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Physiological responses of pakcoy (*Brassica rapa* ssp. *chinensis*) to chitosan application under short-term drought stress

Respons fisiologis pakcoy (*Brassica rapa* ssp. *chinensis*) terhadap aplikasi kitosan pada kondisi cekaman kekeringan jangka pendek

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ABSTRACT
Drought stress is a major environmental factor limiting leafy vegetable productivity in greenhouse cultivation. Chitosan is widely reported as a biostimulant that may enhance stress tolerance through physiological and biochemical regulation. This study aimed to evaluate physiological responses of pakcoy (*Brassica rapa* ssp. *chinensis*) to foliar-applied chitosan under short-term drought stress conditions. The experiment was conducted in a greenhouse using a factorial design with three chitosan concentrations (0, 1, and 2 g L^{-1}) and three drought durations (0, 2, and 4 days without irrigation prior to harvest). Growth parameters, photosynthetic pigments, proline accumulation, hydrogen peroxide (H_2O_2) content, and leaf area were measured to assess plant responses. Results showed that moderate chitosan application (1 g L^{-1}) helped maintain plant growth and physiological stability under mild drought conditions. In contrast, higher chitosan concentrations increased oxidative responses, indicated by elevated H_2O_2 and proline levels. Principal component analysis revealed distinct physiological response patterns associated with chitosan dosage and drought duration. The study provides insights into the dose-dependent role of chitosan in modulating physiological responses of leafy vegetables under short-term water deficit.

ABSTRAK
Cekaman kekeringan menjadi salah satu faktor lingkungan utama yang membatasi produktivitas sayuran daun dalam budidaya rumah kaca. Kitosan dilaporkan sebagai biostimulan yang dapat meningkatkan toleransi tanaman terhadap cekaman melalui regulasi fisiologis dan biokimia. Penelitian ini bertujuan mengevaluasi respons fisiologis pakcoy (*Brassica rapa* ssp. *chinensis*) terhadap aplikasi kitosan secara semprot daun pada kondisi cekaman kekeringan jangka pendek. Percobaan dilakukan di rumah kaca menggunakan rancangan faktorial dengan tiga konsentrasi kitosan (0, 1, dan 2 g L^{-1}) serta tiga durasi cekaman kekeringan (0, 2, dan 4 hari tanpa penyiraman sebelum panen). Parameter yang diamati meliputi pertumbuhan tanaman, pigmen fotosintetik, akumulasi prolin, kandungan hidrogen peroksida (H_2O_2), dan luas daun untuk mengevaluasi respons tanaman. Hasil penelitian menunjukkan bahwa aplikasi kitosan pada konsentrasi moderat (1 g L^{-1}) mampu mempertahankan pertumbuhan dan stabilitas fisiologis tanaman pada kondisi kekeringan ringan. Sebaliknya, konsentrasi kitosan yang tinggi meningkatkan respons oksidatif, ditunjukkan oleh peningkatan kadar H_2O_2 dan prolin. Analisis komponen utama menunjukkan pola respon fisiologis yang berbeda terkait dengan dosis kitosan dan durasi cekaman kekeringan. Penelitian ini memberikan pemahaman mengenai peran kitosan yang bergantung pada dosis dalam memodulasi respons fisiologis sayuran daun pada kondisi defisit air jangka pendek.

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INTRODUCTION

Brassica rapa ssp. chinensis, commonly known as pakcoy, is an economically important leafy vegetable belonging to the Brassicaceae family. The crop is widely consumed due to its high nutritional value and its content of health-promoting phytochemicals, including antioxidants and phenolic compounds (Jeon et al., 2018; Nasiri, 2024). Despite its benefits, pakcoy production is often affected by environmental factors such as drought stress. Drought or water deficit is a major abiotic stress that limits plant growth and agricultural productivity worldwide (Seleiman et al., 2021). Water limitation disrupts plant water relations and reduces water uptake, ultimately affecting essential physiological processes such as photosynthesis, nutrient transport, and cell expansion (Franco-Navarro et al., 2025).

Plants respond to drought through a range of physiological adjustments, including stomatal closure, osmotic regulation, and reduced growth. These responses help maintain cellular homeostasis under short-term stress, while prolonged drought may lead to metabolic disruption and severe physiological damage (Juan et al., 2021; Kaur & Asthir, 2017). Drought stress is also associated with excessive accumulation of reactive oxygen species (ROS). Elevated ROS levels may cause oxidative damage through lipid peroxidation, protein denaturation, and DNA degradation, ultimately reducing plant productivity (Jia et al., 2024). However, ROS can also function as signalling molecules that activate stress-response pathways (Zia et al., 2021). Previous studies have shown that drought stress significantly reduces growth parameters in Chinese cabbage (*Brassica rapa*), including leaf number, leaf area, and biomass accumulation (Shawon et al., 2020). In addition to growth reduction, drought can alter secondary metabolite profiles in Brassicaceae species, including changes in flavonoids, phenolic acids, and glucosinolate content (Park et al., 2021).

