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Research article

Individual and interactive effects of amino acid and paracetamol on growth, physiological and biochemical aspects of *Brassica napus* L. under drought conditions

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ABSTRACT

Drought stress poses a significant threat to *Brassica napus* (L.), impacting its growth, yield, and profitability. This study investigates the effects of foliar application of individual and interactive pharmaceutical (Paracetamol; 0 and 250 mg L^{-1}) and amino acid (0 and 4 ml/L) on the growth, physiology, and yield of *B. napus* under drought stress. Seedlings were subjected to varying levels of drought stress (100% field capacity (FC; control) and 50% FC). Sole amino acid application significantly improved chlorophyll content, proline content, and relative water contents, as well as the activities of antioxidative enzymes (such as superoxide dismutase and catalase) while potentially decreased malondialdehyde and hydrogen peroxide contents under drought stress conditions. Pearson correlation analysis revealed strong positive correlations between these parameters and seed yield (R^2 = 0.8–1), indicating their potential to enhance seed yield. On the contrary, sole application of paracetamol exhibited toxic effects on seedling growth and physiological aspects of *B. napus*. Furthermore, the combined application of paracetamol and amino acids disrupted physio-biochemical functions, leading to reduced yield. Overall, sole application of amino acids proves to be more effective in ameliorating the negative effects of drought on *B. napus*.

1. Introduction

Drought is a common abiotic stress that leads to decreased plant growth and reduced seed yield in agricultural crops [1]. Global climate change is increasing the severity and frequency of drought stress [2]. The soil moisture deficit disrupts the root system of plants, limiting water uptake. This physiological dysfunction of plants results in stomatal closure and decreased cell growth, leading to

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severe yield losses and sometimes complete crop failure [3,4]. In response to long-term drought, plants accumulate compatible such as proline [1], which acts as a stress defender and plays a crucial role in osmoregulation, maintaining cell water balance, and reinforcing protein structures [5]. Similarly, the ability of plants to maintain cell water status is crucial for withstanding drought stress, especially in turgor-driven activities such as turgor pressure, cell growth, and cell expansion [4]. The stability of cell membranes also serves as an indicator of the plant's ability to withstand drought stress. Changes in the integrity of the cell membrane can lead to the outflow of cellular ions and solutes, resulting in cellular dysfunction [6]. Under drought stress conditions, reactive oxygen species (ROS) are produced, which are responsible for oxidative stress in plants [7]. The excessive synthesis and accumulation of ROS may damage cellular membranes, inactivate enzymes, degrade proteins, and create ionic imbalance [8]. To prevent the overproduction of ROS, plants have a highly effective and complex antioxidant defense system [9,10]. These antioxidant defense systems are crucial for mitigating ROS-based damage to cell organelles [11].

Brassica napus (L.) is an important oilseed crop globally, contributing significantly to the world's food security [7]. Renowned for its nutrient-rich oil, it is widely utilized in cooking and various industrial applications within modern agriculture [12]. Additionally, its protein-rich meal serves as valuable livestock feed and holds promise as a potential biofuel, supporting sustainable energy goals and bolstering the livestock industry [13,14]. With its adaptability to climate change and high economic value [15], *B. napus* plays a vital role in crop rotation, pest and disease management, and soil fertility enhancement [16]. Its resilience to environmental stresses, such as drought stress, positions it as a key resource for securing food security and energy supplies amidst changing climatic conditions. Therefore, the adoption of modern techniques to mitigate the adverse effects of abiotic stress, especially drought, is imperative to enhance *B. napus* yield under rain-fed conditions [17,18].

Amino acids play a crucial role in plant metabolism, growth, development, stress tolerance, and the regulation of metabolic pathways and physiological processes. They serve as intermediates in metabolite pathways and protein biosynthesis [19,20]. Acting as osmoprotectants and signal molecules, amino acids are essential for maintaining plant cell integrity under drought stress by regulating cellular turgor pressure [21,22]. Amino acids such as proline, betaine, thiamine, and trehalose are essential for osmoregulation, water balance, and stabilizing protein structures [23,24]. Proline, in particular, is renowned for its crucial role in plant stress defense and drought tolerance, as well as scavenging ROS [22].

Pharmaceutical compounds are synthesized for their potential roles in curing diseases and maintaining health. Paracetamol, also known as acetaminophen, is a widely used analgesic and antipyretic drug. It is considered safe at therapeutic doses and is available over-the-counter in most countries [25]. Paracetamol's antioxidant properties can mitigate the harmful effects of ROS during drought stress, preserving cell integrity and photosynthetic activity of plants. The multifaceted positive influences of paracetamol on plants make it an unconventional tool for enhancing crop resilience to abiotic stresses [26].

The single and interactive effects of amino acids and paracetamol, particularly in terms of promoting proline accumulation and reinforcing the antioxidant defense system, can improve drought resilience of *B. napus* [27]. The present study explores a novel method to enhance drought resilience in *B. napus* through foliar application of amino acids and paracetamol. We hypothesized that the application of amino acids and paracetamol would improve drought resilience in *B. napus* by boosting osmotic regulation, stabilizing cell water content, enhancing membrane integrity, and mitigating oxidative stress, thereby enhancing crop productivity (Fig. 1).

