in a range of 1.00 g/plant and 1.01 g/plant, respectively. Similar but decreasing trend in shoot dry weight was observed in 'Early Milo'. Yield, in 'Early Milo', was higher (65.66 g) in case of normal water spray as compared to thiamine application (43.62 g), whereas, drought along with thiamine exhibited 1.67 g and 2.12 g, respectively. Similar but decreasing trend in yield was observed in 'Laal Pari' (Figure 1).

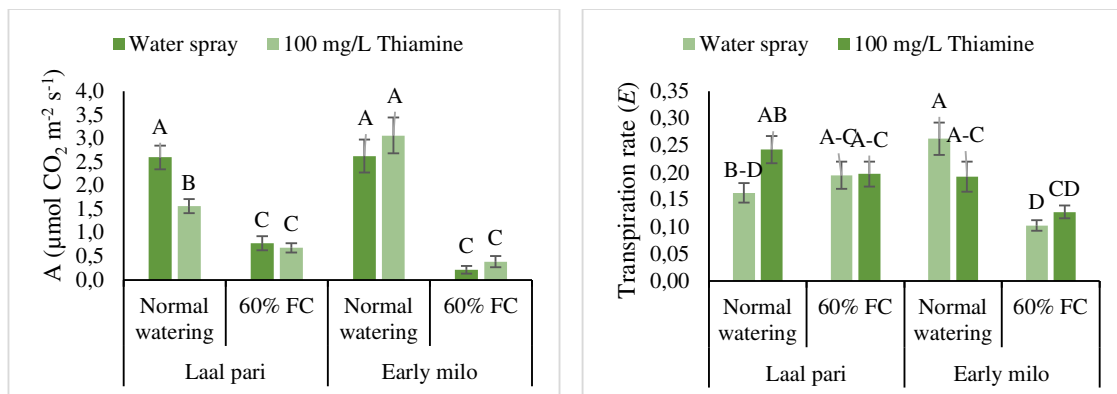


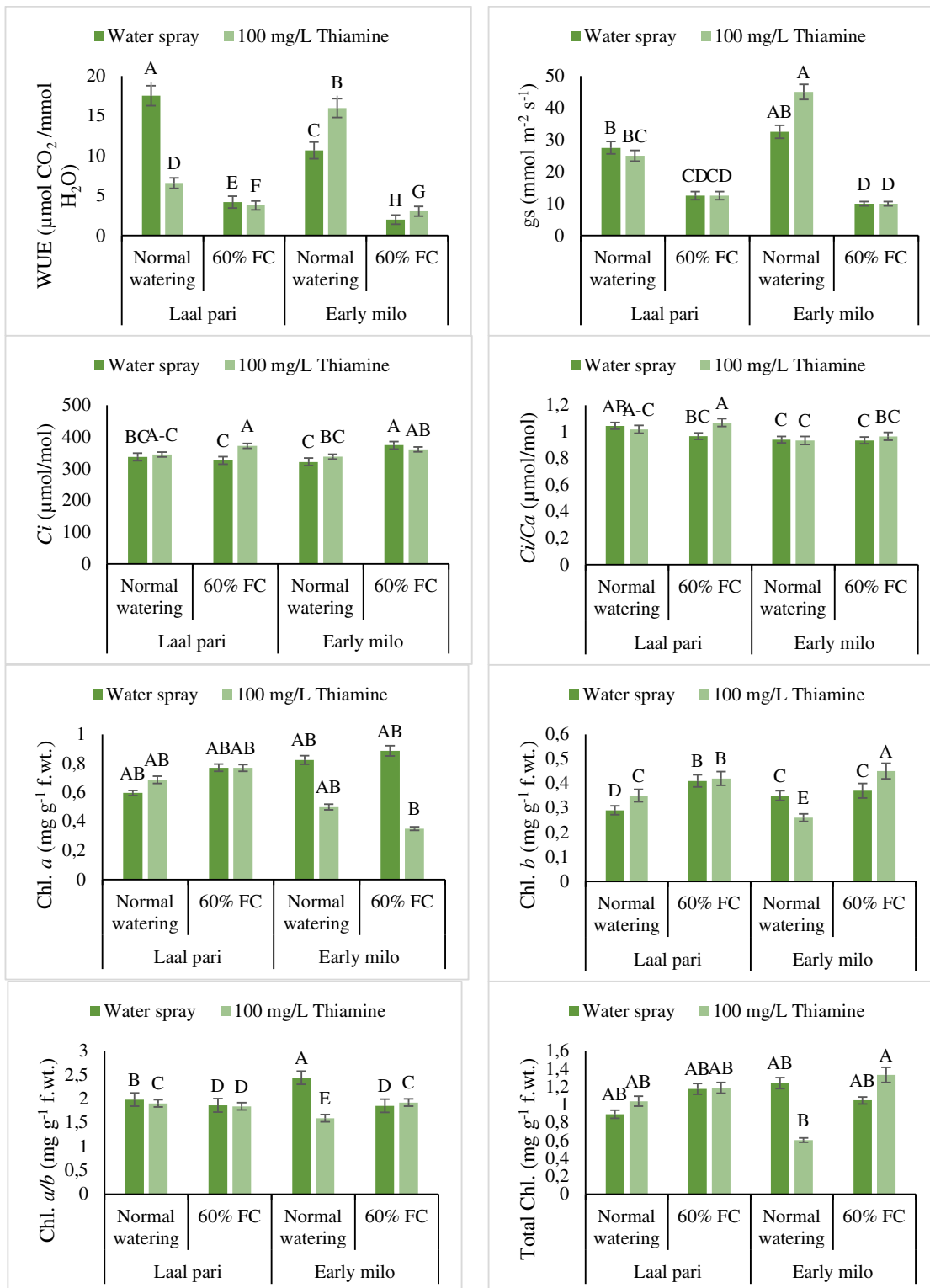
**Figure 1.** Biomass characteristics (in fresh and dry form), crop yield, crop growth parameters of two varieties of radish treated with Thiamine (100 mg/L) under normal, limited water (water spray) and drought conditions. The uppercase letters in the bars demonstrates that there is a significant difference between treatment mean values at  $p \leq 0.05$  (LSD); the values present the mean values and were replicated four times.