Chitosan is widely recognized as a plant biostimulant capable of inducing physiological responses and stimulating defence mechanisms under abiotic stress conditions such as drought. Chitosan is a natural biopolymer derived from chitin and composed of repeating units of N-acetylglucosamine (Aranaz et al., 2021). Chitosan treatment has been reported to stimulate photosynthetic activity, enhance antioxidant enzyme systems, and induce the production of metabolites involved in plant stress responses, including drought stress (Hidangmayum et al., 2019). Previous studies have demonstrated that chitosan enhances drought tolerance in several crops, including potato, rice, white clover, and grapevine, by stimulating antioxidant activity, increasing H_2O_2 levels, and promoting root system development (Sharif et al., 2018). In addition to improving stress tolerance, chitosan has also been reported to enhance plant growth. For example, increased shoot biomass has been observed in basil (*Ocimum basilicum* L.) and lemon balm (*Melissa officinalis* L.) following chitosan application, associated with increased accumulation of phytochemicals (Abobatta, 2023). The interaction between drought stress and chitosan application has also been shown to stimulate secondary metabolite production and increase proline accumulation in thyme under moderate drought conditions (Bistgani et al., 2017).

Although previous studies have investigated the effect of chitosan on *B. rapa ssp. chinensis* under heavy metal stress (Zong et al., 2017), information regarding its interaction with short-term drought stress remains limited. Most existing studies focus on long-term abiotic stress conditions and generally describe chitosan as a stress-mitigating agent. However, the physiological responses of leafy vegetables during short-term drought periods occurring shortly before harvest are still poorly understood. Evaluating these interactions within a short 2–4 day drought window that resembles fluctuations commonly occurring in greenhouse cultivation may provide new insights into the physiological responses of leafy vegetables under transient water deficit conditions.

It was hypothesized that chitosan application could mitigate the detrimental effects of drought stress by improving plant growth, enhancing pigment content, and regulating physiological markers such as H_2O_2 and proline. This study aims to examine the interaction between chitosan application and short-term drought stress in *B. rapa ssp. chinensis* and to evaluate the potential role of chitosan in improving drought tolerance in leafy vegetable crops.

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MATERIALS & METHODS

Study site

The experiment was conducted in a greenhouse and laboratory facility of the National Research and Innovation Agency (BRIN), Serpong, Indonesia.

Plant materials and growing conditions

Seeds of *B. rapa ssp. chinensis* were sown in plastic trays under controlled greenhouse conditions. The initial growing medium was a 1:1 (v/v) mixture of rice husk and manure. Two weeks after sowing, uniform seedlings were individually transplanted into polybags containing a sterilized topsoil, rice husk, and composted cattle manure at a 1:1:1 (v/v/v) ratio. The greenhouse environment was monitored throughout the experimental period. Light intensity ranged from 89 to 676 μmol m^{-2} s^{-1}. The pH of the growing substrate was consistently maintained at an average of 5.8. Plants were uniformly irrigated with tap water daily to maintain optimal moisture levels. In addition, a nutrient solution (AB mix, 50 ppm) was applied via foliar spray once a week until seven weeks after planting.

Experimental design and treatments

The experimental study used a factorial design within a randomized block design (RBD) with four replications. Each replication consisted of one individual plant grown in a single pot, which served as the experimental unit, resulting in a total of 36 experimental units (3 chitosan concentrations (0, 1, 2 g L^{-1}) × 3 drought durations (0, 2, 4 days pre-harvest) × 4 replications). All morphological and biochemical measurements were conducted on individual plants per replication to account for biological variability, and no samples were pooled during the analysis. The chitosan foliar application was initiated at 6 weeks after planting, while drought treatments were imposed at specific intervals (2 and 4 days) leading up to the harvest at 7 weeks after planting, ensuring that the final stages of chitosan application overlapped with the drought period.

Chitosan solutions were prepared by dissolving chitosan powder in 0.1% lactic acid to ensure solubility (Goñi et al., 2017). Control plants (0 g L^{-1}) were treated with an equivalent volume of the lactic acid solution without chitosan. A uniform volume of 10 mL of the respective solution was applied as a foliar spray to both adaxial and abaxial surfaces of all leaves on each plant to ensure comprehensive coverage. Chitosan was applied as a foliar spray three times per week during the 6th and 7th weeks after planting (WAP). Control plants (0-day pre-harvest) were irrigated to maintain 100% field capacity until harvest (7 WAP). Drought stress treatments were applied by withholding irrigation for 2 and 4 days before harvest time. To characterize the intensity of the water deficit, soil moisture was monitored; the soil water content reached approximately 80% of field capacity after 2 days of withholding irrigation (representing moderate stress) and declined to approximately 50% of field capacity after 4 days (representing severe stress). Field capacity was determined using the gravimetric method prior to treatment application.

Growths and environment data

Chlorophyll content index (CCI) was non-destructively measured on the third fully expanded leaf of each plant using SPAD-502Plus chlorophyll meter (Konica Minolta, Japan). Photosynthetically active radiation (PAR), or photosynthetic photon flux (PPF), was monitored daily from 8:00 AM to 1:00 PM using an Apogee MQ-200 quantum sensor (Apogee Instruments, USA), which detects solar radiation within the 400-700 nm spectral range. Soil moisture was monitored using a Takemura DM-5 soil tester (Takemura Electric Works, Japan), which was pre-calibrated against the gravimetric method to ensure the moisture levels corresponded accurately to the target percentages of field capacity. Measurement was performed daily at a depth of 10 cm to ensure uniform drought intensity across all replicates.

To ensure the stability of environmental conditions during the drought period, air temperature and relative humidity (RH) were monitored daily within the greenhouse. The average temperature was maintained at 29 ± 2 °C with a relative humidity of 58 ± 8%. The PPFD levels recorded by the Apogee MQ-200 sensor remained consistent throughout the treatment period

at 409±60 μmol m^{-2} s^{-1}, confirming that the observed physiological changes were primarily driven by soil moisture deficits rather than atmospheric fluctuations. Environmental conditions in the greenhouse during the experimental period are summarized in Table 1.