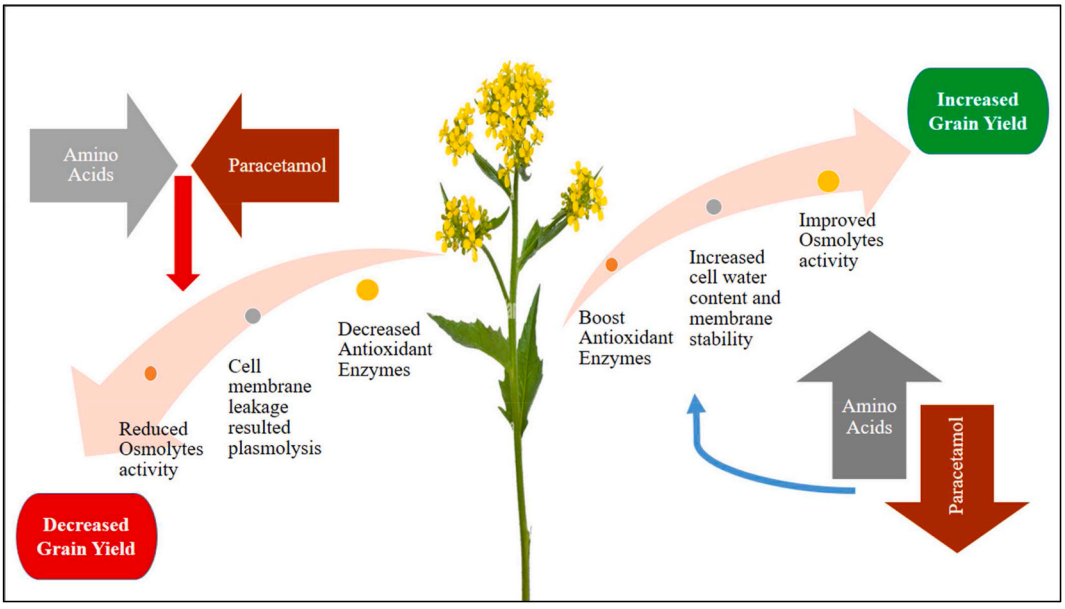

Fig. 1. Graphical visualization of sole and interactive effect of amino acid and paracetamol on Brassica napus (L.) performance under drought conditions.

2. Materials and methods

The study was conducted during the canola-growing season of 2022–23 at the Department of Agronomy, PMAS, Arid Agriculture University Rawalpindi, Pakistan (33.6492°N, 73.0815°E). Isabion, an amino acid-based product, was obtained from Syngenta Pvt. The paracetamol-based product, Panadol, was obtained from the GSK group of companies. The canola variety cv. NARC Sarsoon, commonly grown throughout the country, was obtained from the National Agricultural Research Centre (NARC) Islamabad. Sodium hypochlorite solution (0.1 %) was used to disinfect the seeds for 5 min, followed by thorough washing with distilled water. The pots were filled with 7.5 kg of fine sandy-loam soil. The initial soil profile is given in (Table 1). At the time of sowing, N, P and K were applied at 300, 300 and 200 mg pot^{-1}. The emergence data was recorded by counting daily emerged plants. Fifteen seeds were sown in each pot. Later, five healthy uniform plants were kept for further experimentation. For drought application, seedlings were subjected to two different FC levels (viz. 100 % (control) and 50 %). The foliar application of Isabion (0 and 4 mL/L) and paracetamol (0 and 250 mg L^{-1}) were carried out three and six weeks after germination. The detailed treatment arrangement is presented in Table 2. The experiment was laid out following a Completely Randomized Design (CRD) with three three-factor factorial arrangement. The doses of Isabion and paracetamol were selected according to product recommendations provided by the respective companies. Pots were covered with manually movable, flexible, and transparent polythene sheets to prevent precipitation. Different growth, physiological, and biochemical analyses were carried out as follows.

2.1. Sample collection for biochemical analysis

Leaf samples were collected after two weeks of the treatments' application. The collected samples were immediately frozen in liquid nitrogen for biochemical analysis.

2.2. Total free proline

The total free proline was analyzed following the standard method [28]. Firstly, 500 mg fresh leaf samples were meshed with 4 mL of 3 % sulphosalicylic acid, and centrifuged at 1000 rpm for 15 min. Next, 2 mL of supernatant was collected in 5 mL pipette, which was then mixed with 2 mL of glacial acetic acid and 2 mL of acid ninhydrin. The resultant solutions were then heated at 100 °C for 1 h in a water bath. After cooling to room temperature, the mixture was stirred for 1 min using a vortex stirrer, with addition of 5 mL of toluene. The upper layer containing toluene was separated and transferred to a new tube. The absorbance of the toluene layer and toluene as a blank was measured using a spectrophotometer (wavelength: 350–1200 nm; D2 lamp: 1500 h, Fencia) at 520 nm. Proline concentration was estimated using a standard curve.

2.3. Lipid peroxidation/malondialdehyde

About 500 mg of fresh leaf was homogenized with 1.5 mL of 5 % trichloroacetic acid (w/v). The mixture was then centrifuged at 1500 rpm for 15–20 min. Later, 2 mL of filtrate was mixed with 2 mL of 0.5 % thiobarbituric acid (TBA) (w/v). The mixture was incubated in a water bath at 100 °C for 30 min and centrifuged again at 1500 rpm for 10 min. The absorbance was measured at 450 nm, 532 nm and 600 nm using a spectrophotometer [29].

2.4. Hydrogen peroxide accumulation

Hydrogen peroxide (H_2O_2) was determined using trichloroacetic acid (TCA) and potassium iodide (KI) based method of [20]. Fresh leaf sample (0.1 g) was homogenized in 1 mL of 0.1 % TCA solution and centrifuged at 12,000 rpm for 15 min. 0.5 mL of supernatant was obtained and mixed with 1 mL of 1 M KI and 0.5 mL of 2.5 mM potassium buffer. The solution was then kept in the dark for 60 min. Afterward, the mixture was vortexed for 1–2 min, and absorbance was obtained at 390 nm using a spectrophotometer. A standard curve was used for estimating the concentration of H_2O_2 [30].