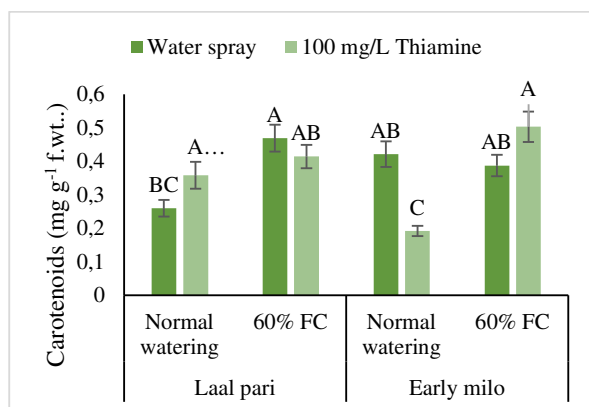
#### Physiological attributes

In ‘Early Milo’, net  $\text{CO}_2$  assimilation ( $A$ ) was higher ( $3.06 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) where normal water along with thiamine was applied followed by normal water spray ( $2.67 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), whereas, net  $\text{CO}_2$  assimilation ( $A$ ) was assessed lower ( $0.38 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ,  $0.21 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) in case of drought + thiamine and drought + normal water application, respectively. Decreasing trend in net  $\text{CO}_2$  assimilation was observed in ‘Laal Pari’ except in drought + water spray and drought + thiamine. In ‘Early Milo’, transpiration rate was higher ( $0.26 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) where normal water spray was applied followed by normal water + thiamine ( $0.19 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), drought + thiamine ( $0.12 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) over drought + water spray ( $0.10 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ). Increasing trend in transpiration rate was observed in ‘Laal Pari’ except in normal water spray

treatment. In ‘Laal Pari’, WUE ( $17.52 \mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$ ) was higher where normal water along with thiamine was applied followed by normal water spray ( $6.58 \mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$ ) whereas, WUE was assessed lower ( $3.78 \mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$ ,  $4.19 \mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$ ) in case of drought and thiamine application, respectively. Similar but decreasing ( $15.97 \mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$ ,  $10.68 \mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$ ) trend in WUE was observed in ‘Early Milo’. In ‘Early Milo’, stomatal conductance ( $g_s$ ) was higher ( $45 \text{ mmol m}^{-2} \text{ s}^{-1}$ ) where normal water along with thiamine was applied followed by normal water spray ( $32.5 \text{ mmol m}^{-2} \text{ s}^{-1}$ ) whereas, stomatal conductance ( $g_s$ ) was assessed lower ( $12.5 \text{ mmol m}^{-2} \text{ s}^{-1}$ ,  $12.5 \text{ mmol m}^{-2} \text{ s}^{-1}$ ) in case of drought + water spray and drought + thiamine application, respectively. Decreasing trend in stomatal conductance ( $g_s$ ) was observed in Laal Pari except in drought + water spray and drought + thiamine. In ‘Laal Pari’, higher substomatal conductance ( $c_i$ ) ( $371.48 \mu\text{mol mol}^{-1}$ ) was observed where drought + thiamine was applied followed by normal watering + thiamine ( $344.25 \mu\text{mol mol}^{-1}$ ), normal water spray ( $336.8 \mu\text{mol mol}^{-1}$ ) and drought + water spray ( $326.07 \mu\text{mol mol}^{-1}$ ). Decreasing trend was observed in Early Milo with respect to substomatal conductance ( $c_i$ ) except in drought + water spray. In ‘Laal Pari’,  $C_i/C_a$  was higher ( $1.06 \mu\text{mol mol}^{-1}$ ) in case of drought + thiamine application followed by normal water spray ( $1.04 \mu\text{mol mol}^{-1}$ ), normal water + thiamine ( $1.07 \mu\text{mol mol}^{-1}$ ) and drought + water spray ( $0.96 \mu\text{mol mol}^{-1}$ ). Decreasing trend in  $C_i/C_a$  was observed in ‘Early Milo’. In ‘Laal Pari’, Chl a was high ( $0.76 \text{ mg g}^{-1} \text{ f.wt.}$ ) where normal water along with drought was applied followed by drought + thiamine ( $0.76 \text{ mg g}^{-1} \text{ f.wt.}$ ) normal water + thiamine ( $0.68 \text{ mg g}^{-1} \text{ f.wt.}$ ), and normal water spray ( $0.59 \text{ mg g}^{-1} \text{ f.wt.}$ ). Decreasing trend in Chl a was observed in ‘Early Milo’ in all treatments except normal water spray and drought + water spray. In ‘Laal Pari’, Chl b was high ( $0.42 \text{ mg g}^{-1} \text{ f.wt.}$ ) where drought along with thiamine was applied followed by drought + water spray ( $0.41 \text{ mg g}^{-1} \text{ f.wt.}$ ), normal water + thiamine ( $0.35 \text{ mg g}^{-1} \text{ f.wt.}$ ) and normal water spray ( $0.29 \text{ mg g}^{-1} \text{ f.wt.}$ ). Decreasing trend in Chl b was observed in ‘Early Milo’ in all treatments except normal water spray and drought + thiamine. In ‘Early Milo’, Chl a/b was higher ( $2.44 \text{ mg g}^{-1} \text{ f.wt.}$ ) where normal water spray was used followed by drought + thiamine ( $1.92 \text{ mg g}^{-1} \text{ f.wt.}$ ), drought + water spray ( $1.85 \text{ mg g}^{-1} \text{ f.wt.}$ ) and normal watering + thiamine ( $1.59 \text{ mg g}^{-1} \text{ f.wt.}$ ). Increasing trend in Chl a/b was observed in ‘Laal Pari’ in all treatments except in normal water spray. In ‘Early Milo’, higher total chlorophyll contents ( $1.33 \text{ mg g}^{-1} \text{ f.wt.}$ ) was observed where drought + thiamine was applied followed by normal watering spray ( $1.24 \text{ mg g}^{-1} \text{ f.wt.}$ ), drought + water spray ( $1.04 \text{ mg g}^{-1} \text{ f.wt.}$ ) and thiamine + normal watering ( $0.60 \text{ mg g}^{-1} \text{ f.wt.}$ ). Decreasing trend was observed in ‘Laal Pari’ with respect to total chlorophyll. Contents except in drought + water spray and normal watering + thiamine. In ‘Laal Pari’, carotenoids was high ( $0.46 \text{ mg g}^{-1} \text{ f.wt.}$ ) where normal water along with drought was applied followed by drought + thiamine ( $0.41 \text{ mg g}^{-1} \text{ f.wt.}$ ), whereas, carotenoids was assessed lower ( $0.35 \text{ mg g}^{-1} \text{ f.wt.}$ ,  $0.25 \text{ mg g}^{-1} \text{ f.wt.}$ ) in case of water spray + thiamine and normal water spray application, respectively. Increasing trend in carotenoids was observed in ‘Early Milo’ in all treatments except drought + thiamine and normal water spray (Figure 2).