Table 1. Average environmental conditions during the greenhouse experiment.

Time	PPFD (μmol m^{-2} s^{-1})	Temperature (°C)	Relative humidity (%)
Morning	166 ± 71.77	26 ± 2	70 ± 10
Day	506 ± 102.15	31 ± 3	55 ± 8
Afternoon	557 ± 28.73	32 ± 3	50 ± 6
Mean	409 ± 60	29 ± 2	58 ± 8

Note: Values represent mean ± SD.

Plant growth parameters, including shoot length (cm), canopy width (cm), and leaf area (cm^2) were measured using a digital caliper or measuring tape. To ensure consistency between physiological and morphological data, chlorophyll content index (CCI) and plant growth parameters (shoot length, canopy width, and leaf area) were measured concurrently during the 6^{th} and 7^{th} weeks after planting. This simultaneous sampling approach was adopted to accurately correlate the photosynthetic capacity with the cumulative biomass development at the same developmental stage. After harvesting (7 WAP), each plant was carefully separated into shoots and roots to measure fresh weight (g) and, following oven-dried at 50 °C until a constant dry weight was achieved, dry weight (g).

Chlorophyll analysis

Leaf chlorophyll concentrations were analysed following Lichtenthaler and Welburn method (Lichtenthaler & Wellburn, 1983). Leaves of each plant were ground with liquid nitrogen, and 0.1 g of ground leaf tissue were soaked in 5 ml 80% acetone to extract the pigments. The samples were incubated for 1 h at 4° C and homogenized with vortex. Then, the extraction solvents were centrifuged and read (supernatant) with a spectrophotometer at 663, 645, and 470 nm as a raw data. Pigment content was calculated based on the following equation:

$$\text{Chlorophyll a } (\mu g\ g^{-1} \text{ fresh weight}) = (12.21 \times A_{663}) - (2.81 \times A_{646}) \quad (1)$$

$$\text{Chlorophyll b } (\mu g\ g^{-1} \text{ fresh weight}) = (20.13 \times A_{646}) - (5.03 \times A_{663}) \quad (2)$$

$$\text{Total Chlorophyll } (\mu g\ g^{-1} \text{ fresh weight}) = (7.18 \times A_{663}) + (17.32 \times A_{646}) \quad (3)$$

Carotenoid analysis

Carotenoid analysis based on Lichtenthaler and Wellburn method using acetone as a solvent (Bayram et al., 2015). Leaves of each plant were ground with liquid nitrogen and 0.1 g of the samples were soaked in 5 ml 80% acetone. The samples were incubated for 60 min at 4° C and were homogenized using vortex. The extraction solvents were centrifuged (5,000 rpm, 10 min, 4° C) and read (supernatant) with spectrophotometer at 663, 645, and 470 nm as a raw data. Pigment content was calculated based on the following equation:

$$\text{Carotenoids } (\mu g\ g^{-1} \text{ fresh weight}) = (1000\ A_{470} - 1.82 \text{ Chlorophyll a} - 85.02 \text{ Chlorophyll b}) / 198 \quad (4)$$

Anthocyanin analysis

Anthocyanin content was determined using the pH differential method with methanol acid as a solvent (Taghavi et al., 2022). Leaf samples of each plant were ground with liquid nitrogen, and the pigments were extracted by soaking 0.1 g of

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the samples in 5 mL 80% methanol acid. The samples were incubated for 60 min at 4° C and homogenized with vortex. The extraction solvents were then centrifuged (5,000 rpm, 10 min, 4° C) and the supernatant was measured as raw data at 530, and 657 nm using a spectrophotometer.

Proline analysis

Proline content was determined following the ninhydrin method described by Bates et al. (1973). Leaf samples from of each plant were ground in liquid nitrogen, 0.2 g of the samples that has been ground were soaked in 3 ml 70% methanol, homogenized (shaken for 5 min), and centrifuged (5,000 rpm, 10 min, 4° C). After centrifugation, 700 µL of reagent solution (1:1 of ninhydrin Acid 1% and glacial acetic acid) were added to each blank (Ethanol 70%), standards, and samples. The solvents were incubated for 1 h at 90° C using water bath and were cooling down for 5 min before spectrophotometer read. Then, the supernatant was read and measured with spectrophotometer at 520 nm.

H_2O_2 analysis

Hydrogen peroxide (H_2O_2) content was determined using a colorimetric method described by Jana and Choudhuri (1982). Each plant's leaves were ground using liquid nitrogen, then 0.2 g of the ground samples were soaked in 2.5 ml 0.1% TCA, homogenized (shaken for 5 min), then centrifuged (5,000 rpm, 10 min, 4° C). After centrifugation, 0.5 mL of reagent solution (1:2 of phosphate buffer and potassium iodide) were added to each blank (aquadest), standards, and samples. The solvents were homogenized using vortex and incubated for 1 h on a dark condition. Then, the absorbance of the supernatant was measured at 520 nm using spectrophotometer.

Data analysis

Data were subjected to a two-way analysis of variance (ANOVA) using the general linear model (GLM) to evaluate the main effects of chitosan concentration, drought duration, and their interaction. The experiment followed a 3 × 3 factorial structure within a Randomized Block Design. When the ANOVA indicated significant differences ($p < 0.05$), a post-hoc comparison was performed using Tukey's honestly significant difference (HSD) test to separate the means. All statistical analyses were conducted at a significance level of $\alpha = 0.05$ using Minitab 20. Results in tables and figures are presented as mean ± standard deviations (SD). Each of the parameters was also presented as principal component analysis (PCA) biplot graphic using Rstudio 4.5.0. PCA was performed using replicate-level data to evaluate the multivariate relationships between growth parameters and biochemical markers. In this plot, the original data were represented by principal components that explain the majority of the data variance using the loading vectors and PC scores.

