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RESEARCH ARTICLE

Evaluation of foliar-applied biostimulants and nutrients for enhancing drought tolerance in canola (*Brassica napus* L.)

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ABSTRACT

Due to the adverse effects of climate change, drought has become a global phenomenon that significantly impacts agricultural productivity. This study focused on stimulating tolerance of canola (*Brassica napus* L.) plants against drought stress by applying different doses of sodium silicate (Na_2SiO_3), potassium silicate (K_2SiO_3), melatonin, ascorbic acid, salicylic acid, potassium nitrate (KNO_3), and zinc sulfate ($ZnSO_4$). The canola plants were grown under control (80% water holding capacity) and drought-stressed (50%) conditions and were sprayed three times at the three-leaf stage. The experiment was planned by a completely randomized design with six replicates. Morphological (plant height, fresh and dry biomass, and leaf area), physiological traits (chlorophyll content, leaf temperature, relative water content, and electrolyte leakage), and stomata properties were investigated. Drought depressed the growth of the canola plant, decreasing leaf area, plant fresh and dry weight. Plant fresh weight decreased by 35% and leaf area by 25%. Increased electrolyte leakage and stomata number were also observed under drought conditions. Electrolyte leakage was higher in drought (25.7%) than in well-water conditions (11.2%). However, KNO_3 treatments effectively mitigated the adverse effects of drought stress on plant growth by regulating chlorophyll content, relative water content, and electrolyte leakage. Principal component and hierarchical cluster analysis have revealed that plant fresh weight, dry weight, leaf area and relative water content were primarily affected by drought, and stomata properties and electrolyte leakage are secondarily critical traits in the drought tolerance of canola plants. In conclusion, foliar applications of KNO_3 should be recommended to induce plant growth in canola under drought stress. Stomata properties, particularly stomata number, may also be considered as selection criteria for drought-tolerant canola plants.

Key words: *Brassica napus* L., KNO_3, principal component, stomata, water deficit

INTRODUCTION

Oilseed rape (*Brassica napus* L.) is an important oilseed crop widely cultivated in temperate and cool continental regions of Europe. It is particularly valued for its healthy, high-quality oil and its high oil content in seeds. Numerous rapeseed varieties with a high erucic acid content are used in lubricants, plastics production, and other industries. Others, however, have a high oleic acid content suitable for human consumption and biodiesel (known as canola) and exhibit winter hardiness, making them ideal for autumn sowing (Ulhassan et al., 2019; Alamri et al., 2020; Batool et al., 2023; Azimi Gandomani, 2024). In Türkiye, canola cultivation has recently been replaced by sunflower, as it enables higher productivity and earlier harvesting compared to other oilseeds. However, early sowing (late August-September) to obtain plants at the rosette stage (4-6 leaves) before the first autumn frost has led to a risk of drought.

Water scarcity during sowing, often exacerbated by delayed autumn rainfall or erratic rainfall patterns associated with climate change, impacts negatively on germination, seedling emergence, and growth, limiting canopy development and consequently reducing yield potential (Batool et al., 2023; Anwar et al., 2023; Gatasheh et al., 2023). Early sowing in arid and semi-arid regions is often accompanied by insufficient soil moisture, which makes it difficult for crops to establish successfully (Bukhari et al., 2021; Azimi Gandomani, 2024). Recent studies show that a lot of compounds have shown promising results in stress modulation of Si-based compounds (e.g., sodium and potassium silicates) (Bukhari et al., 2021), phytohormone analogs like melatonin (Ulhassan et al., 2019) and lipoic acid (Javeed et al., 2021), and micronutrients including potassium nitrate and zinc sulfate (Ibraheem and Alsultan, 2025). For example, Bukhari et al. (2021) and Gatasheh et al. (2023) reported that Si application reinforces cellular structures, maintains leaf turgor, and improves antioxidative defenses in *B. napus* seedlings under limited water availability. Salicylic acid, known for its role in systemic acquired resistance, enhances drought tolerance by modulating stomatal closure and reactive oxygen species (ROS) scavenging (Tanveer et al., 2023; Anwar et al., 2023; Siddiqui et al., 2023). Melatonin, a multifunctional plant regulator, not only delays senescence but also boosts root architecture, osmolyte accumulation, and antioxidant enzyme activity, thereby improving stress adaptation (Gatasheh et al., 2023; Rafique et al., 2024). Notably, the combined or synergistic applications of these substances have led to even greater improvements in drought resilience, particularly in the vulnerable seedling phase (Javeed et al., 2021; Rafique et al., 2024). Despite these advances, there is still a lack of empirical data on which treatments are more effective in in enhancing drought tolerance of winter canola during early growth stages remain limited (Alamri et al., 2020; Batool et al., 2023). This knowledge gap emphasizes the need for targeted research to assess the physiological, biochemical and agronomic effects of these compounds under water-deficit conditions (Ulhassan et al., 2019; Gatasheh et al., 2023; Rafique et al., 2024). Therefore, this study aimed to mitigate the adverse effects of drought stress on canola plants by using optimal dosages of various agents whose efficacy has been demonstrated in other plants.

MATERIALS AND METHODS

Preparation of experimental materials

The experiment was conducted under controlled environment conditions at the Field Crops Department of Eskişehir Osmangazi University, Türkiye, in 2024. Five seeds of winter canola (*Brassica napus* L.) 'KWS Riccardo' were sown in pots (12 cm depth, 10 cm diameter) filled with uniformly mixed soil, peat, vermiculite, and sand in a 3:1:1:1 ratio. Soil structure was clay-loam with low organic matter (1.1%) and P. Plants were grown under a temperature regime of 22 °C during the day and 16 °C at night, with a light (500 µmol m^{-2} s^{-1} 18 h light and 6 h dark) in 50%-60% relative humidity. Once the seedling had emerged, three plants were allowed to grow in each pot. To simulate drought stress (50% water-holding capacity) and non-stress conditions (80% water-holding capacity), the plants were subjected to two irrigation regimes when they were at the three-leaf stage. Each pot was weighed to determine water loss at two-day intervals, and distilled water was applied until they reached their desired water-holding capacity.

Different doses of sodium silicate (2, 4, and 6 mM Na_2SiO_3), potassium silicate (15 and 30 mM K_2SiO_3), melatonin (10, 50, 100, and 500 µM), ascorbic acid (0.5, 1, and 2 mM), salicylic acid (0.25, 0.5, and 1 mM), potassium nitrate (250, 500, and 1000 mg L^{-1} KNO_3), and zinc sulfate (0.1 and 0.2 mg L^{-1} $ZnSO_4$), which were selected using previous studies conducted in various plant species, were sprayed onto the leaves of canola plants three times with 4 d intervals when the plants were at the three-leaf stage. A hand-held manual sprayer was used for the applications, with 5 mL solution sprayed per plant. Distilled water was sprayed on the control plants. The plants were harvested 7 d after the final foliar application. Treatments are summarized in Table 1.