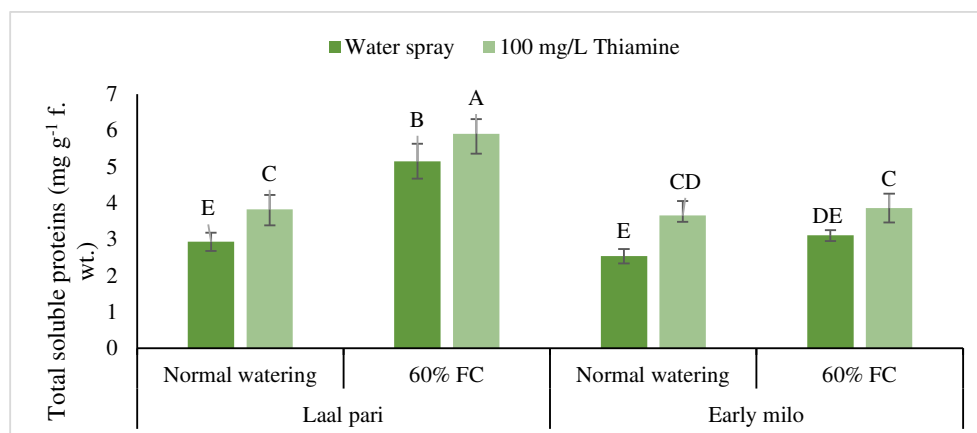




**Figure 2.** Physiological attributes of two varieties of radish treated with Thiamine ( $100 \text{ mg L}^{-1}$ ) under normal, limited water supply (water spray) and drought conditions. The uppercase letters on the bar graphs present a significant difference between the mean values of the treatments at  $p \leq 0.05$  (LSD); the values demonstrate the mean values and were replicated four times.

#### *Osmolytic attribute*

Total soluble proteins, in 'Laal Pari', were higher ( $5.91 \text{ mg g}^{-1}$ ) in case of drought along with thiamine application followed by drought environment ( $5.15 \text{ mg g}^{-1}$ ). Whereas, normal watering in combination with thiamine exhibited  $3.82 \text{ mg g}^{-1}$  total soluble protein contents as compared to normal water spray ( $2.93 \text{ mg g}^{-1}$ ). Similar but decreasing trend in total soluble protein content was observed in 'Early Milo' (Figure 3).