2.5. Enzymatic extraction

Fresh leaf tissues (0.5 g) were homogenized in 10 mL of 50 mM phosphate butter, with a pH range of 6.8–7, and mixed with 1 mM

Table 1
Initial soil profile before experimentation.

Soil parameters	Unit	Value
pH	–	8.1 ± 0.4
Ec	μS cm^{-1}	11.8 ± 1.7
Bulk density	Mg m^{-3}	1.33 ± 0.03
Porosity	%	46.7 ± 1.5
Mineral nitrogen	mg kg^{-1}	24.1 ± 0.19
Available phosphorus	mg kg^{-1}	8.7 ± 0.01
Available potassium	mg kg^{-1}	12.6 ± 0.24

Table 2
Various morphological and yield related aspects of *Brassica napus* L under amino acid and paracetamol treatments under normal and drought stress conditions.

Field capacity	Amino acids	Paracetamol	Shoot length (cm)	Root length (cm)	Dry weight (g)	No. of siliques per plant	No. of seeds per siliques
100	0	0	89.3 ± 0.78b	14.7 ± 0.30a	6.6 ± 0.33 ab	35.3 ± 0.09b	11.6 ± 0.30b
	250	0	98.2 ± 0.68a	16.1 ± 0.52 ab	7.6 ± 0.33a	47.8 ± 0.13a	13.8 ± 0.44a
	0	4	86.8 ± 0.55cd	13.5 ± 0.28bc	6.3 ± 0.33abc	34.6 ± 0.08bc	9.5 ± 0.23bc
	250	4	86.4 ± 0.63bc	14.1 ± 0.29b	6.4 ± 0.33abc	38.9 ± 0.18cd	10.2 ± 0.60bcd
50	0	0	77.5 ± 0.51e	9.6 ± 0.20d	5.0 ± 0.57bc	23.4 ± 0.07de	7.9 ± 036de
	250	0	83.1 ± 0.58d	12.2 ± 0.25c	5.6 ± 0.88abc	29.5 ± 0.20cd	8.1 ± 0.60cd
	0	4	73.7 ± 0.62f	7.1 ± 0.15d	4.6 ± 0.33c	20.2 ± 0.10de	6.1 ± 0.44e
	250	4	75.2 ± 0.60ef	8.8 ± 0.67e	5.1 ± 0.57bc	21.6 ± 0.68e	6.5 ± 0.28e

FC100, 100 % field capacity; FC50, 50 % field capacity. The presented values are the average of three replications (mean ± S.E). The interactions are analyzed by using 3-way ANOVA. The pairwise comparisons are evaluated by using Tukey HSD test ($p < 0.05$). The significant difference was presented by different alphabet lettering. The control treatment showed that there is no foliar application under normal and drought stress conditions.

EDTA and 1 % PVP. This mixture was then centrifuged at 12,000 rpm for 15 min. The filtrated solution was subsequently used for SOD and CAT estimation.

2.5.1. Superoxide dismutase

For SOD determination, 0.02 mL of extract solution was mixed with 50 mM NBT, 50 mM phosphate buffer, 1 mM riboflavin, 14 mM methionine, 0.03 % Triton, 100 mM EDTA and 0.1 M tris-HCl. The illumination process was conducted under fluorescent light for 7 min. Subsequently, lights were turned off to stop the reaction, and immediate riboflavin was added. Absorbance was measured at 560 nm using a spectrophotometer [31].

2.5.2. Catalase

The degradation of H_2O_2, resulting in the reduction of absorbance at 240 nm, is utilized to analyze CAT activity. A 3 mL reaction mixture was prepared by adding 0.1 mL of collected enzymatic extract, 50 mM potassium phosphate butter with a pH of 7.8, and 59 mM H_2O_2. The reaction was halted by measuring the absorbance at 240 nm using a spectrophotometer [31].

2.6. Chlorophyll content

The acetone–based method was used for chlorophyll estimation. A fresh leaf sample (20 mg) was homogenized in 80 % acetone solution (v/v). Then, the mixture was centrifuged at 10000 rpm for 20 min, and the supernatant was collected. Absorbance readings were taken at 470 nm, 645 nm and 663 nm using a spectrophotometer. An equation, as per a previous study [32], was applied to calculate the chlorophyll content.

2.7. Relative water content

A fresh, weighted leaf sample was immersed in distilled water for 5 h at room temperature. Subsequently, turgid weight was measured immediately, followed by the determination of dry weight [33].

2.8. Membrane thermostability index

Leaf samples were washed with distilled water to remove excess ions. Then, the samples were placed in a water bath at 100 °C for 30 min, and electrical conductivity was measured. Later, the same samples were again kept in a water bath for 10 min, and a second measurement of electrical conductivity was recorded [34].

2.9. Total free amino acids

Total free amino acids were estimated using the ninhydrin method. Leaf extract was obtained by homogenizing 0.2 g sample in sodium phosphate buffer. To 1 mL of leaf extract, 1 mL of 10 % pyridine and 1 mL of 2 % ninhydrin were added. The solution was then incubated in a water bath for half 30 min. Subsequently, absorbance was recorded at 570 nm. Total free amino acids were calculated using the formula employed in a previous study [35].

3. Statistical analysis

The data collected from the laboratory after chemical analysis were organized in MS excel and analyzed by applying a three-way factorial ANOVA using R programming software. Pairwise comparisons were conducted using the Tukey HSD test ($p < 0.05$). Pearson correlation analysis was performed using R programming software to assess the relationships between variables.