Measurement of morphological characteristics

Leaf number, leaf area, plant fresh and dry weights of the canola plants were measured on the 7th day after the last application. Leaf area was measured by analyzing scanned photos of each plant's leaves using the ImageJ program (Cosmulescu et al., 2020).

Table 1. Treatments of different agents and doses used in the canola plant.

No	Treatments
T1	50% Water holding capacity (WHC) (Drought)
T2	80% WHC (Control)
T3	50% WHC + 2 mM Na_2SiO_3
T4	50% WHC + 4 mM Na_2SiO_3
T5	50% WHC + 6 mM Na_2SiO_3
T6	50% WHC + 15 mM K_2SiO_3
T7	50% WHC + 30 mM K_2SiO_3
T8	50% WHC + 10 µM Melatonin
T9	50% WHC + 50 µM Melatonin
T10	50% WHC + 100 µM Melatonin
T11	50% WHC + 500 µM Melatonin
T12	50% WHC + 0.5 mM Ascorbic acid
T13	50% WHC + 1 mM Ascorbic acid
T14	50% WHC + 2 mM Ascorbic acid
T15	50% WHC + 0.25 mM Salicylic acid
T16	50% WHC + 0.50 mM Salicylic acid
T17	50% WHC + 1.00 mM Salicylic acid
T18	50% WHC + KNO_3 250 mg L^{-1}
T19	50% WHC + KNO_3 500 mg L^{-1}
T20	50% WHC + KNO_3 1000 mg L^{-1}
T21	50% WHC + $ZnSO_4$ 0.1 mg L^{-1}
T22	50% WHC + $ZnSO_4$ 0.2 mg L^{-1}

Measurement of physiological characteristics

Leaf chlorophyll content was measured using the SPAD-502 meter (Konica Minolta, Tokyo, Japan). Leaf temperature was recorded with an infrared thermometer (BP21, Trotec, Heinsberg, Germany). Relative water content (RWC) was determined by Barrs and Weatherley (1962), and electrolyte leakage (EL) was evaluated as an indicator of cell membrane integrity (Gulen and Eris, 2004). To determine RWC, two leaves from the top of each plant (2^{nd} and 3^{rd}) were removed from each replicate and weighed immediately to determine their fresh weight (FW). The leaves were then floated in distilled water in a Falcon tube for 24 h to reach turgidity. After quantifying turgor weight (TW), the leaves were dried at a constant temperature of 80 °C for 24 h to detect the dry weight (DW). The RWC was then calculated using the following formula:

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

Electrolyte leakage was determined using eight 5 mm discs taken from the true leaves. These were rinsed with distilled water and then dried gently with paper towels. The discs were then placed in a 50 mL glass test tube containing 30 mL distilled water. After incubation at 20 °C for 24 h, the first EL measurement was performed using a conductivity meter (3.15i; WTW, Weilheim, Germany) (ECf). They were transferred to a water bath at 90 °C for 60 min to kill the tissue. After cooling for 1 h at room temperature, the second measurement of EL (ECs) was read. The EL was calculated as follows:

$$EL (\%) = (ECf/ECs) \times 100.$$

Measurement of stomata properties

The number, width, length, and size of the stomata on the abaxial side of the third leaf from the top of the plants were quantified using a microscope. The direct forceps peeling was used to take images of the stomata. The center of the leaf was carefully stripped using a fine tweezer. Extracted samples were first immersed in acetone, then washed with distilled water and stained with safranin dye. A light microscope (Axiophot microscope, 40×10; Zeiss, Oberkochen, Germany) in conjunction with the image acquisition and digitizing program AxioVision 4.3 software (Zeiss), along with EOS camera eyepieces (Canon, Tokyo, Japan). Stomata number (stomata number per mm^2 leaf area) was counted using an ocular micrometer calibrated with an object micrometer described by Kaya (2021), and stomata dimensions in the photographs were measured.

Statistical analysis

The experimental design was a completely randomized design (CRD) with six replicates per treatment, with three plants in each replicate. All data were analyzed using ANOVA, and the differences between the means were compared using Tukey's HSD test at a significance level of $p < 0.05$. To classify the treatments, principal component (PC) and hierarchical cluster analyses (HCA) were conducted using the JMP 13.0 program. The HCA was performed using Ward's method of diversity analysis. Data averaged from the replicates of each treatment were used.

RESULTS

The imposition of drought stress at 50% water holding capacity resulted in a significant reduction in leaf number compared to the well-watered control at 80% water holding capacity. Among the treatments, KNO_3, particularly at concentrations of 250 and 500 mg L^{-1}, significantly increased leaf number under drought conditions (Table 2). Leaf number also increased with the application of 2 mM ascorbic acid and 10 µM melatonin. However, these treatments were less effective than K_2SiO_3 (30 mM). Drought resulted in a decrease in the number of fully expanded leaves per plant. All levels of KNO_3 significantly alleviated this decrease, resulting in leaf numbers comparable to or even exceeding those under non-stressed conditions.

Table 2. Morphological characteristics of canola plants treated with different agents under drought conditions. Mean ± SE of replicates connected with the same letter(s) in the same column are not significantly different by the Tukey test at $p \le 0.05$. *, **Significant at 5% and 1%, respectively. T1: 50% water holding capacity (WHC); T2: 80% WHC; T3: 50% WHC + 2 mM Na_2SiO_3; T4: 50% WHC + 4 mM Na_2SiO_3; T5: 50% WHC + 6 mM Na_2SiO_3; T6: 50% WHC + 15 mM K_2SiO_3; T7: 50% WHC + 30 mM K_2SiO_3; T8: 50% WHC + 10 µM melatonin; T9: 50% WHC + 50 µM melatonin; T10: 50% WHC + 100 µM melatonin; T11: 50% WHC + 500 µM melatonin; T12: 50% WHC + 0.5 mM ascorbic acid; T13: 50% WHC + 1 mM ascorbic acid; T14: 50% WHC + 2 mM ascorbic acid; T15: 50% WHC + 0.25 mM salicylic acid; T16: 50% WHC + 0.50 mM salicylic acid; T17: 50% WHC + 1.00 mM salicylic acid; T18: 50% WHC + KNO_3 250 mg L^{-1}; T19: 50% WHC + KNO_3 500 mg L^{-1}; T20: 50% WHC + KNO_3 1000 mg L^{-1}; T21: 50% WHC + $ZnSO_4$ 0.1 mg L^{-1}; T22: 50% WHC + $ZnSO_4$ 0.2 mg L^{-1}.