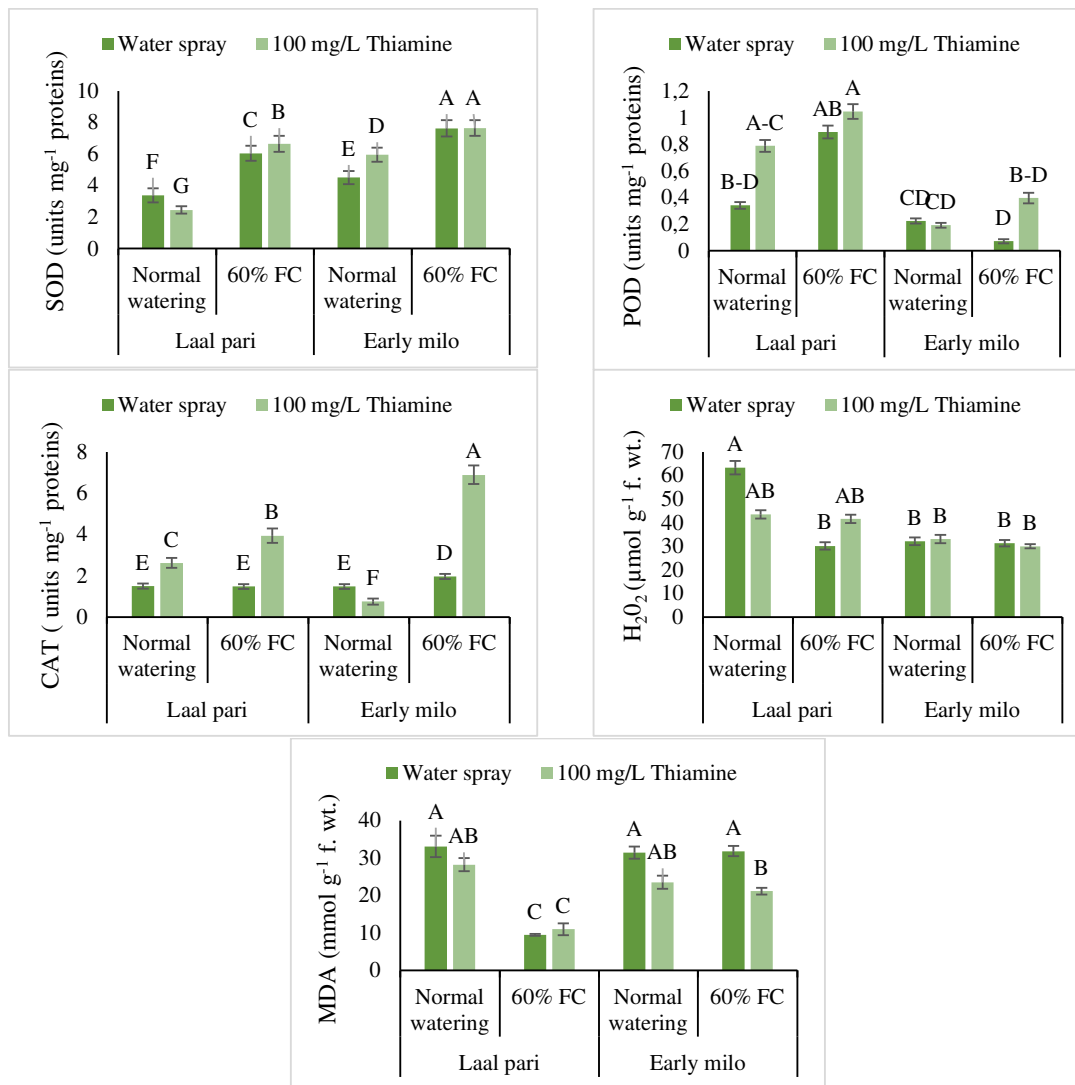


**Figure 3.** Osmolytic attributes of two varieties of radish treated with Thiamine ( $100 \text{ mg L}^{-1}$ ) under normal, water spray (limited water) and drought circumstances. The uppercase letters in the bars denote significant differences between treatment means at  $p \leq 0.05$  (LSD); the values represent the means and were replicated four times

#### *Antioxidants attributes*

SOD activities, in 'Early Milo', were higher ( $7.65 \text{ units mg}^{-1} \text{ protein}$ ) in drought + thiamine application followed by drought + water spray ( $7.64 \text{ units mg}^{-1} \text{ protein}$ ) and normal watering + thiamine ( $5.96 \text{ units mg}^{-1} \text{ protein}$ ) over normal water spray ( $4.51 \text{ units mg}^{-1} \text{ protein}$ ). Similar but decreasing trend, in 'Laal Pari', was observed regarding SOD except in normal watering + thiamine treatment. Regarding POD activities, 'Laal Pari' exhibited higher POD activities ( $1.04 \text{ units mg}^{-1} \text{ protein}$ ) where drought + thiamine was applied followed by drought + water spray ( $0.89 \text{ units mg}^{-1} \text{ protein}$ ), normal watering + thiamine ( $0.78 \text{ units mg}^{-1} \text{ protein}$ ) and normal water spray ( $0.34 \text{ units mg}^{-1} \text{ protein}$ ). Similar but decreasing trend regarding POD was observed in