RESULTS & DISCUSSION

Growth responses to chitosan and drought interaction

To investigate the role of chitosan in regulating plant growth under water deficit conditions, *B. rapa ssp. chinensis* plants were observed at the final growth stage when moderate (2 days) to severe drought (4 days) occurred before harvest. The analysis emphasized the impact of different chitosan concentrations on shoot and root biomass distribution, as well as changes in leaf area as drought duration increased. The 4-day drought treatment significantly reduced shoot biomass, root biomass, and leaf area compared with the well-watered control (Figure 1).

This indicates that water deficit directly reduces the overall biomass and water content of the shoot system of these plants. Similar to the shoots, both root fresh weight and dry weight also significantly decreased with prolonged drought (4 days). This showed that the negative impact of drought extends to the root system, which is critical for water absorption (Figure 1D and Figure 1E). Prolonged drought can significantly inhibit root growth and reduce photosynthesis leading to diminished biomass accumulation (Dao et al., 2025).

Drought stress commonly induces hormonal signalling such as abscisic acid (ABA), which regulates plant responses to water deficit (Castro-Valdecantos et al., 2023). Leaf area also showed a significant decrease under the longest drought treatment compared to control (4 days) (Figure 1C). This is a common plant response to water stress, as reducing leaf area

helps to minimize water loss through transpiration. The reduction in leaf area represents one of the adaptive strategies in plants to avoid drought stress through the suppression of evapotranspiration and water consumption (Ke et al., 2025). Water stress significantly inhibits cell expansion and growth due to reduced turgor pressure (Yang et al., 2021).

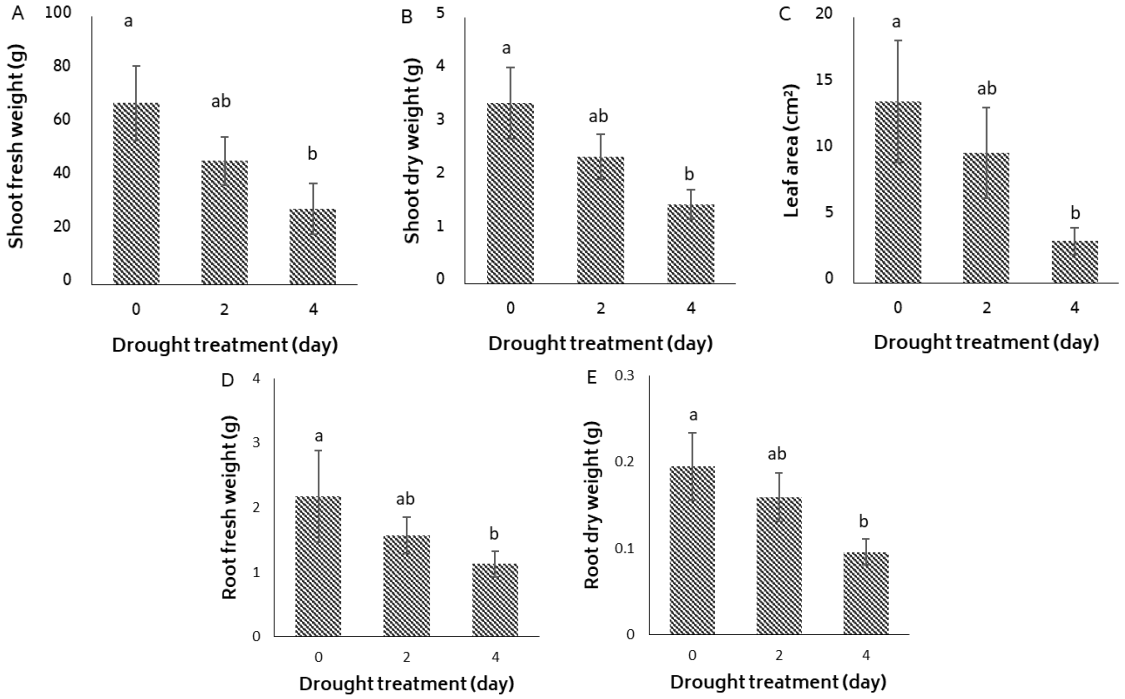

Figure 1. Effects of drought duration on vegetative growth parameters of *B. rapa* ssp. *chinensis*: (A) shoot fresh weight, (B) shoot dry weight, (C) leaf area, (D) root fresh weight, and (E) root dry weight. Different letters indicate significant differences according to Tukey's test ($p < 0.05$). Data are presented as mean ± SD (n = 4).

The interaction plot between chitosan and drought treatments showed a complex pattern (Figure 2A and Figure 2B). While drought stress generally reduced shoot fresh weight, the application of chitosan at both 1 g L^{-1} and 2 g L^{-1} concentrations appeared to mitigate this effect. Under both moderate (2 days) and severe (4 days) drought conditions, chitosan treatment at 1 g L^{-1} and 2 g L^{-1} showed that chitosan partially mitigated the reduction in shoot biomass (Figure 2A and Figure 2B). No statistically significant differences were observed between the two chitosan concentrations, as indicated by the shared statistical groupings. Therefore, both concentrations appear to contribute similarly to alleviating drought-induced biomass reduction. In contrast, the interaction between chitosan concentration and drought duration did not show a statistically significant effect on leaf area, root fresh weight, and root dry weight ($p > 0.05$); as a result, these parameters are not presented as interaction plots. This suggests that the ameliorative effect of chitosan in this study was more pronounced in the aerial biomass than in the root system or leaf expansion during the short-term stress window. Overall, the data demonstrate that chitosan application helps maintain growth under water-limited conditions, highlighting chitosan's potential as a drought-mitigating agent.