N°	Leaf number	Fresh weight	Dry weight	Leaf area
	Nr $plant^{-1}$	g $plant^{-1}$	g $plant^{-1}$	cm^2
T1	4.5 ± 0.29^{bcd}	5.10 ± 0.23^{fg}	0.22 ± 0.01^{m}	153 ± 3.71^{i}
T2	5.0 ± 0.00^{ab}	7.85 ± 0.18^{a}	0.37 ± 0.01^{a}	204 ± 1.41^{a}
T3	4.5 ± 0.29^{bcd}	6.34 ± 0.27^{bcd}	0.31 ± 0.01^{ghi}	163 ± 2.32^{gh}
T4	4.7 ± 0.25^{abc}	5.88 ± 0.12^{de}	0.32 ± 0.00^{fg}	159 ± 2.66^{hi}
T5	4.7 ± 0.25^{abc}	6.33 ± 0.25^{bcd}	0.35 ± 0.00^{bc}	174 ± 1.76^{cde}
T6	4.7 ± 0.25^{abc}	5.91 ± 0.23^{de}	0.31 ± 0.00^{hi}	171 ± 4.92^{c-f}
T7	5.2 ± 0.25^{a}	5.97 ± 0.37^{cde}	0.36 ± 0.01^{ab}	166 ± 3.19^{fgh}
T8	5.0 ± 0.00^{ab}	6.47 ± 0.10^{bc}	0.34 ± 0.01^{de}	175 ± 1.60^{cd}
T9	4.7 ± 0.25^{abc}	5.84 ± 0.29^{de}	0.32 ± 0.01^{fgh}	163 ± 2.59^{gh}
T10	4.7 ± 0.25^{abc}	4.22 ± 0.11^{h}	0.19 ± 0.00^{n}	131 ± 2.88^{k}
T11	4.0 ± 0.00^{d}	4.63 ± 0.20^{gh}	0.19 ± 0.00^{n}	131 ± 2.17^{k}
T12	4.5 ± 0.29^{bcd}	5.42 ± 0.12^{ef}	0.25 ± 0.00^{l}	154 ± 2.06^{i}
T13	4.2 ± 0.25^{cd}	5.99 ± 0.29^{cde}	0.29 ± 0.00^{j}	168 ± 4.00^{d-g}
T14	5.0 ± 0.00^{ab}	6.36 ± 0.11^{bcd}	0.32 ± 0.00^{ef}	173 ± 1.94^{c-f}
T15	4.5 ± 0.29^{bcd}	4.99 ± 0.27^{fg}	0.27 ± 0.01^{k}	167 ± 2.87^{efg}
T16	4.7 ± 0.25^{abc}	6.08 ± 0.08^{bcd}	0.32 ± 0.00^{fgh}	174 ± 0.72^{cde}
T17	4.5 ± 0.29^{bcd}	5.47 ± 0.08^{ef}	0.28 ± 0.00^{jk}	164 ± 2.01^{gh}
T18	5.0 ± 0.00^{ab}	6.52 ± 0.17^{bc}	0.35 ± 0.00^{bc}	185 ± 1.61^{b}
T19	5.0 ± 0.00^{ab}	6.50 ± 0.12^{bc}	0.36 ± 0.00^{ab}	175 ± 1.53^{cd}
T20	4.7 ± 0.25^{abc}	6.59 ± 0.16^{b}	0.36 ± 0.00^{ab}	175 ± 2.00^{cd}
T21	4.2 ± 0.25^{cd}	5.26 ± 0.13^{f}	0.29 ± 0.00^{j}	139 ± 1.38^{i}
T22	4.2 ± 0.25^{cd}	5.07 ± 0.20^{fg}	0.30 ± 0.00^{i}	144 ± 0.89^{j}
p	$< 0.03^{*}$	$< 0.01^{**}$	$< 0.01^{**}$	$< 0.01^{**}$

Foliar application of 10-100 µM melatonin also increased leaf number, suggesting a protective effect on meristematic activity and leaf expansion. The fresh weight of the canola plant, a direct indicator of plant water status and turgor, decreases significantly under drought stress. However, KNO_3 applications, especially at 1000 mg L^{-1}, significantly improved plant fresh weight, reflecting better osmotic adjustment and water uptake. In addition, the application of Na_2SiO_3 at 2 and 6 mM and melatonin at 10 µM also promoted plant fresh weight compared to the drought-stressed plants (T1), indicating improved hydration and reduced water loss. Drought stress restricts DM accumulation, resulting in lower yields in untreated canola plants under drought conditions. The KNO_3 applications produced the highest dry biomass, indicating a greater ability to sustain C assimilation and structural growth. Additionally, K_2SiO_3 (30 mM) enhanced DM, although to a lesser extent. Leaf area diminished significantly in response to water deficit, which limited photosynthetic area. The KNO_3 applications, especially at 250 mg L^{-1}, enlarged leaf area compared to drought-stressed plants, indicating the maintenance of cell expansion. Sodium silicate (6 mM) and melatonin (10 µM) also induced leaf area expansion, suggesting a role in maintaining cell turgor.

Drought stress induced the chlorophyll content (SPAD), reflecting a decrease in leaf area and an increase in dry weight. Zinc sulfate at 0.2 mg L^{-1} produced SPAD values (50.2 SPAD) similar to those of drought-stressed plants (50.8 SPAD) (Table 3). Sodium silicate applications also supported the maintenance of chlorophyll content, indicating reduced oxidative stress and stabilized chloroplast structure. Elevated leaf temperatures were recorded under drought stress due to reduced transpirational cooling. Treatments with salicylic acid and $ZnSO_4$ considerably lowered leaf surface temperatures, suggesting better stomatal control and evaporative cooling ability. Under water-limited conditions, relative water content (RWC) dramatically dropped; however, KNO_3 (250 and 1000 mg L^{-1}) successfully recovered RWC to levels close to those of the control group, indicating enhanced osmotic adaptation. Sodium silicate and melatonin also improved RWC, supporting their role in cell water retention and membrane permeability. Drought-induced membrane damage resulted in increased electrolyte leakage (EL). The application of 500 mg L^{-1} KNO_3 resulted in the lowest EL values. Potassium silicate treatments also reduced ion leakage from leaf tissues, consistent with strengthened cell structure and reduced lipid peroxidation.