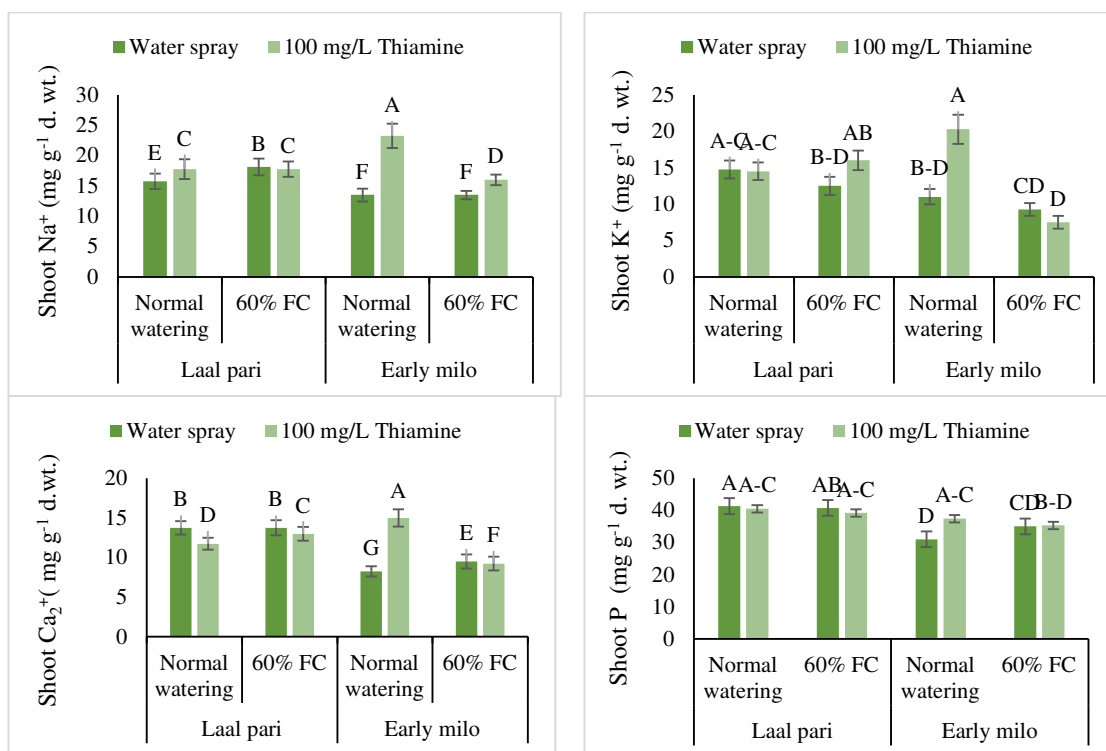
'Early Milo' except normal water spray. Catalase activity, in 'Early Milo', was higher (6.9 units mg<sup>-1</sup> protein) in case of drought + thiamine application followed by drought + water spray (1.97 units mg<sup>-1</sup> protein) and normal water spray (1.48 units mg<sup>-1</sup> protein) over thiamine + normal watering (0.75 units mg<sup>-1</sup> protein). Similar but increasing trend was observed in 'Laal Pari' regarding catalase activity. In 'Laal Pari', higher H<sub>2</sub>O<sub>2</sub> activity (63.38 μmol g<sup>-1</sup>) was observed where normal water spray was applied followed by normal watering + thiamine (43.54 μmol g<sup>-1</sup>), drought + thiamine (41.65 μmol g<sup>-1</sup>) and drought + water spray (30.18 μmol g<sup>-1</sup>). Decreasing trend was observed in 'Early Milo' with respect to H<sub>2</sub>O<sub>2</sub>. Normal water spray exhibited higher MDA level (33.10 mmol g<sup>-1</sup>) in 'Laal Pari' followed by normal watering + thiamine (28.23 mmol g<sup>-1</sup>), drought + thiamine (10.99 mmol g<sup>-1</sup>) over drought + water spray (9.51 mmol g<sup>-1</sup>). In 'Early Milo', on the other hand, drought + water spray exhibits high level of MDA activity (31.85 mmol g<sup>-1</sup>) followed by normal water spray (31.44 mmol g<sup>-1</sup>), thiamine + normal watering (23.55 mmol g<sup>-1</sup>) and drought + thiamine (21.18 mmol g<sup>-1</sup>) (Figure 4).



**Figure 4.** Antioxidant attributes of two varieties of radish treated with Thiamine (100 mg L<sup>-1</sup>) under normal, water spray (limited water) and drought circumstances. The uppercase letters in the bars denote significant differences between treatment means at  $p \leq 0.05$  (LSD); the values represent the means and were replicated four times.

### Ionic attributes

In 'Early Milo', Na<sup>+</sup> contents in shoot were higher (18.12 mg g<sup>-1</sup>) where thiamine and water spray was applied over normal water application (13.5 mg g<sup>-1</sup>), whereas, Na<sup>+</sup> contents in shoot were 16 mg g<sup>-1</sup> in case of drought condition combined with thiamine application followed by drought environment in combination with water spray (13.5 mg g<sup>-1</sup>). On the other hand, in 'Laal Pari', increasing trend in Na<sup>+</sup> contents in shoot were assessed except drought + thiamine application. K<sup>+</sup> contents, in 'Early Milo', were higher (20.25 mg g<sup>-1</sup>) in case of combined application of thiamine + normal watering followed by water spray (11 mg g<sup>-1</sup>), however, K<sup>+</sup> contents were 9.25 mg g<sup>-1</sup> where drought conditions prevails and 7.5 mg g<sup>-1</sup> K<sup>+</sup> contents were observed in case of combined application of drought + thiamine. Similar but increasing trend in K<sup>+</sup> contents was observed in 'Laal Pari' except drought + thiamine application. In 'Laal Pari', Ca<sup>2+</sup> contents in shoot were higher (13.75 mg g<sup>-1</sup>) where normal water and drought + normal water was applied followed by thiamine + drought (13 mg g<sup>-1</sup>) application over thiamine + normal watering (11.75 mg g<sup>-1</sup>). On the other hand, in 'Early Milo', decreasing trend in Ca<sup>2+</sup> contents in shoot were assessed except normal water + thiamine application. In 'Laal Pari', P contents in shoot were higher (41.34 mg g<sup>-1</sup>) where normal water applied and contents were 40.77 mg g<sup>-1</sup> in case of drought + normal water followed by thiamine + normal watering (40.49 mg g<sup>-1</sup>) application over thiamine + drought (39.25 mg g<sup>-1</sup>). Similarly, in 'Early Milo', decreasing trend in P contents in shoot was assessed except normal water + thiamine application (Figure 5).