Recent studies also reported that foliar application of chitosan stimulates growth and increasing leaf numbers, root length, and plant dry weight on *Allium sativum* L. compared with untreated plants in drought conditions (Abdelaal et al., 2021) These results suggest that the effectiveness of chitosan in leafy vegetables may depend on the timing of application relative to drought exposure. Chitosan treatments increased key activities of nitrogen metabolism such as nitrate reductase, glutamine synthetase, and protease, and enhanced nitrogen transport in plant leaves which stimulated plant growth and development (Malekpoor et al., 2016). While the structural growth was visibly impaired by drought, the

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underlying physiological shifts, particularly in photosynthetic and protective pigments, provide further insight into the plant's adaptive capacity.

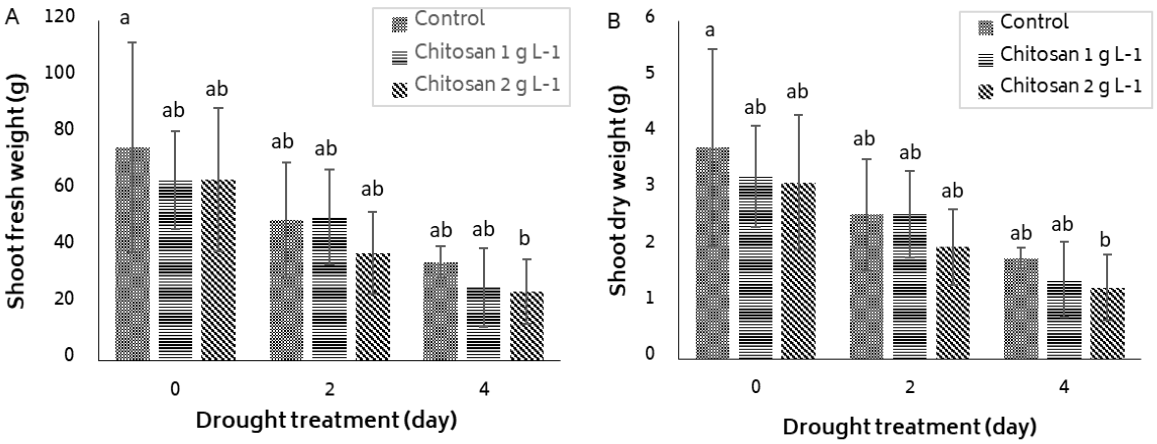

Figure 2. Effects of chitosan and drought interaction on shoot biomass of *B. rapa* ssp. *chinensis*: (A) shoot fresh weight and (B) shoot dry weight. Different letters indicate significant differences according to Tukey's test ($p < 0.05$). Data are presented as mean ± SD (n = 4).

Pigment responses to chitosan and drought interaction

To obtain a more comprehensive understanding, further analyses were conducted on the pigment content of *B. rapa ssp. chinensis*, with particular emphasis on chlorophyll and anthocyanin contents. As the concentration of chitosan increased from 0 g L^{-1} to 2 g L^{-1}, the total anthocyanin content in the plant's fresh weight showed a general decreasing trend (Figure 3A). The highest anthocyanin level was observed in the untreated control plants (0 g L^{-1}), although there was no significant difference between the control and 1 g L^{-1} treatments. The lowest anthocyanin level was recorded at 2 g L^{-1} chitosan, which was significantly different from the control. This suggests that high concentrations of chitosan may suppress anthocyanin accumulation compared to control.

These results suggest that higher chitosan concentrations may reduce anthocyanin accumulation under non-stress conditions. Chitosan treatment has been reported to induce reactive oxygen species (ROS) signalling, which can promote the synthesis of defence compounds such as anthocyanins. However, excessive ROS accumulation may also lead to oxidative damage, which may explain the reduced metabolite levels observed at higher chitosan concentrations (García-García et al., 2023). The interaction between chitosan concentration and drought duration significantly influenced anthocyanin content (Figure 3B). Plants without chitosan treatment showed the highest anthocyanin content after 4 days of drought, which was significantly higher than plants treated with 1 g L^{-1} chitosan. The lower anthocyanin levels in chitosan-treated plants under moderate drought (2 days) may indicate a potential reduction in oxidative stress compared with the untreated control (Ji et al., 2022).

Our results indicated that anthocyanin content showed no significant variation under drought alone compared to the control but responded markedly to chitosan and its interaction. Similarly, chlorophyll levels remained relatively stable under chitosan treatment alone but were significantly affected by drought. In summary, chitosan application differentially affects chlorophyll and anthocyanin contents depending on chitosan concentration and the level of drought stress. Overall, chitosan applications (1 g L^{-1} and 2 g L^{-1}) help maintain chlorophyll content under 2-day and 4-day drought stress, indicating its potential function as an elicitor that supports plant defence mechanisms.