Table 3. Physiological parameters of canola plants treated with different agents under drought conditions. Mean ± SE of replicates connected with the same letter(s) in the same column are not significantly different by the Tukey test at $p \leq 0.05$. **Significant at 1%, respectively. T1: 50% water holding capacity (WHC); T2: 80% WHC; T3: 50% WHC + 2 mM Na_2SiO_3; T4: 50% WHC + 4 mM Na_2SiO_3; T5: 50% WHC + 6 mM Na_2SiO_3; T6: 50% WHC + 15 mM K_2SiO_3; T7: 50% WHC + 30 mM K_2SiO_3; T8: 50% WHC + 10 µM melatonin; T9: 50% WHC + 50 µM melatonin; T10: 50% WHC + 100 µM melatonin; T11: 50% WHC + 500 µM melatonin; T12: 50% WHC + 0.5 mM ascorbic acid; T13: 50% WHC + 1 mM ascorbic acid; T14: 50% WHC + 2 mM ascorbic acid; T15: 50% WHC + 0.25 mM salicylic acid; T16: 50% WHC + 0.50 mM salicylic acid; T17: 50% WHC + 1.00 mM salicylic acid; T18: 50% WHC + KNO_3 250 mg L^{-1}; T19: 50% WHC + KNO_3 500 mg L^{-1}; T20: 50% WHC + KNO_3 1000 mg L^{-1}; T21: 50% WHC + $ZnSO_4$ 0.1 mg L^{-1}; T22: 50% WHC + $ZnSO_4$ 0.2 mg L^{-1}.

No	Leaf temperature	Chlorophyll content	Relative water content	Electrolyte leakage
	°C	SPAD	%	%
T1	23.7 ± 0.70^{a}	50.8 ± 0.28^{a}	76.5 ± 1.11^{ghi}	25.7 ± 0.36^{a}
T2	23.5 ± 0.39^{ab}	44.6 ± 0.55^{h-k}	83.2 ± 1.07^{a}	11.2 ± 0.21^{cde}
T3	22.9 ± 0.18^{abc}	47.6 ± 0.53^{bcd}	76.6 ± 0.52^{ghi}	13.1 ± 0.31^{b}
T4	22.8 ± 0.16^{a-d}	48.2 ± 0.56^{b}	78.8 ± 0.69^{bcd}	10.6 ± 0.09^{d-g}
T5	22.4 ± 0.27^{b-f}	47.5 ± 0.79^{bcd}	77.5 ± 0.70^{d-h}	9.6 ± 0.58^{h}
T6	23.1 ± 0.36^{abc}	46.5 ± 0.72^{b-g}	77.9 ± 0.49^{c-g}	9.4 ± 0.10^{hi}
T7	22.9 ± 0.57^{abc}	47.8 ± 0.39^{bc}	76.4 ± 0.25^{g-j}	8.7 ± 0.21^{ij}
T8	21.7 ± 0.59^{efg}	44.9 ± 0.43^{ghi}	76.7 ± 0.40^{ghi}	9.9 ± 0.40^{gh}
T9	23.4 ± 0.52^{ab}	45.6 ± 0.49^{e-h}	76.1 ± 1.06^{e-i}	10.5 ± 0.24^{fg}
T10	22.8 ± 0.36^{a-e}	43.5 ± 0.80^{ijk}	74.9 ± 0.33^{j}	10.9 ± 0.14^{c-f}
T11	22.0 ± 0.26^{c-g}	43.0 ± 0.89^{k}	78.4 ± 0.15^{cde}	10.6 ± 0.03^{d-g}
T12	21.8 ± 0.31^{d-g}	46.0 ± 0.35^{c-h}	77.8 ± 0.17^{c-g}	10.6 ± 0.11^{d-g}
T13	23.0 ± 0.37^{abc}	47.2 ± 0.29^{b-e}	77.4 ± 0.30^{d-h}	10.5 ± 0.28^{efg}
T14	22.0 ± 0.51^{c-g}	46.5 ± 0.79^{b-g}	76.8 ± 0.29^{f-i}	10.7 ± 0.18^{def}
T15	21.3 ± 0.54^{f-i}	43.1 ± 0.87^{jk}	75.6 ± 0.61^{ij}	11.4 ± 0.18^{c}
T16	20.3 ± 0.38^{ij}	46.8 ± 0.40^{b-f}	75.9 ± 0.34^{hij}	10.4 ± 0.13^{fg}
T17	20.5 ± 0.30^{hij}	45.1 ± 0.27^{f-i}	76.9 ± 0.41^{e-i}	11.2 ± 0.39^{c-f}
T18	21.6 ± 0.42^{fgh}	45.8 ± 0.61^{d-h}	79.1 ± 0.19^{bc}	9.6 ± 0.12^{h}
T19	21.3 ± 0.25^{f-i}	44.8 ± 0.93^{g-j}	76.6 ± 0.41^{ghi}	8.6 ± 0.24^{j}
T20	22.1 ± 0.35^{c-g}	47.4 ± 0.14^{bcd}	80.2 ± 0.35^{b}	9.9 ± 0.16^{gh}
T21	21.0 ± 0.45^{g-j}	45.2 ± 0.35^{fgh}	72.3 ± 0.38^{c-f}	11.3 ± 0.34^{cd}
T22	20.1 ± 0.16^{j}	50.2 ± 1.16^{a}	75.6 ± 0.15^{ik}	10.6 ± 0.15^{d-g}
p	< 0.01**	< 0.01**	< 0.01**	< 0.01**

The number of stomata was higher in drought-stressed plants, probably as an adaptive mechanism to maximize CO_2 uptake during brief stomatal openings. The application of 30 mM K_2SiO_3 and salicylic acid (0.25 and 0.50 mM) helped normalize the number of stomata, thus supporting optimal stomatal behavior under drought stress (Table 4). Drought imposed a decrease in the width of stomata. However, KNO_3 at 250 mg L^{-1} and $ZnSO_4$ maintained wider stomata openings, allowing continued gas exchange and water vapor release without excessive loss. The reduction in stomata length was observed in canola plants subjected to drought, which may compromise transpiration control. The KNO_3 and $ZnSO_4$ maintained longer stomata, facilitating more efficient regulation of water loss. Stomata size was significantly reduced by drought, but 250 mg L^{-1} KNO_3 maintained a larger stomata size, allowing for balanced gas exchange and an effective response to atmospheric demands. The pictures of stomata belonging to canola foliar-treated plants under drought stress were displayed in Figure 1.