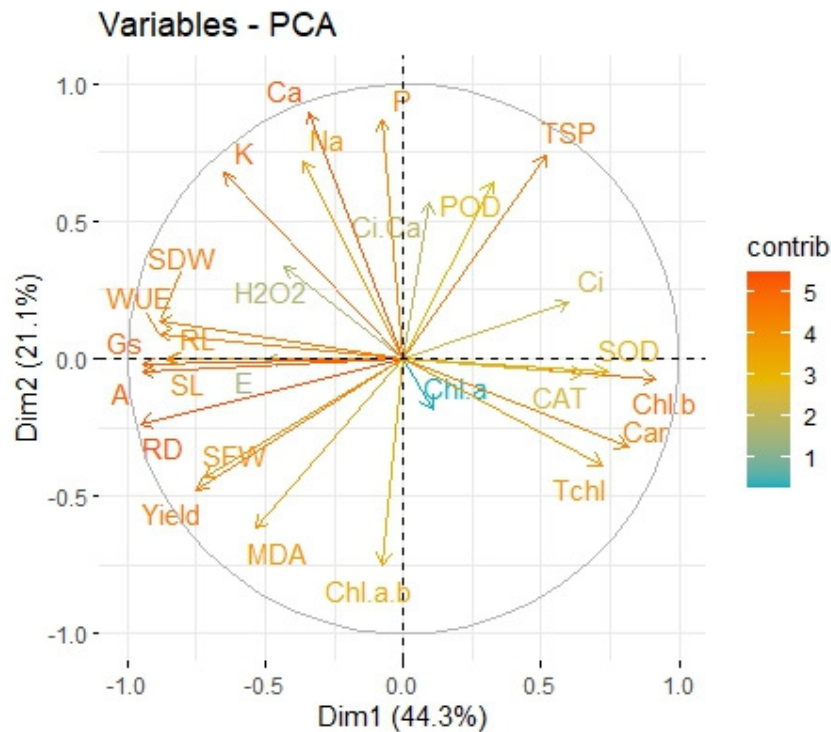


**Figure 5.** Ionic parameters of two varieties of radish treated with thiamine (100 mg L<sup>-1</sup>) under normal, water spray (limited water) and drought circumstances. The uppercase letters in the bars denote significant differences between treatment mean values at  $p \leq 0.05$  (LSD); the values represent the mean values and were replicated four times.

### Principal Component Analysis

Principal component analysis (PCA) was performed to detect the combination pattern between the measured parameters based on agronomic, physiological, ionic, antioxidant and yield-related characteristics in response to drought and thiamine application. Primary components viz. Dim1 and Dim2 made the highest

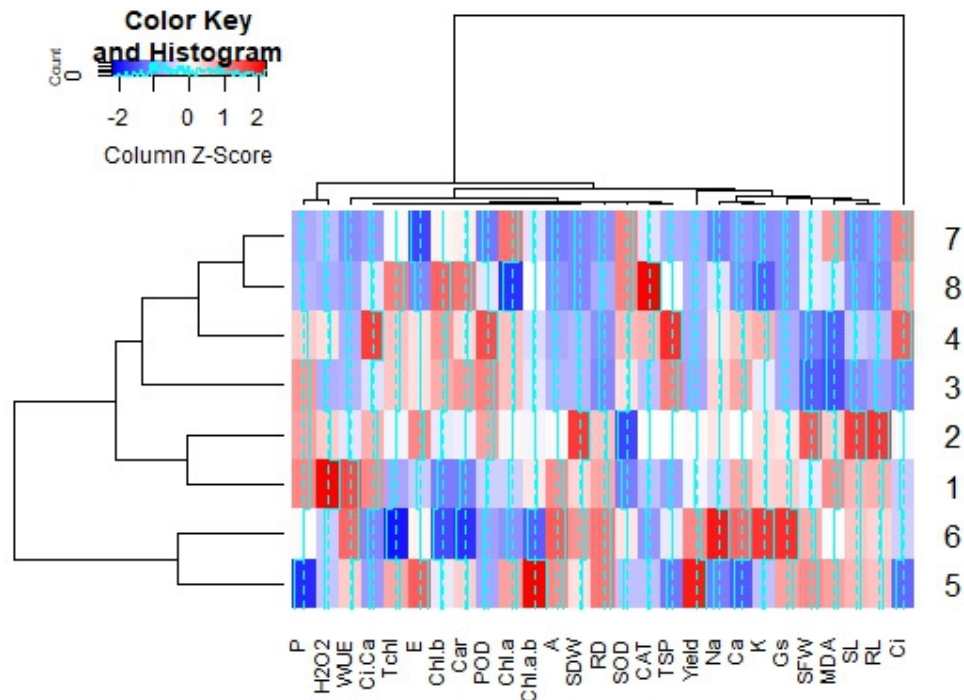
percentage and account for 65.4% of total database. Dim1 contributes 44.3% (x-axis), whereas, Dim2 contributes 21.3% (y-axis) of database (Figure 6).



**Figure 6.** Principal component analysis plot showing correlations between variables for two varieties of radish treated with Thiamine (100 mg L<sup>-1</sup>) under normal, water spray (limited water) and drought circumstances. RD presents Root diameter, Yield, RL presents Root length, SL presents Shoot length, SFW presents Shoot Fresh Weight, SDW presents Shoot Dry Weight, A presents Net Carbon Assimilation, E presents Transpiration Rate, Gs presents Stomatal Conductance, WUE presents Water Use Efficiency, T.Chl presents Total Chlorophyll Contents, Chl a presents Chlorophyll a, Chl b presents Chlorophyll b, Chl ab presents Chlorophyll a/b, CAT presents Catalase, POD presents Peroxidase, SOD presents Superoxide dismutase, H<sub>2</sub>O<sub>2</sub> presents Hydrogen Peroxide, TSP presents Total Soluble Protein.