Drought treatment influenced the chlorophyll content index (SPAD value), as shown in Figure 3C. The 2-day drought treatment exhibited the highest SPAD value ('a'), which was significantly higher than the 4-day treatment ('b'). Although a numerical decrease was observed as drought progressed to 4 days, the SPAD value under severe drought remained statistically comparable to the control (0 days), as indicated by the shared 'b' letter in their significance labels. These results suggest that short-term drought may trigger adaptive responses that temporarily maintain or enhance chlorophyll content, whereas prolonged drought leads to a gradual decline (Nimnuan et al., 2025).

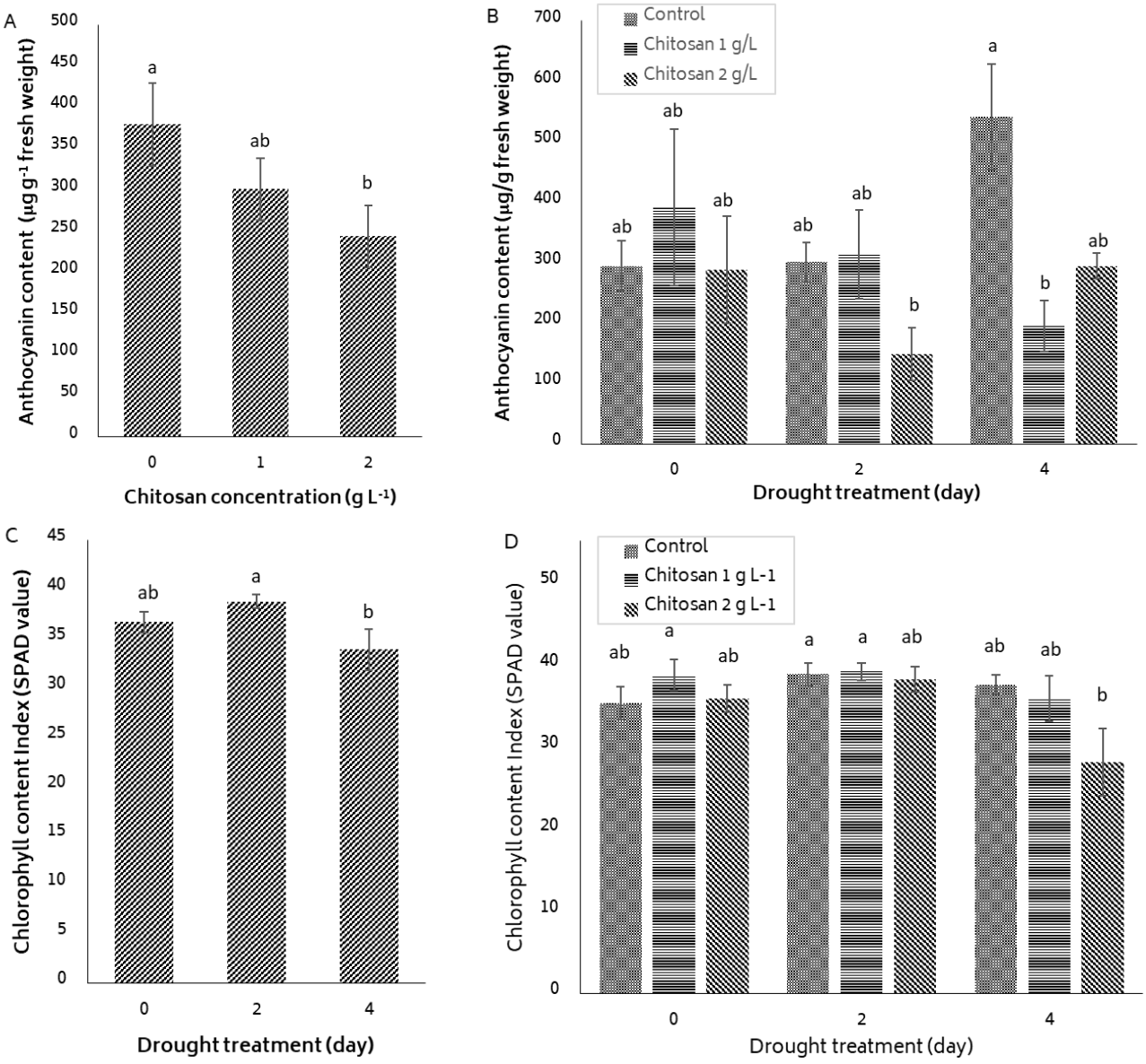

Figure 3. Effects of chitosan concentration and drought treatment on pigment-related traits of *B. rapa* ssp. *chinensis*: (A) anthocyanin content under different chitosan concentrations, (B) anthocyanin content under chitosan–drought interaction, (C) chlorophyll content index (SPAD) under drought treatments, and (D) chlorophyll content index (SPAD) under chitosan–drought interaction. Different letters indicate significant differences according to Tukey's test ($p < 0.05$). Data are presented as mean ± SD (n = 4).

The interaction between chitosan and drought treatment (Figure 3D) revealed that chitosan application generally helps maintain chlorophyll content under water deficit. Under 2-day drought, both 1 g L^{-1} and 2 g L^{-1} chitosan treatments showed

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numerically higher SPAD values compared to the control, though they were statistically comparable (all labeled 'a' or 'ab'). However, under severe drought (4 days), a different trend emerged; while 1 g L^{-1} chitosan maintained a SPAD value similar to the control, the 2 g L^{-1} concentration showed a numerical decline (labeled 'b'), although it remained statistically non-significant compared to the control (labeled 'ab') due to the shared 'b' letter. This suggests that while chitosan supports chlorophyll stability, its effectiveness may vary depending on the concentration and the severity of the drought. This result suggests that *B. rapa ssp. chinensis* can maintain chlorophyll stability during short-term drought stress. Other studies reported that basil plants supplemented with chitosan also maintained chlorophyll content (Pirbalouti et al., 2017). Chitosan application enhanced chlorophyll content and photosynthetic activity by increasing mineral uptake (Berliana et al., 2020). Chitosan is thought to support chlorophyll stability, potentially by facilitating the uptake of minerals such as iron and magnesium (Haghaninia et al., 2024).