Table 4. Stomata properties of canola plants treated with different agents under drought conditions. Mean ± SE of replicates connected with the same letter(s) in the same column are not significantly different by the Tukey test at $p \leq 0.05$. **Significant at 1%, respectively. T1: 50% water holding capacity (WHC); T2: 80% WHC; T3: 50% WHC + 2 mM Na_2SiO_3; T4: 50% WHC + 4 mM Na_2SiO_3; T5: 50% WHC + 6 mM Na_2SiO_3; T6: 50% WHC + 15 mM K_2SiO_3; T7: 50% WHC + 30 mM K_2SiO_3; T8: 50% WHC + 10 µM melatonin; T9: 50% WHC + 50 µM melatonin; T10: 50% WHC + 100 µM melatonin; T11: 50% WHC + 500 µM melatonin; T12: 50% WHC + 0.5 mM ascorbic acid; T13: 50% WHC + 1 mM ascorbic acid; T14: 50% WHC + 2 mM ascorbic acid; T15: 50% WHC + 0.25 mM salicylic acid; T16: 50% WHC + 0.50 mM salicylic acid; T17: 50% WHC + 1.00 mM salicylic acid; T18: 50% WHC + KNO_3 250 mg L^{-1}; T19: 50% WHC + KNO_3 500 mg L^{-1}; T20: 50% WHC + KNO_3 1000 mg L^{-1}; T21: 50% WHC + $ZnSO_4$ 0.1 mg L^{-1}; T22: 50% WHC + $ZnSO_4$ 0.2 mg L^{-1}.

No	Stomata number	Stomata length	Stomata width	Stomata size
	Nr mm^{-2}	µm	µm	$µm^2$
T1	243 ± 1.02^{h}	19.8 ± 0.12^{hi}	13.52 ± 0.08^{kl}	210 ± 1.35^{ij}
T2	298 ± 4.32^{d}	20.2 ± 0.01^{gh}	14.58 ± 0.10^{d}	236 ± 1.66^{g}
T3	193 ± 0.84^{k}	22.6 ± 0.05^{d}	14.54 ± 0.03^{de}	259 ± 2.02^{c}
T4	315 ± 0.87^{b}	19.6 ± 0.04^{i}	14.12 ± 0.08^{f}	218 ± 0.39^{h}
T5	240 ± 1.94^{h}	21.3 ± 0.10^{f}	13.82 ± 0.05^{hi}	231 ± 0.79^{g}
T6	260 ± 1.80^{f}	20.3 ± 0.04^{gh}	13.66 ± 0.08^{jk}	218 ± 2.23^{h}
T7	317 ± 1.83^{b}	20.4 ± 0.09^{g}	13.39 ± 0.06^{lm}	215 ± 1.32^{hi}
T8	217 ± 1.40^{i}	23.2 ± 0.22^{bc}	13.71 ± 0.05^{ij}	249 ± 2.98^{d}
T9	178 ± 2.65^{l}	22.8 ± 0.15^{cd}	13.73 ± 0.05^{ij}	247 ± 0.20^{d}
T10	205 ± 1.82^{j}	21.8 ± 0.07^{e}	14.08 ± 0.06^{fg}	242 ± 2.00^{f}
T11	253 ± 2.90^{g}	19.1 ± 0.07^{jk}	13.78 ± 0.05^{ij}	207 ± 0.43^{jk}
T12	268 ± 0.59^{e}	21.4 ± 0.09^{ef}	14.42 ± 0.03^{e}	242 ± 1.32^{f}
T13	307 ± 0.71^{c}	19.6 ± 0.08^{ij}	14.06 ± 0.08^{fg}	216 ± 0.81^{h}
T14	270 ± 1.73^{e}	20.0 ± 0.12^{ghi}	13.31 ± 0.06^{m}	209 ± 1.74^{j}
T15	333 ± 4.00^{a}	18.8 ± 0.29^{k}	13.96 ± 0.05^{gh}	206 ± 1.52^{jk}
T16	307 ± 0.71^{c}	18.8 ± 0.20^{k}	13.68 ± 0.07^{ijk}	202 ± 1.98^{k}
T17	241 ± 1.65^{h}	19.6 ± 0.35^{ij}	13.14 ± 0.01^{n}	202 ± 0.96^{k}
T18	200 ± 3.48^{j}	23.0 ± 0.41^{bcd}	15.31 ± 0.04^{b}	277 ± 2.35^{b}
T19	250 ± 2.38^{g}	21.6 ± 0.19^{ef}	14.14 ± 0.02^{f}	244 ± 1.54^{ef}
T20	202 ± 3.27^{j}	21.5 ± 0.15^{ef}	14.83 ± 0.02^{c}	251 ± 2.54^{d}
T21	206 ± 1.91^{j}	23.3 ± 0.13^{b}	15.20 ± 0.03^{b}	278 ± 2.04^{b}
T22	189 ± 1.44^{k}	25.2 ± 0.24^{a}	16.08 ± 0.03^{a}	318 ± 2.29^{a}
p	$< 0.01^{**}$	$< 0.01^{**}$	$< 0.01^{**}$	$< 0.01^{**}$