4. Results

4.1. Malondialdehyde

Drought stress, paracetamol, and amino acid application significantly influenced the MDA contents, however, the interactive influence was non-significant (Table 3). The maximum MDA contents were recorded under drought stress (50 % FC), which was 25.63 % higher than the control ($p < 0.00$; Fig. 2). However, under drought, amino acid application significantly reduced MDA content by 4.68 % ($p < 0.01$), over control. Moreover, paracetamol application under drought conditions showed no significant variation in MDA content when compared to the respective control under the same stress environment (Fig. 2). In addition, a slight reduction of 2.72 % in MDA content was observed under the combined application of amino acid and paracetamol under drought conditions compared to control plants without their application.

4.2. Hydrogen peroxide

Drought stress significantly increased H_2O_2 content by 71.22 % under drought stress, over control plants without amino acid or paracetamol application. However, a significant ($p < 0.05$) decrease of 9.11 % was noted under the sole application of amino acid than untreated plants under drought conditions. Contrary, sole paracetamol application increased H_2O_2 content by 6 % compared with untreated plants under drought conditions (Fig. 2). A negligible decline in H_2O_2 (1.33 %) was recorded under co-application of amino acid and paracetamol than untreated plants under drought stress conditions.

4.3. Superoxide dismutase activity

The results showed that SOD activity was significantly ($p < 0.001$) increased by 90.78 % in plants exposed to drought stress (50 % FC) compared with unstressed plants without amino acid and paracetamol application (Fig. 2). However, sole amino acid application increased SOD activity by 6.97 % over none treated plants under drought stress. Contrary, the sole application of paracetamol and combined application of paracetamol and amino acid decreased SOD activity by 40.76 % and 19.36 %, respectively, under drought stress (Fig. 2).

4.4. Catalase (CAT) activity

Drought stress resulted in a 21.33 % increase in CAT activity when compared with control ($p < 0.00$, Fig. 2). However, under drought stress, sole amino acid application increased CAT activity by 2.98 % over untreated stressed plants. Sole application of paracetamol and combined application of these resulted in a significant decline in SOD activity by 15.88 % and 13.84 %, respectively, then untreated plants (control) under drought stress (Fig. 2).

4.5. Proline content

For drought treatments, significantly higher proline contents were recorded under drought conditions as compared with irrigated

Table 3
Data are mean square (MS) values from ANOVA.

SOV	Amino acids	Paracetamol	Field capacity	Amino acids × Paracetamol	Amino acids × Field capacity	Paracetamol × Field capacity	Amino acids × Paracetamol × Field capacity
df	1	1	1	1	1	1	1
H2O2	*	*	***	ns	.	ns	ns
MDA	**	ns	***	*	*	ns	ns
MTSI	***	.	***	*	ns	ns	ns
RWC	**	***	***	ns	ns	ns	ns
SOD	.	***	***	ns	ns	***	ns
CAT	ns	***	***	ns	ns	***	*
Chl a	***	***	***	***	ns	ns	ns
Chl b	***	***	***	***	ns	ns	ns
TFAA	***	***	***	***	***	***	***
Shoot length	***	***	***	***	.	*	.
Root length	***	***	***	.	*	*	*
Plant dry weight	*	***	*	ns	ns	ns	ns
No of siliques per plant	***	***	***	***	***	***	***
No of seeds per silique	***	***	***	***	ns	ns	ns