#### Heatmap Analysis

The cluster heat map analysis summarized the responses of the osmolytes, physiological parameters, antioxidants and agronomic characteristics of the radish varieties under thiamine and drought circumstances (Figure 7). In the context of traits association, the heat map divided the varieties ('Laal Pari', 'Early Milo') along with tested treatments into various dendrograms with respect to their combination with thiamine and drought regimes. In column, 1 to 4 depicts 'Laal Pari' combinations with drought and thiamine in limited water application. While, 4 to 8 shows same combinations of treatments with 'Early Milo'. All the traits illustrated differential associations varying from positive to negative extremes in both genotypes with respect to used treatments, as demonstrated in Figure 7.



**Figure 7.** Heatmap analysis showing response of variables for two varieties of radish treated with Thiamine ( $100 \text{ mg L}^{-1}$ ) under normal, limited water (water spray) and drought conditions. Here RD presents Root diameter, Yield, RL presents Root length, SL presents Shoot length, SFW presents Shoot Fresh Weight, SDW presents Shoot Dry Weight, A presents Net Carbon Assimilation, E presents Transpiration Rate, Gs presents Stomatal Conductance, WUE presents Water Use Efficiency, TchI presents Total Chlorophyll Contents, ChI a presents Chlorophyll a, ChI b presents Chlorophyll b, ChI ab presents Chlorophyll a/b, CAT presents Catalase, POD presents Peroxidase, SOD presents Superoxide dismutase,  $\text{H}_2\text{O}_2$  presents Hydrogen Peroxide and TSP presents Total Soluble Protein.

## Discussion

Drought stress can have a significant impact on radish (*Raphanus sativus* L.) varieties. Radishes are a cool-season vegetable that prefers moderate temperatures and consistent moisture in the soil (Henschel *et al.*, 2022). Similar findings were also noticed that prolonged drought conditions, radishes may experience several physiological and developmental changes, affecting their growth, yield, and overall quality (Tuver *et al.*, 2022). Findings of the current study also depicted the similar findings that at 60% field capacity all the growth, biomass, physiological and antioxidant attributes are significantly affected. When radish varieties were subjected to water scarcity, they experience reduced growth rates and stunted development. This might be due to lack of sufficient water availability that hampers the uptake of essential nutrients and slows down vital metabolic processes, such as photosynthesis (Lacerda *et al.*, 2022). As a result, the both varieties exhibit smaller and thinner leaves, limiting their ability to harness sunlight for energy. Additionally, drought stress negatively affects root development, causing the radish roots to become smaller and less fleshy (Matamoros *et al.*, 2022). This reduced root growth leads to a decline in overall yield as the plants allocate their limited resources towards survival rather than reproduction (Manzoor *et al.*, 2021). It was detected that the thiamine application (vitamin B1), has shown potential in improving the growth attributes of radish plants (El-Shazoly *et al.*, 2019) under drought stress conditions in both varieties (Figure 1). Improvements in the radish crop growth attributes using thiamine might be due to the involvement in an essential coenzyme in various metabolic processes,

particularly in energy production and carbohydrate metabolism (Ghafar *et al.*, 2019). Similarly, when radish plants are subjected to drought stress, their energy production may be compromised due to reduced photosynthetic activity and nutrient uptake (Kausar *et al.*, 2023). Thiamine application has been found to enhance the activity of key enzymes involved in photosynthesis, as ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco), thus improving carbon fixation and overall plant growth (Amjad *et al.*, 2021). Moreover, mitigation of drought stress by the use of thiamine might be due to the action as an antioxidant, mitigating oxidative stress caused by drought, and protecting cellular components from damage and improving the growth and development of both radish varieties under limited water regimes conditions (Zhang *et al.*, 2023).

As water becomes scarce, the plants undergo various physiological adjustments to cope with the challenging conditions (Ahmad *et al.*, 2022). One of the most noticeable responses that was observed in the radish varieties is the closure of stomata, small pores on the leaf surface responsible for gas exchange and overall limiting the photosynthetic rate (Lv *et al.*, 2020). Moreover, abrupt change in the chlorophyll and carotenoids contents were observed at 60% field capacity as compared to control as explained by the (Abrar *et al.*, 2020). This mechanism reduces water loss through transpiration but also restricts the uptake of carbon dioxide, impairing photosynthesis and consequently diminishing overall plant growth (Ansari *et al.*, 2019). The reduced photosynthetic activity leads to a decline in the production of sugars and other essential metabolites, affecting energy availability for growth and development (Malik *et al.*, 2021). Additionally, application of thiamine improved the physiological attributes of the radish under drought stress (Figure 2) that might be due to the regulation in the balance of various hormones, including abscisic acid (ABA), which plays a pivotal role in the plant's response to drought stress (Jabeen *et al.*, 2022). Thiamine application has been linked to a reduction in ABA levels, which can help to moderate the negative impacts of drought-stress on radish plants, such as stomatal closure and reduced photosynthesis (Naheed *et al.*, 2022). The drought-stress triggers the growth of harmful reactive oxygen species within the plant cells, causing oxidative damage to cellular components and further impeding growth (Zhang *et al.*, 2023). To counteract the oxidative stress, radish plants often increase the production of antioxidants as a defense mechanism (Vendruscolo *et al.*, 2022). Additionally, drought-stressed radishes may experience alterations in hormone levels, such as abscisic acid (ABA), which regulates various stress responses, including stomatal closure (Baba Rabi *et al.*, 2019). Furthermore, thiamine aids in maintaining proper membrane permeability and ion balance within the plant, which is essential for nutrient uptake and overall cellular functioning (Ramos *et al.*, 2023). Through its antioxidant and hormone-regulating properties, thiamine contributes to improving the physiological resilience of radish plants under drought stress, supporting their survival and productivity in water-limited conditions (Omar *et al.*, 2020).