Under severe drought (4 days), the application of 2 g L^{-1} chitosan resulted in a numerically lower SPAD value compared to the 1 g L^{-1} treatment and the control. However, statistically, the 2 g L^{-1} treatment (b) was comparable to both the control and the 1 g L^{-1} treatment (ab) as they shared the 'b' significance letter. This suggests that while chitosan generally supports pigment stability, higher concentrations under prolonged stress may not yield further benefits, possibly due to the metabolic cost of high-dose elicitation (Mueangnak et al., 2025) The observed trends in chlorophyll and anthocyanin contents remain closely linked to the plant's oxidative status and adaptive strategies under drought.

Principal component analysis of growth and physiological traits

Principal component analysis (PCA) was performed to evaluate the multivariate relationships between growth parameters and biochemical markers. The PCA biplot explained 65.4% of the total variance, with Dim1 and Dim2 accounting for 42.5% and 22.9%, respectively (Figure 4). Dim1 was primarily defined by structural growth parameters, which showed the highest loading scores (e.g., RootFW and ShootLeng). The PCA results were consistent with the two-way ANOVA results (see Supplementary Information, Table S1), where drought was identified as the dominant factor affecting biomass. For example, drought explained 59.2% of the variance in shoot fresh weight ($\eta p^2 = 0.595$, $p = 0.001$), corresponding to the separation of treatments along the Dim1 axis. The well-watered treatments (K0D0, K1D0, K2D0) were clustered on the right side of the biplot and were associated with growth-related variables, indicating higher vegetative growth under well-watered conditions. As drought severity increased (D2 to D4), the treatment clusters shifted to the left along Dim1, reflecting the negative impact of drought on biomass accumulation. In contrast, Dim2 was characterized by physiological variables, including number of leaves, CCI, and photosynthetic pigments. Although most biochemical parameters showed no significant response to chitosan alone in the ANOVA, anthocyanin exhibited a significant interaction between chitosan and drought ($p = 0.009$, $\eta p^2 = 0.573$). The treatments with chitosan (K1 and K2) were positioned differently along the Dim2 axis compared with non-chitosan treatments (K0), suggesting that chitosan influenced plant physiological responses independently of drought-induced growth reduction.

Separation along Dim2 was associated with physiological responses, particularly pigment-related variables. Treatments with chitosan (K1 and K2) were positioned differently along Dim2 compared with non-chitosan treatments, suggesting that chitosan influenced plant physiological responses independently of drought-induced growth reduction. For example, the K1D2 treatment was located near chlorophyll and carotenoid vectors, indicating a relatively higher pigment profile under moderate drought stress. Increased accumulation or maintenance of photosynthetic pigments under stress conditions is commonly associated with improved photosynthetic stability and stress tolerance in plants (Khalil & Badr Eldin, 2021; Haghaninia et al., 2024). In contrast, the K2D4 treatment appeared in a different region of the biplot, suggesting a distinct physiological response under severe drought conditions. The proximity of H_2O_2 to the K1D2 treatment should be interpreted cautiously, as ANOVA results showed no significant main effect of chitosan on H_2O_2 levels ($p = 0.764$). Nevertheless, H_2O_2 is widely recognized as an important signalling molecule involved in plant responses to abiotic stress and in the activation of antioxidant defence pathways (Ávila et al., 2023; Rao et al., 2025). Therefore, the observed PCA

pattern may reflect a potential signalling role of H_2O_2 in stress adaptation rather than a direct physiological outcome of chitosan application.