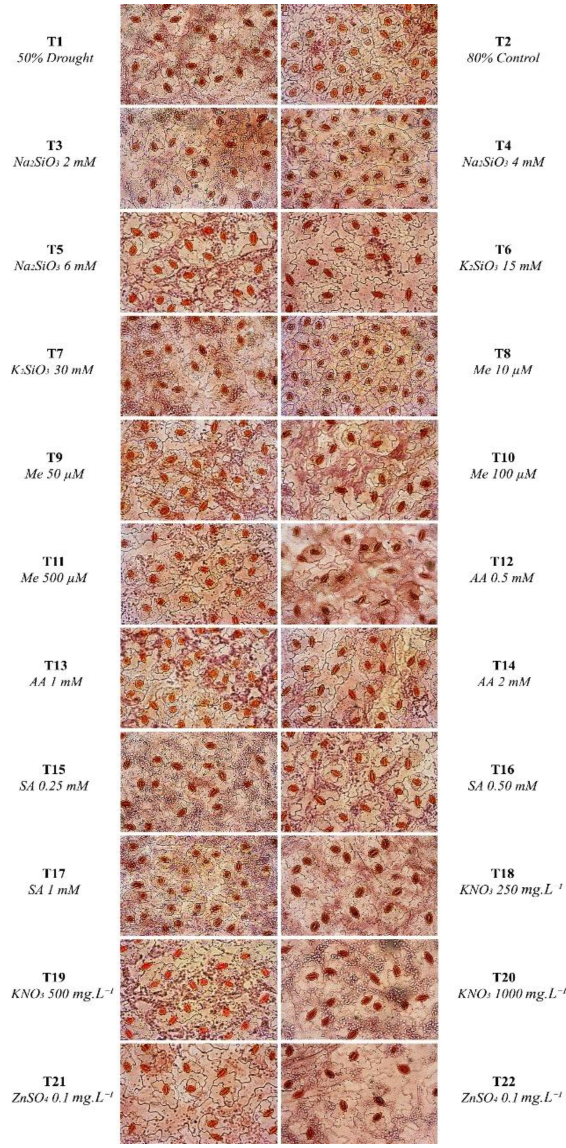

Figure 1. Stomata pictures of foliar-treated canola plants with different chemicals under drought stress. T1: 50% water holding capacity (WHC); T2: 80% WHC; T3: 50% WHC + 2 mM Na_2SiO_3; T4: 50% WHC + 4 mM Na_2SiO_3; T5: 50% WHC + 6 mM Na_2SiO_3; T6: 50% WHC + 15 mM K_2SiO_3; T7: 50% WHC + 30 mM K_2SiO_3; T8: 50% WHC + 10 µM melatonin; T9: 50% WHC + 50 µM melatonin; T10: 50% WHC + 100 µM melatonin; T11: 50% WHC + 500 µM melatonin; T12: 50% WHC + 0.5 mM ascorbic acid; T13: 50% WHC + 1 mM ascorbic acid; T14: 50% WHC + 2 mM ascorbic acid; T15: 50% WHC + 0.25 mM salicylic acid; T16: 50% WHC + 0.50 mM salicylic acid; T17: 50% WHC + 1.00 mM salicylic acid; T18: 50% WHC + KNO_3 250 mg L^{-1}; T19: 50% WHC + KNO_3 500 mg L^{-1}; T20: 50% WHC + KNO_3 1000 mg L^{-1}; T21: 50% WHC + $ZnSO_4$ 0.1 mg L^{-1}; T22: 50% WHC + $ZnSO_4$ 0.2 mg L^{-1}.

Principal component analysis (PCA)

Polygenic variation is measured by determining the relative contribution of each trait to overall divergence. The variability of all traits can be complex and difficult to interpret. The PCA simplifies this process by extracting critical traits, thereby facilitating a better understanding of and ability to implement cluster analysis (Kaya et al., 2019). Furthermore, PCA measures the importance and contribution of each component to the total variance. This approach enables an independent assessment of the impact of particular traits, with the number of extracted components corresponding to the number of analyzed variables.

In this study, the PCA grouped the 11 drought tolerance traits, except for leaf number, into 11 principal components (PCs), explaining the total (100%) variability among the treatments (Table 5). The present analysis exhibited 3 principal components with eigenvalues greater than 1.0, which collectively account for 77.2% of the total variability among the treatments. The highest eigenvalue (3.57) was obtained from PC1, which explains 32.5% of the variability (Table 5 and Figure 2). It was followed by PC2 with an eigenvalue of 3.05, PC3 with an eigenvalue of 1.86, and PC4 (0.94), respectively.

Table 5. Eigenvalues, proportion of variance, and cumulative effect of ten principal components. **Significant at 1%.

Components	Eigenvalue	Percent	Cumulative	Chi square	Probability
PC1	3.57	32.5	32.5	248.5	< 0.0001**
PC2	3.05	27.8	60.3	210.4	< 0.0001**
PC3	1.86	16.9	77.2	163.2	< 0.0001**
PC4	0.94	8.6	85.8	123.0	< 0.0001**
PC5	0.66	6.0	91.8	99.0	< 0.0001**
PC6	0.43	4.0	95.8	76.3	< 0.0001**
PC7	0.30	2.8	98.6	54.9	< 0.0001**
PC8	0.06	0.6	99.2	24.2	< 0.0098**
PC9	0.05	0.5	99.7	20.0	< 0.0027**
PC10	0.03	0.3	99.9	13.9	< 0.0018**
PC11	0.003	0.03	100.0	-	-

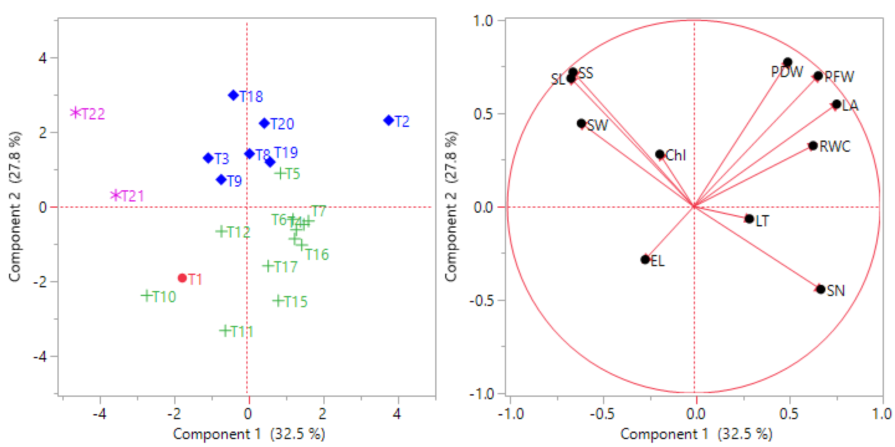

Figure 2. Determination of suitable treatment(s) and characteristics for drought tolerance in canola using Principal Component and Hierarchical Cluster analysis, with different colors indicating cluster numbers: 1 red, 2 green, 3 blue, and 4 purple. PFW: Plant fresh weight; PDW: plant dry weight; LA: leaf area; Chl: chlorophyll content; LT: leaf temperature; RWC: relative water content; EL: electrolyte leakage; SS: stomata size; SN: stomata number; SL: stomata length; SW: stomata width; T1: 50% water holding capacity (WHC); T2: 80% WHC; T3: 50% WHC + 2 mM Na_2SiO_3; T4: 50% WHC + 4 mM Na_2SiO_3; T5: 50% WHC + 6 mM Na_2SiO_3; T6: 50% WHC + 15 mM K_2SiO_3; T7: 50% WHC + 30 mM K_2SiO_3; T8: 50% WHC + 10 µM melatonin; T9: 50% WHC + 50 µM melatonin; T10: 50% WHC + 100 µM melatonin; T11: 50% WHC + 500 µM melatonin; T12: 50% WHC + 0.5 mM ascorbic acid; T13: 50% WHC + 1 mM ascorbic acid; T14: 50% WHC + 2 mM ascorbic acid; T15: 50% WHC + 0.25 mM salicylic acid; T16: 50% WHC + 0.50 mM salicylic acid; T17: 50% WHC + 1.00 mM salicylic acid; T18: 50% WHC + KNO_3 250 mg L^{-1}; T19: 50% WHC + KNO_3 500 mg L^{-1}; T20: 50% WHC + KNO_3 1000 mg L^{-1}; T21: 50% WHC + $ZnSO_4$ 0.1 mg L^{-1}; T22: 50% WHC + $ZnSO_4$ 0.2 mg L^{-1}.