It was observed that significant increase in the antioxidants of various radish varieties under the limited water regimes conditions, which activate various defense mechanisms to combat the damaging effects of reactive oxygen species (ROS), which accumulate due to the stress as explained by Mehrabad Pour-Benab *et al.* (2019). As a response to drought, the production of enzymatic antioxidants, such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), increases significantly (Sahay *et al.*, 2019). These enzymes play a crucial role in neutralizing ROS and preventing oxidative damage to cellular components (Tiepo *et al.*, 2020). However, thiamine acts as an essential coenzyme in various enzymatic antioxidant pathways, facilitating the activities of key enzymes like superoxide dismutase (SOD) and catalase (CAT) (Jabeen *et al.*, 2022). By enhancing the enzymatic antioxidant defense system, thiamine helps radish plants efficiently neutralize ROS, preventing cellular damage and maintaining cell integrity. Superoxide dismutase converts harmful superoxide radicals into less toxic hydrogen peroxide ( $H_2O_2$ ), which is then further detoxified by catalase and ascorbate peroxidase (Rady *et al.*, 2021). Peroxidase also contributes to the detoxification process by reducing hydrogen peroxide and other peroxides (Sultan *et al.*, 2023). The upregulation of these enzymatic antioxidants in response to drought stress is an essential adaptation for radish plants to enhance their tolerance to oxidative

stress. Moreover, thiamine application has been found to increase the expression of genes associated with antioxidant defense in radish plants, leading to heightened levels of antioxidant compounds and enzymes, even under drought stress conditions (Seifi *et al.*, 2023) (Figure 4). Similar findings were also observed in the current study that application of thiamine improved the antioxidant potential of the radish varieties (Atif and Perveen, 2023). As a result, thiamine supplementation contributes significantly to improving the antioxidant attributes of radish plants, making them better equipped to withstand the adverse effects of drought stress and promoting healthier growth and productivity (Kaya *et al.*, 2020).

Drought stress has a notable impact on the ionic status of radish varieties. As water becomes limited, the availability and uptake of essential nutrients in the soil are reduced, affecting the overall ion balance within the plant (Ahmad *et al.*, 2022). The scarcity of water limits the movement of ions through the root system, leading to changes in the concentrations of various ions within the plant tissues (Ramos *et al.*, 2023). For instance, the concentrations of ions such as potassium ( $K^+$ ), calcium ( $Ca^{2+}$ ) and phosphorous may decrease, as they rely on adequate water for their transport to the roots and subsequent absorption (Atif and Perveen, 2023). However, from the findings of current study it was observed that thiamine application has been found to positively influence the activities of certain ion transporters and channels, enhancing the uptake and movement of crucial ions like potassium, calcium, and magnesium (Ramos *et al.*, 2023). These ions play vital roles in maintaining cell turgor, enzymatic activities, and overall cellular health, which are crucial for radish plants to endure drought stress (Ramos *et al.*, 2023). Conversely, the concentrations of certain ions like sodium ( $Na^+$ ) and chloride ( $Cl^-$ ) may increase due to their passive uptake in the absence of sufficient water. The imbalance in ion concentrations can disrupt several physiological processes, including enzyme activities and membrane functions (Ahmad *et al.*, 2022). Consequently, this disruption may impair nutrient transport, protein synthesis, and overall plant metabolism, leading to reduced growth, wilted appearance, and decreased productivity of radish plants under drought stress (Kaya *et al.*, 2020). Thiamine's involvement in various metabolic pathways can also aid in the efficient utilization of available ions, ensuring optimal nutrient absorption and utilization by the plant (Jabeen *et al.*, 2022). Moreover, thiamine's role as an antioxidant can alleviate oxidative stress-induced disruptions in ion homeostasis (Naheed *et al.*, 2022). By maintaining a balanced ionic status, thiamine supplementation helps radish plants better cope with drought stress, thereby promoting improved growth, biomass production, and overall plant health (Rodrigues De Queiroz *et al.*, 2023) (Figure 5).

## Conclusions

Drought conditions linearly reduced plant growth, yield and biomass in both varieties by minimizing water use efficiency as compared to thiamine application where these attributes enhanced significantly. Physiological, antioxidants and osmoprotectants improved in case of thiamine application against drought stress in both varieties. Based on the empirical evidence derived from this study, it can be inferred that the supplementation of thiamine exhibits a greater efficacy in mitigating the deleterious consequences associated with drought conditions. Current findings offer a promising approach to alleviate the drought stress impacts, attaining the Sustainable Development Goals (SDGs) aiming for good health and zero hunger. However, further research is needed to determine the precise mechanisms by which thiamine influences ion regulation under drought stress and to optimize its application methods for different radish varieties and environmental conditions.

### Authors' Contributions

Conceptualization, M.S.; R.I.; methodology, R.I.; M.Z.M.; software, K.A.; K.I.; formal analysis, R.A.; investigation, M.S.; resources, M.S., and R.I.; data curation, R.I.; writing—original draft preparation, M.Z.M., I.K., U.T. and A-R.Z.G.; writing—review and editing, M.S., Q.Z., K.A., M.H.S. and M.S.H.; supervision, M.S.; project administration, M.S.; funding acquisition, M.H.S., A-R.Z.G and M.S.H. All authors read and approved the final manuscript.

### Ethical approval (for researches involving animals or humans)

Not applicable.

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### Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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