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

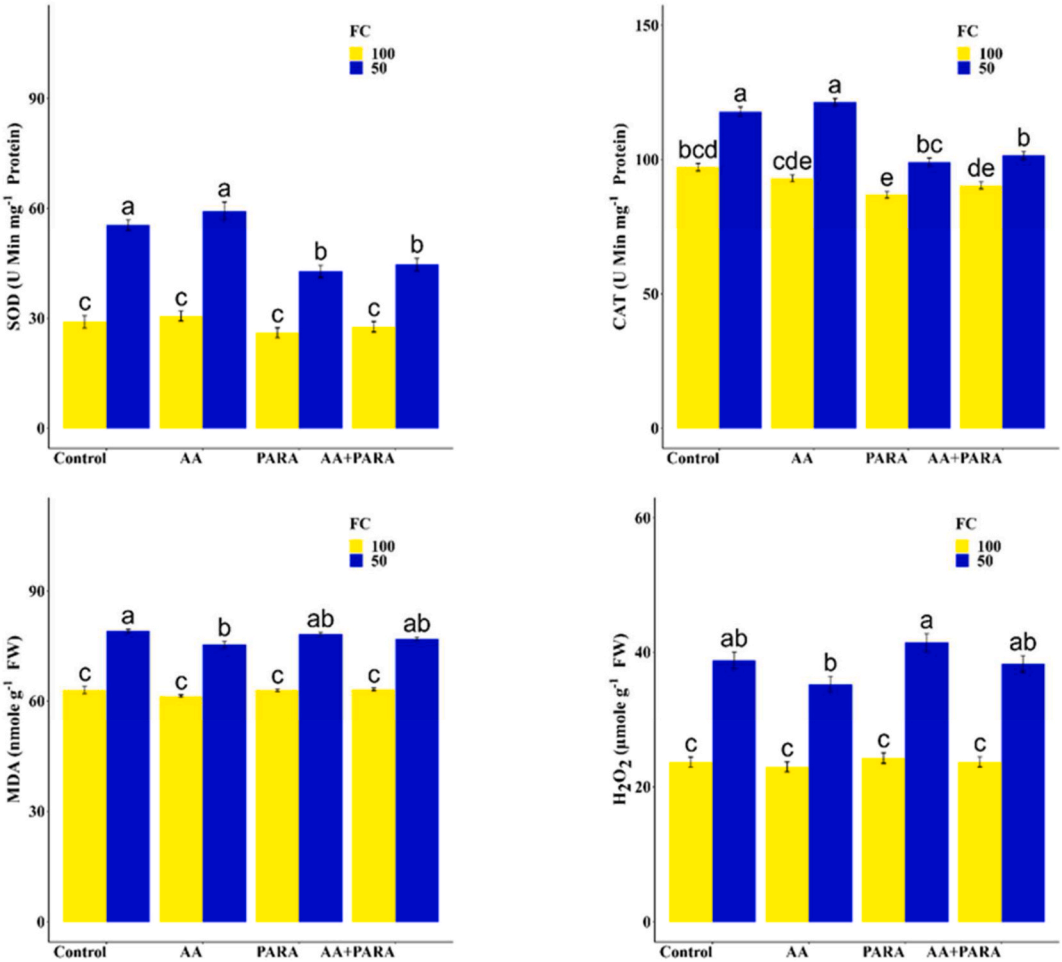

Fig. 2. Effect of foliar application of amino acid (AA) and paracetamol (PARA) on antioxidants and reactive oxygen species under normal and drought conditions. FC100, 100 % field capacity; FC50, 50 % field capacity. The error bar shows the standard errors. The values are the means of three replications. The interactions were analyzed using three-way ANOVA. The pairwise comparisons were evaluated by using Tukey HSD test ($p < 0.05$). The significant differences were made by different alphabets. The control treatment showed that no foliar application was done under normal- and drought-conditions.

plants (100 % FC). For chemical amendments, the sole application of amino acid further improved proline content by 23.87 % in drought-exposed plants, over respective control. Nonetheless, under well-watered conditions, foliar application of amino acid showed no significant effect on proline contents. The application of paracetamol as sole or combined with amino acid significantly ($p < 0.01$) decreased proline content when compared with untreated plants under drought conditions (Fig. 3).

4.6. Chlorophyll content

Drought stress depicted a significant decrease in chlorophyll (Chl) a and b contents, compared with the control treatment. However, under drought stress, the sole application of amino acid improved Chl a and b contents by 72.93 % and 14.31 %, respectively, compared with untreated plants under stress conditions. On the other hand, sole paracetamol application exhibited a negative effect on Chl a and b contents under drought stress. Interestingly, the combined application of amino acid and paracetamol increased Chl a content by 6.54 %, when compared with untreated plants subjected to drought stress (Fig. 3).

4.7. Total free amino acids

Drought stress significantly reduced amino acid contents as compared with well-watered conditions. Nonetheless, sole amino acid application significantly ($p < 0.05$) increased amino acid contents by 125.69 % under control and drought conditions, over control treatment. Moreover, sole paracetamol application reduced the amino acid accumulation. The combined application of paracetamol and amino acid also increased amino acid accumulation than untreated plants under drought stress conditions (Fig. 3).

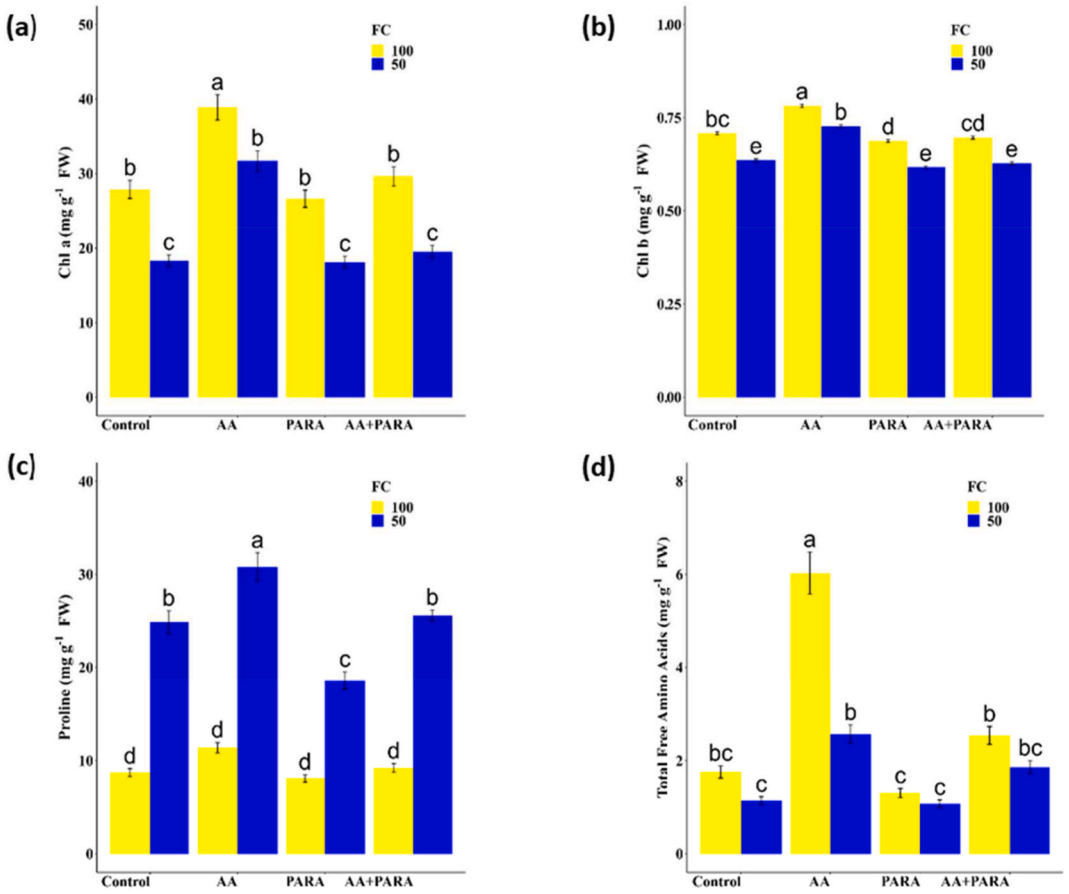

Fig. 3. Effect of foliar application of amino acid (AA) and paracetamol (PARA) on (a,b) chlorophyll contents (chl a and chl b), (c) proline and (d) total free amino acids under normal and drought conditions. FC100, 100 % field capacity; FC50, 50 % field capacity. The error bar shows the standard errors. The values are the means of three replications. The interactions were analyzed using three-way ANOVA. The pairwise comparisons were evaluated by using Tukey HSD test ($p < 0.05$). The significant differences were made by different alphabets. The control treatment showed that no foliar application was made under normal- and drought-conditions.