Principal component and hierarchical cluster analysis were performed using the mean values of the parameters of canola plants under drought conditions. It was revealed that four main groups were constituted (Figure 3). Drought-stressed plants (T1) were placed on the negative sides of PC1 and PC2. The effective treatments were categorized as follows: T18, T19, and T20 (250, 500, and 1000 mg L^{-1} KNO_3), T8 and T9 (melatonin at 10 µM and 50 µM), and T5 (6 mM Na_2SiO_3), which were found on the positive side of both PC1 and PC2. This finding confirms the superiority of the KNO_3 treatment in alleviating drought stress in canola. Low dosages of melatonin and a high level of Na_2SiO_3 were also beneficial in increasing drought tolerance, according to PCA. Among the parameters investigated, stomata properties were found to be the effective in enhancing drought tolerance in canola, along with chlorophyll content and plant fresh and dry weight. Electrolyte leakage was also a significant trait for drought tolerance, but it showed a negative correlation.

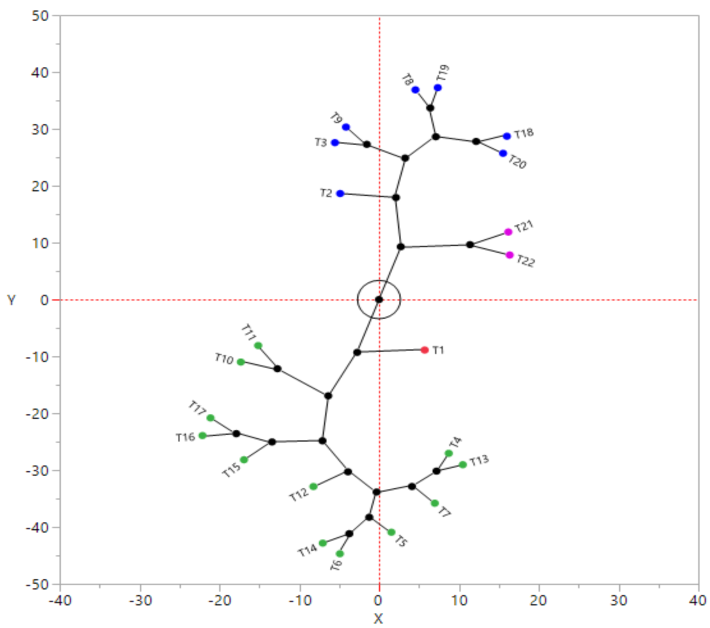

Figure 3. Constellation plot of canola plants grown under drought stress with 22 treatments. T1: 50% water holding capacity (WHC); T2: 80% WHC; T3: 50% WHC + 2 mM Na_2SiO_3; T4: 50% WHC + 4 mM Na_2SiO_3; T5: 50% WHC + 6 mM Na_2SiO_3; T6: 50% WHC + 15 mM K_2SiO_3; T7: 50% WHC + 30 mM K_2SiO_3; T8: 50% WHC + 10 µM melatonin; T9: 50% WHC + 50 µM melatonin; T10: 50% WHC + 100 µM melatonin; T11: 50% WHC + 500 µM melatonin; T12: 50% WHC + 0.5 mM ascorbic acid; T13: 50% WHC + 1 mM ascorbic acid; T14: 50% WHC + 2 mM ascorbic acid; T15: 50% WHC + 0.25 mM salicylic acid; T16: 50% WHC + 0.50 mM salicylic acid; T17: 50% WHC + 1.00 mM salicylic acid; T18: 50% WHC + KNO_3 250 mg L^{-1}; T19: 50% WHC + KNO_3 500 mg L^{-1}; T20: 50% WHC + KNO_3 1000 mg L^{-1}; T21: 50% WHC + $ZnSO_4$ 0.1 mg L^{-1}; T22: 50% WHC + $ZnSO_4$ 0.2 mg L^{-1}.

Hierarchical cluster analysis

Hierarchical cluster and PCA of 22 treatments based on morphological, physiological, and stomata parameters revealed four distinct clusters. The analysis produced a constellation plot of cluster analysis that framed the four clusters (Figure 3). In cluster analysis, a constellation plot is a graphical representation that helps visualize the clustering structure and relationships among the clusters. As seen in Figure 3, cluster 1 (blue) and cluster 4 (purple) are on one side, while cluster 2 (green) and cluster 3 (red) are on the other side. Well-watered plants (T2) were placed in the same group as T3, T8, T9, T18, T19, and T20, while T1 (drought stress) was placed in a separate cluster (Figure 3).

DISCUSSION

Drought stress limits the growth and development of plants at all stages of their life cycles by creating an imbalance in the amount of water in their tissues compared to those of well-watered plants. This study tested various drought-mitigating agents to identify effective treatments for canola plants. Treatments including 250 and 500 mg L^{-1} potassium nitrate (KNO_3) significantly enhanced leaf numbers, reaching levels comparable to or exceeding those of non-stressed plants. This result confirms the findings of Seleiman et al. (2021), who reported increased vegetative growth and leaf proliferation with K supplementation, even in water-limited conditions. Foliar application of 10 µM melatonin also increased leaf number, suggesting a protective effect on meristematic activity and leaf expansion, consistent with observations on chlorophyll preservation and leaf morphology by El-Yazied et al. (2022). Leaf fresh weight, a direct indicator of plant water status and turgor, decreased significantly under drought. However, KNO_3 (especially at 1000 mg L^{-1}) significantly improved this parameter, reflecting better osmotic adjustment and water uptake. Under similar conditions, Gimeno et al. (2014) demonstrated an improvement in biomass with KNO_3, confirming these improvements. Sodium silicate (Na_2SiO_3) at 2 mM and melatonin at 10 µM also increased leaf fresh weight compared to the stressed control, indicating improved hydration and reduced water loss, attributes previously associated with increased aquaporin activity and cell wall elasticity (Seham et al., 2021; Cao et al., 2022). Under drought conditions, DM accumulation was reduced in untreated plants. Applications of KNO_3 resulted in the highest dry biomass, suggesting a greater ability to sustain C assimilation and structural growth. This finding is consistent with a study by Kaur (2011) that emphasized the role of KNO_3 in promoting the allocation of photosynthates to structural tissues under water-deficit conditions. Other treatments, such as potassium silicate (K_2SiO_3) (30 mM), also provided improvements, although to a lesser extent. Leaf area decreased significantly in response to water deficit, limiting photosynthetic area. Similar results were reported by Kazemi Oskuei et al. (2023), who observed a reduction in leaf area as drought severity increased. The KNO_3 at 250 mg L^{-1} significantly enlarged leaf area compared to the drought-stressed control, indicating maintenance of cell expansion. This observation is supported by Moradi et al. (2024), who demonstrated that K and Si treatments can preserve cell expansion and maintain leaf structure under high evaporative demand. Sodium silicate (6 mM) and melatonin (10 µM) also increased leaf area, demonstrating their role in maintaining cell turgidity and integrity.