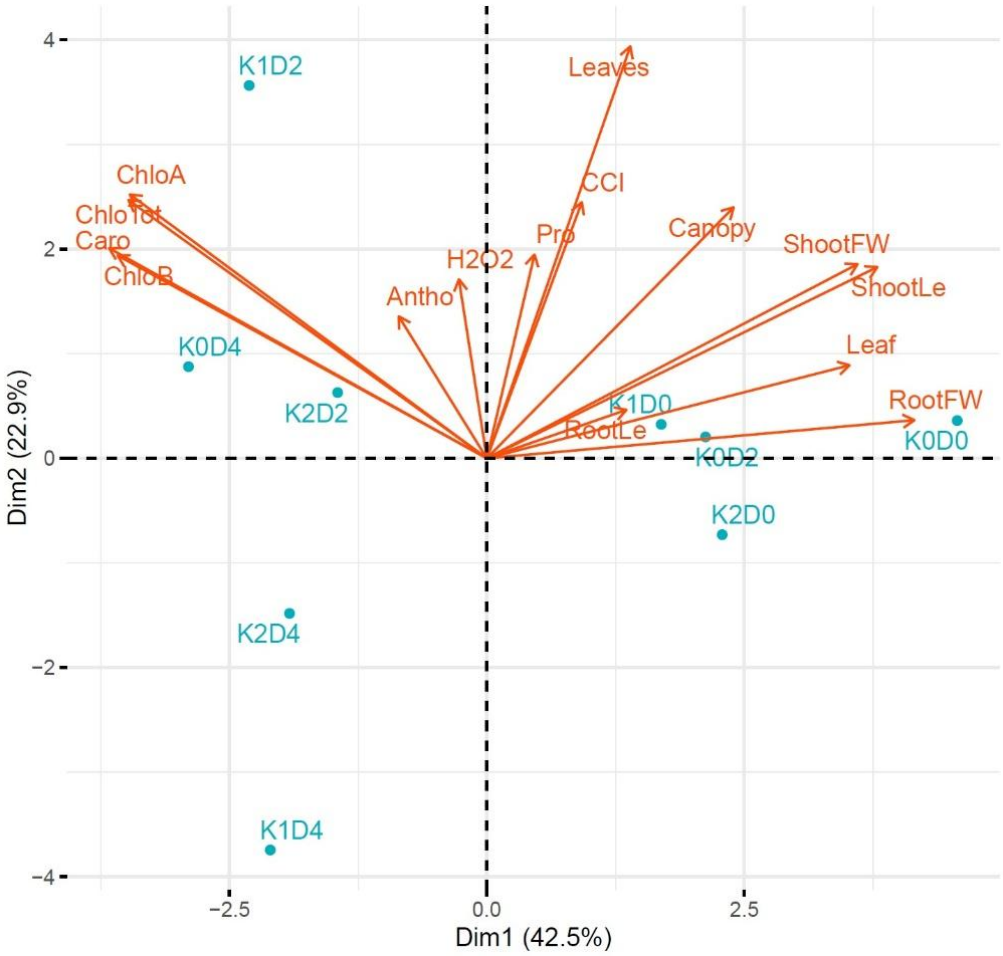

Figure 4. Principal component analysis (PCA) biplot showing the relationships among growth traits, pigment-related variables, and stress-related metabolites in *B. rapa* ssp. *chinensis* under different drought and chitosan treatments. Treatments are coded as K0 (0 g L^{-1} chitosan), K1 (1 g L^{-1} chitosan), and K2 (2 g L^{-1} chitosan), combined with drought durations of 0, 2, and 4 days prior to harvest (D0, D2, D4). Arrows represent variable loadings, and points indicate treatment combinations. Abbreviations: ChloA, chlorophyll a; ChloB, chlorophyll b; ChloTot, total chlorophyll; Caro, carotenoids; Antho, anthocyanin; Pro, proline; CCI, chlorophyll content index; LeafAr, leaf area; RootLeng, root length; ShootLeng, shoot length; ShoFW, shoot fresh weight; RoFW, root fresh weight; ShoDW, shoot dry weight; RoDW, root dry weight.

As expected, all the growth parameters (fresh/dry weights, lengths, leaf area) were clustered together, indicating they are highly correlated. The Chlorophyll Content Index (CCI) is also positively correlated with growth, showing that plants with higher chlorophyll content have better overall growth. Specifically, the proximity of the CCI vector to canopy and leaf variables indicates that chlorophyll content is positively associated with vegetative growth. Higher chlorophyll content could absorb more light energy required for photosynthesis (Ort et al., 2015). The biplot shows a strong positive correlation among Chlorophyll A, B, Total Chlorophyll, and Carotenoids. These vectors were positioned in the upper-left quadrant, opposite to the majority of the growth vectors, suggesting that drought-stressed plants, particularly those with chitosan treatment (like K1D2), showed a different metabolic profile with potentially higher pigment concentrations relative to their lower biomass.

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Chitosan application may contribute to maintaining photosynthetic pigments under drought stress (Khalil & Badr Eldin, 2021). Interestingly, anthocyanin (Antho) and proline (Pro) were also located in the upper half of the biplot, consistent with their role as stress-induced metabolites, but their correlation with the other variables was not as strong. The presence of Proline (Pro) in the upper quadrant, although showing a moderate correlation, may be associated with osmotic adjustment. Proline accumulation is commonly associated with osmotic adjustment under drought stress, helping the plant maintain cellular turgor and protect membrane integrity, which partially mitigates the reduction in shoot and root biomass (Dim1) observed during drought progression. The vector for H_2O_2 is distinct, located between the growth and pigment vectors, suggesting its role as a key intermediate in the stress response. H_2O_2 is proposed to be one of the key signaling molecules for plant growth during drought conditions by chitosan application (Ávila et al., 2023).

The PCA biplot clearly demonstrated that drought stress is the primary factor limiting growth and biomass accumulation. However, chitosan application, particularly at the 1 g L^{-1} concentration under moderate drought (K1D2), leads to a unique physiological response characterized by higher levels of photosynthetic pigments and a distinct stress-metabolite profile. This suggests that while chitosan may not fully reverse the effects of drought on growth, it effectively modulates the plant's physiological and biochemical responses to stress, potentially enhancing its resilience and survival.

CONCLUSIONS

In conclusion, drought stress was the primary factor limiting vegetative growth of *B. rapa* ssp. *chinensis*, resulting in significant reductions in shoot biomass, root biomass, and leaf area. Chitosan application at a moderate concentration (1 g L^{-1}) partially mitigated these effects by maintaining higher biomass and supporting physiological stability under water deficit conditions. Plants treated with chitosan also showed improved pigment retention during drought stress, indicating enhanced physiological tolerance. Multivariate analysis further indicated that drought severity primarily influenced growth-related parameters, while chitosan modified several physiological responses associated with stress adaptation. These results suggest that chitosan may represent a promising strategy to improve drought tolerance in leafy vegetables. Future studies should evaluate the effectiveness of chitosan under field conditions and further investigate the physiological and molecular mechanisms underlying plant responses to chitosan under water deficit.

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