4.8. Membrane injury index

The results showed that plants exposed to drought stress exhibited an increase of 18.05 % in proline content as compared to well-watered conditions, under the absence of chemical amendments. However, the sole amino acids application reduced MTSI by 6 %, whereas sole paracetamol application depicted an increase of about 1.5 % when compared with control treatments (Fig. 4). As similar to sole amino acid, the combined application of these also decreased MTSI by 2 %.

4.9. Relative water content

The plants subjected to drought stress exhibited a reduction in RWC compared with well-watered conditions. Nonetheless, the sole application of amino acid improved RWC contents (7.80 %) under drought conditions, over control. Contrary, individual paracetamol application and its combined application with amino acid significantly ($p < 0.001$) decreased RWC under stress conditions (Fig. 4).

4.10. Plant morphological traits

Drought stress significantly decreased shoot and root length compared with well-watered conditions ($p < 0.00$). However, the sole amino acids application increased the shoot and root length by 7.2 % and 27.08 %, respectively, over non-treated stressed plants ($p < 0.05$). For sole paracetamol application, a significant decrease in shoot and root length over non-treated stressed plants ($p < 0.05$) was depicted. Similar results were recorded for combined paracetamol and amino acid application under stress conditions ($p < 0.05$; Table 2). Drought stress also significantly ($p < 0.01$) decreased shoot dry weight compared with control in the absence of chemical treatments (Table 2). The foliar application of paracetamol further decreased the dry weights of stressed plants. However, the amino acid application markedly ($p < 0.05$) increased shoot dry weight by 12 % over non-treated stressed plants. On the other hand, under

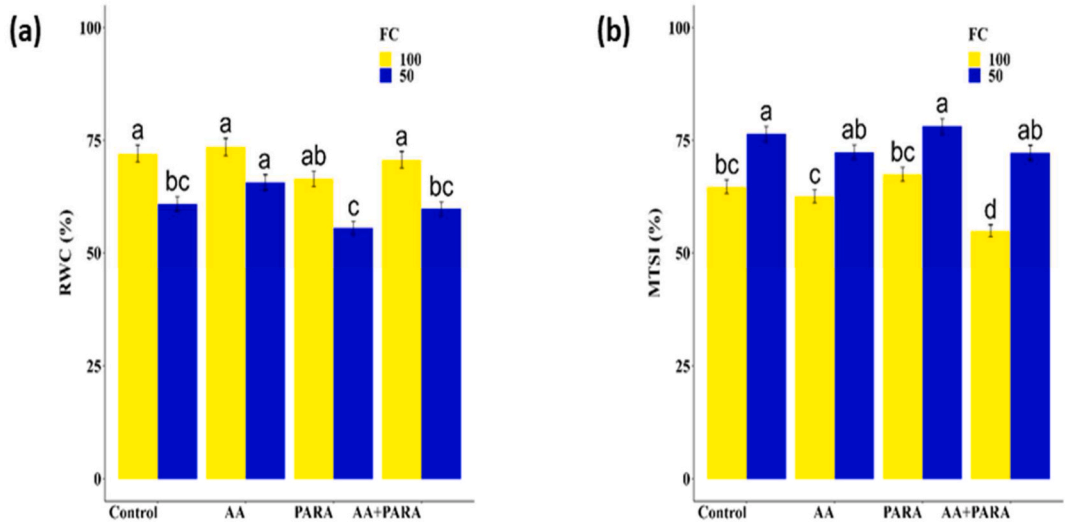

Fig. 4. Effect of foliar application of amino acid (AA) and paracetamol (PARA) on (a) relative water content (RWC) and (b) membrane thermostability index (MTSI) under normal and drought conditions. FC100, 100 % field capacity; FC50, 50 % field capacity. The error bar shows the standard errors. The values are the means of three replications. The interactions were analyzed using three-way ANOVA. The pairwise comparisons were evaluated by using Tukey HSD test ($p < 0.05$). The significant differences were made by different alphabets. The control treatment showed that no foliar application was made under normal- and drought-conditions.

stress conditions, combined paracetamol and amino acid application decreased shoot dry weight (2 %) over non-treated stressed plants (Table 2).

4.11. Yield related traits

As compared with drought stress, significantly ($p < 0.001$) higher siliques per plant and number of seeds per siliques were recorded under well-watered conditions without amino acid and paracetamol application (Table 2). However, sole amino acid application under drought stress significantly increased siliques per plant and number of seeds per siliques by 28.03 % and 12.97 %, respectively, over non-treated plants under drought stress conditions. Surprisingly, sole paracetamol application decreased both traits compared with non-treated stressed plants (Table 2). Similar findings were recorded for combined amino acid and paracetamol application.

4.12. Correlation analysis

The Pearson Correlation analysis revealed that seed yield was significantly affected by drought stress in relation to various parameters (Fig. 5). Under drought stress, seed yield showed a positive correlation with proline and RWC, SOD and CAT, Chl a, b, and total free amino acids (Fig. 5).