Chlorophyll content generally decreases under drought stress conditions. However, SPAD readings indicated higher levels of chlorophyll in drought-stressed canola plants than in control plants (Umer et al., 2026). This is mainly due to the reduced water content in leaf tissues. Furthermore, this difference may stem from two main reasons. First, drought reduced the water content of plant tissues, leading to an increased chlorophyll rate. Second, older leaves turn yellow first, followed by younger leaves. In our study, we measured the chlorophyll rate of the second leaf from the top of plants that had not yet turned yellow. It is well known that drought first affects the lowest leaves and then moves to the upper leaves. However, Kazemi Oskuei et al. (2023) found an increase in total chlorophyll content of canola cultivars when drought severity increased. In this study, the treatment of zinc sulfate ($ZnSO_4$) at 0.2 mg L^{-1} produced the highest SPAD values under drought stress, probably through improved N assimilation and chloroplast protection, as reported by El-Yazied et al. (2022) in potato and Ullah et al. (2025) in corn. Sodium silicate also supported the maintenance of chlorophyll content, indicating reduced oxidative stress and stabilized chloroplast structure. Increased leaf temperature was recorded in canola plants exposed to drought stress due to reduced transpirational cooling. Salicylic acid (0.50 and 1.00 mM) and $ZnSO_4$ (0.2 mg L^{-1}) treatments significantly reduced leaf surface temperatures, indicating improved stomatal regulation and evaporative cooling capacity. Relative water content (RWC) decreased significantly under water-limited conditions. Potassium nitrate (250 and 1000 mg L^{-1}) effectively restored RWC to levels approaching those of the control group, suggesting improved osmotic adaptation. Sodium silicate and melatonin also improved RWC values, supporting their role in cell water retention and membrane permeability, which has also been demonstrated in recent controlled studies by Lee et al. (2020), Sattar et al. (2023), and Sima et al. (2024). Drought-induced membrane damage resulted in increased electrolyte leakage (EL). Application of 500 mg L^{-1} KNO_3 resulted in the lowest EL value, reflecting strong membrane stabilization. This protective effect is supported by findings in spinach by Bukhari et al. (2021). Potassium silicate and Na_2SiO_3 treatments also reduce leakage, consistent with strengthened cell structure and reduced lipid peroxidation. The number of stomata was increased in drought-stressed plants, probably as an adaptive mechanism to maximize CO_2 uptake during brief stomatal openings. In line with a study on shallot (Indarwati et al., 2021), treatment with salicylic acid helped normalize stomata number, thereby supporting optimal stomatal behavior under stress. Drought stress caused a decrease in stomata width. However, KNO_3 and $ZnSO_4$ maintained wider stomata openings, allowing continued gas exchange and water vapor release without excessive loss (Vazin, 2012; Gimeno et al., 2014). Reductions in stomata length were observed under stress, which may compromise transpiration control. Zinc sulfate and KNO_3 maintained longer stomata, facilitating more efficient regulation of water loss (Avila et al., 2022; Mannan et al., 2022). Stomata size was significantly smaller in canola plants exposed to drought stress. Zinc sulfate applications maintained larger stomata size, enabling balanced gas exchange and an effective response to atmospheric demands (Shahsavari and Dadrasnia, 2016). Principal component analysis (PCA) showed that canola plants grown under drought stress were placed on the negative side and in a distinct group according to hierarchical cluster analysis (HCA). All doses of KNO_3 (T18, T19, T20), melatonin (T8 and T9), and 2 mM Na_2SiO_3 (T3) were administered to the positive sides of PCA1 and PCA2, showing that they were effective treatments in improving plant growth under drought conditions because they were within well-watered plants. This result approved the beneficial effects of the foliar treatments in canola grown under drought stress. Using PCA and HCA, Kaya et al. (2012) successfully classified ten flax genotypes according to their salinity tolerance levels. In a similar vein, Kaya et al. (2019) distinguished between safflower and sunflower in terms of salt tolerance. Yadav et al. (2025) successfully classified 300 rapeseed and mustard genotypes using a constellation plot in cluster analysis with respect to seed vigor traits.

CONCLUSIONS

Drought is the most critical phenomenon affecting agricultural production worldwide. It negatively impacts germination and plant growth, thereby reducing crop yield. Consequently, many recent studies have focused on mitigating the adverse effects of drought stress. In our study, we tested the efficacy of certain proven-effective foliar chemicals in canola. In particular, potassium nitrate (KNO_3) effectively promoted the growth of canola plants subjected to drought by primarily adapting stomata properties to drought conditions and maintaining tissue water balance. Along with plant fresh and dry weight, leaf area, and relative water content, stomata traits, primarily stomata number, were identified as the most important parameters for drought tolerance in canola. It is recommended that these traits be considered in future studies related to drought in the canola plant. These findings suggest that KNO_3 has the potential to improve drought tolerance in canola seedlings under controlled conditions.

Author contributions

Conceptualization: M.D.K. Data curation: M.D.K., N.E., E.G.K. Formal analysis: M.D.K., N.E., E.Y. Funding acquisition: M.D.K. Investigation: M.D.K., N.E., E.G.K., E.Y. Methodology: M.D.K. Project administration: M.D.K. Resources: M.D.K. Software: N.E., E.Y. Supervision: M.D.K. Validation: M.D.K., N.E. Visualization: N.E., E.Y. Writing-original draft: M.D.K., N.E. Writing-review & editing: M.D.K., E.G.K. All co-authors reviewed the final version and approved the manuscript before submission.

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