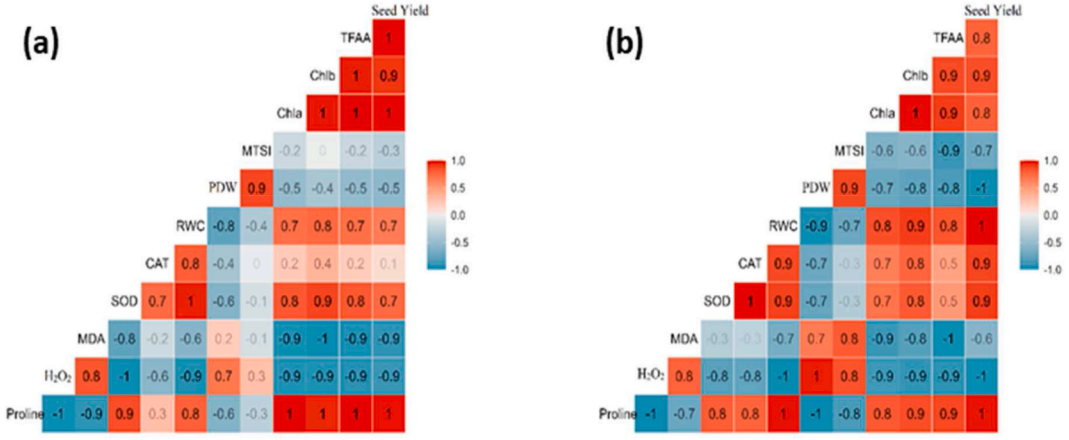

Fig. 5. The Pearson correlation for all parameters under 100 % field capacity (FC) (a) and under 50 % FC (b).

5. Discussion

Drought is the most significant abiotic factor, negatively influences crop growth, development, and overall global food security [36]. Drought also alters crop-water relations, impedes plant growth, and ultimately plant mortality. Herein, the foliar application of amino acid significantly improved crop performance under drought stress, while paracetamol exhibited toxic effects. This outcome did not support our hypothesis that amino acid and paracetamol could mitigate the adverse effects of drought by improving physiological and biochemical traits under drought conditions.

Drought stress induces oxidative stress in plants [2]. Oxidative stress arises from the overproduction of reactive oxidative species (ROS) including singlet oxygen (O_2), hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), and hydroxyl radical (OH^-) under water deficit conditions [37]. ROS causes cell damage through lipid peroxidation, DNA damage, disruption of enzyme systems, and promotion of programmed cell death [38,39]. Hydrogen peroxide, a key player in redox reactions, can induce long-distance oxidative damage and rapidly oxidize target proteins [40]. Our study demonstrated an increase in H_2O_2 levels due to drought stress. This increase could be attributed to its relationship with the SOD enzyme, which converts superoxide (O_2^-) into H_2O_2, as reported by Ahad et al. [41]. In our study, amino acids decreased the level of H_2O_2, indicating their potential in mitigating oxidative stress in *B. napus* L [42]. Notably, paracetamol further increased oxidative stress by increasing H_2O_2, and its interaction with amino acids did not show any variation.

Oxidative stress associated with drought leads to lipid peroxidation, cell membrane damage, and protein denaturation [43]. Plants reduce MDA levels to combat oxidative stress, as it is a marker for lipid peroxidation in response to stress. Generally, plants with lower amounts of MDA under drought conditions are considered more tolerant to drought [44]. Our results showed that MDA levels increased in response to drought conditions. Sundas et al. [45] documented that drought stress increases MDA content in *B. napus*. It is also well documented that amino acids, when applied to *B. napus*, decrease ROS production and lipid peroxidation, highlighting its role in enhancing drought stress tolerance [46,47]. In addition, the negative effects of MDA are decreased by the exogenous application of amino acids under stress conditions [48]. There was no variation in MDA with the application of paracetamol under drought stress. Interestingly, the combined effect of amino acids and paracetamol showed a minor reduction in MDA, which could be attributed to the drought tolerance-promoting effect of amino acids. The beneficial effects of amino acids on decreasing MDA under drought stress were also documented by Hebat-Allah et al. [49].

Proline accumulation plays a crucial role in osmotic adjustment, aiding plants in coping with moisture deficit stress by lowering cell water potential, maintaining the integrity of protein and membrane composition, and scavenging ROS [50]. Ghorban et al. [51] documented that proline level increases in drought-resistant and sensitive cultivars of *B. napus* under stress conditions. In our study, foliar-applied amino acids led to increased proline accumulation in plant cells, likely decreasing cell water potential and aiding in water uptake through roots via osmotic adjustment. Maryam et al. [52] also observed an increase in proline content with the exogenous application of amino acids. However, paracetamol decreased proline accumulation. Similar observations were noted by previous researchers in wheat seedlings under drought stress conditions [19,53].

Antioxidant biosynthesis is associated with tolerance against oxidative damage [9]. Enzymes like SOD and CAT play vital roles in minimizing oxidative stress in plants. SOD acts as the initial defense against ROS by converting the superoxide anion (O_2^-) into H_2O_2, thereby reducing their accumulation [54,55]. In our work, SOD levels were significantly increased in response to drought stress. This finding is consistent with the study by Habib et al. [43], who also reported an increase in SOD levels in oat under drought stress compared to the control. Our study showed a minor increment in SOD activity with the application of amino acids under drought stress compared to the control. A similar trend was observed by Walquiria et al. [56]. Paracetamol exhibited an inhibitory effect on SOD activity when subjected to drought stress. Similarly, the combined effects of amino acid and paracetamol also decreased SOD activity under drought conditions, possibly due to the presence of paracetamol in the treatment mixture.

Catalase, an antioxidant enzyme, facilitates the conversion of H_2O_2 to H_2O and O_2 [57]. We observed an increase in CAT activity under drought stress. A similar trend was noted by Nosheen et al. [58], who reported a decrease in H_2O_2 levels with the increase of CAT activity under drought stress. In our study, CAT activity was increased under foliar-applied amino acids when plants were subjected to drought stress. Maryam et al. [52] also reported the potential of amino acids in increasing CAT activity in cabbage under drought stress conditions. Conversely, both sole and combined applications of paracetamol decreased CAT activity in canola under drought stress.

Chlorophyll is crucial for photosynthesis [59]. Our study revealed a significant decrease in chlorophyll contents under drought stress conditions. A similar trend was recorded by Habib et al. [43] in oat and Sundas et al. [45] in canola under drought conditions. Interestingly, we found that amino acids helped to improve chlorophyll contents under drought stress conditions. This improvement could be attributed to glutamic acid, which is a nitrogen-based amino acids needed for the biosynthesis of chlorophyll [60,61]. In another study, Naheeda et al. [62] also observed an improvement in chlorophyll content with amino acid application under drought conditions. On the other hand, paracetamol showed toxicity in chlorophyll content under drought stress. Jiri et al. [26] noted that paracetamol is responsible for reducing photosynthesis by decreasing chlorophyll contents. This toxicity could be attributed to an excess dose of paracetamol, causing a detrimental effect on chlorophyll content formation and its biosynthesis.

Relative water content estimates water availability under drought conditions relative to the maximum amount of water content that cells can retain [6]. Our findings revealed that RWC was affected by drought conditions, leading to stomatal closure and reduced transpiration. Sundas et al. [45] also observed a decrease in RWC of wheat under drought conditions. Interestingly, we observed that RWC increased with foliar-applied amino acids, which corresponds to the results of Walquiria et al. [63] who recorded higher RWC with the application of amino acid proline and glutamate under drought conditions. Contrary to amino acid, RWC was lower with application of paracetamol under drought conditions. This might be due to the decrease in proline content, which acted as an osmoregulatory and helps to reduce transpiration water loss.

The plant cell membrane, responsible for regulating solute passage, is compromised by drought stress, leading to solute leakage. The MTSI indicates the degree of damage caused by abiotic stress, with higher stability indicating greater drought tolerance. Studies by Masoumeh et al. [64] and Habib et al. [43] reported a decline in MTSI under drought conditions compared to normal irrigated plants. Amino acids helped decrease the MTSI-based cellular damage under stressful conditions. Previously, Bin et al. [65] observed an increase in membrane stability with the exogenous application of amino acid under drought conditions. These results suggest that amino acids play a vital role in stabilizing cell membranes and enhancing their integrity under drought stress. However, paracetamol application, either alone or in combination with amino acid, did not show improvement in cell membrane integrity under drought conditions, indicating that paracetamol did not contribute to improving MTSI in *B. napus*.

Amino acids, an essential component of proteins, are known to enhance morphological and yield-related traits by maintaining turgor pressure, stimulating photosynthesis, and enhancing nutrient uptake [19,20]. We observed an increase in various plant morphological and yield-related attributes under drought stress. However, paracetamol, a non-plant component, can disrupt metabolism, induce oxidative stress, and interfere with hormonal regulation, potentially reducing yield traits [66,67]. The correlation study indicated that the increment in yield attributes is clearly associated with pigments, biochemical and osmolytes activities.

6. Conclusion

This pioneer study examined the effects of amino acids and paracetamol on the physiology, antioxidant activity and yield traits of *B. napus* under drought and normal irrigated conditions. Amino acids supplementation showed positive results in terms of improved antioxidant activity and yield traits of *B. napus*. However, paracetamol application had a detrimental effect on the studied traits. The combined use of amino acids and paracetamol also showed negative effects on antioxidant and yield traits. The findings suggest that amino acid supplementation can enhance *B. napus* yield, but caution should be exercised when using paracetamol. Overall, the study underscores the potential of amino acid supplementation for enhancing *B. napus* growth and yield in rainfed conditions.

Future recommendations

This study suggests that farmers and researchers should consider amino acids supplementation as a sustainable practice for improving *B. napus* cultivation. Understanding the potential interactions between substances is crucial to avoiding unintentional adverse effects on crop growth and development. Future research should explore the molecular mechanisms governing the positive effects of amino acids on *B. napus*, explore environmentally friendly alternatives to chemical compounds like paracetamol, and investigate the long-term effects of amino acids supplementation on soil health and crop resilience to drought stress. This research opens doors for optimizing crop management strategies that enhance productivity and environmental sustainability. Further exploration into the molecular mechanisms governing the beneficial effects of amino acids on *B. napus* and the search for sustainable alternatives to chemical compounds are also essential. Future research is suggested to explore the potential role of other pharmaceutical products to mitigate the negative effects of drought stress on plants. This could involve investigating the effects of different pharmaceutical compounds on plant physiology, biochemistry, and yield traits under drought stress conditions. By expanding our understanding of these interactions, we can develop more effective and environmentally friendly strategies to enhance crop resilience and productivity in the face of changing environmental conditions.

CRediT authorship contribution statement

Habib Ali: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Imran Mahmood:** Writing – review & editing, Writing – original draft, Validation, Software, Resources, Methodology, Investigation. **Muhammad Faizan Ali:** Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Alishba Waheed:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Investigation, Formal analysis, Data curation. **Husnain Jawad:** Writing – review & editing. **Sadam Hussain:** Writing – review & editing. **Fozia Abasi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Conceptualization. **Usman Zulfiqar:** Writing – review & editing. **Manzer H. Siddiqui:** Funding acquisition. **Saud Alamri:** Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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