

Original article

Species-dependent Salinity Tolerance in *Brassicaceae* Microgreens: Growth, Ion Homeostasis, Photosynthetic Performance, and Phenolic–flavonoid Antioxidant Responses

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Abstract

Salinity is a major constraint on plant growth, although moderate salinity may enhance antioxidant-related metabolites in microgreens. This study evaluated species-specific physiological and biochemical responses of four *Brassicaceae* microgreens, radish (*Raphanus sativus* L.), broccoli (*Brassica oleracea* var. *italica*), red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*), and rocket (*Eruca sativa* Mill.), exposed to 0, 50, 100, and 150 mM NaCl. Increasing salinity progressively inhibited growth, with the greatest reductions at 150 mM NaCl. Shoot length decreased by 35.6% in radish and 35.9% in broccoli, while stronger reductions occurred in red cabbage and rocket, reaching 43.3% and 43.8%, respectively. Salinity also disrupted ion homeostasis by increasing Na⁺ accumulation and the Na⁺/K⁺ ratio, particularly in rocket, where this ratio reached 1.33 at 150 mM NaCl. Severe salinity reduced total chlorophyll and Fv/Fm, indicating impaired photosynthetic performance. Despite this decline, radish and broccoli maintained higher pigment content and PSII efficiency. Moderate salinity, especially 100 mM NaCl, stimulated antioxidant-related metabolism, with radish showing the highest phenolic content, flavonoid content, and antioxidant activity. These findings suggest that moderate salinity may improve functional biochemical quality, whereas severe salinity limits growth.

Keywords: Microgreens, *Brassicaceae*, Ion Homeostasis, Chlorophyll Fluorescence, Phenolic Compounds, Flavonoids, Antioxidant Activity, Salinity Tolerance

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INTRODUCTION

Increasing environmental constraints and the progressive degradation of agricultural lands have intensified the need to develop sustainable production systems capable of maintaining both crop productivity and nutritional quality (Godfray et al., 2010; Akın and Aygöl, 2022; Hashemi et al., 2022; Jeyachandra, 2026). Among the various environmental stressors affecting plant growth, soil salinity has emerged as one of the most critical factors limiting agricultural productivity worldwide (Maryum et al., 2022; Melino and Tester, 2023). It is estimated that more than 20% of irrigated agricultural lands are affected by salinity, and this proportion is expected to increase due to climate change, improper irrigation practices, and rising groundwater levels (Aydoğan and Turhan, 2020; Mishra et al., 2023; Shabala et al., 2025). Excessive salt accumulation in the root zone disrupts plant water uptake and induces ionic toxicity, which in turn impairs physiological processes such as photosynthesis, nutrient balance, and metabolic activity (Munir et al., 2022). For this reason, plant responses to salinity have become a central topic in plant physiology and crop science research.

Plants exposed to saline conditions exhibit a range of physiological and biochemical responses aimed at maintaining cellular homeostasis and mitigating oxidative damage (Fathi et al., 2025). One of the primary consequences of high salinity is the overproduction of reactive oxygen species (ROS), which can lead to lipid peroxidation, protein degradation, and membrane damage if not effectively regulated (Shabbir et al., 2026). To counteract these harmful effects, plants activate various defense mechanisms, including the accumulation of compatible solutes such as proline and the synthesis of antioxidant compounds such as phenolics and flavonoids (Ali et al., 2020; Dikilitas et al., 2020; Bano et al., 2022; Khalid et al., 2022; Colak, 2024). These biochemical adjustments play a crucial role in protecting cellular structures and maintaining metabolic stability under stressful conditions (Mehla et al., 2017; Hussain et al., 2019; Rajput et al., 2021).

In recent years, increasing attention has been directed toward microgreens as emerging functional foods with high nutritional value. Microgreens are young seedlings harvested shortly after germination, typically when cotyledons are fully expanded and before the development of the first true leaves (Kyriacou et al., 2016). Despite their small size, microgreens are recognized as concentrated sources of vitamins, minerals, and various phytochemicals, particularly phenolic compounds and antioxidants. Several studies have demonstrated that microgreens may contain significantly higher concentrations of bioactive compounds compared to their mature plant counterparts, making them attractive ingredients for health-oriented diets (Dhaka et al., 2023; Moinuddin et al., 2025). Their short growth cycle, minimal production requirements, and suitability for controlled environment cultivation further enhance their potential for sustainable urban and indoor agriculture.

Among the plant families commonly used for microgreen production, the *Brassicaceae* family holds particular importance due to its rich phytochemical profile. Species such as radish (*Raphanus*

sativus), broccoli (*Brassica oleracea* var. *italica*), red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*), and rocket (*Eruca sativa*) are widely cultivated as microgreens and are known for their high concentrations of phenolic compounds, glucosinolates, and antioxidant metabolites (Baldelli et al., 2025). These bioactive compounds have attracted considerable scientific interest due to their potential health benefits, including antioxidant, anti-inflammatory, and anticancer properties (Narra et al., 2025).

In addition to their nutritional importance, microgreens also represent a useful experimental system for investigating plant responses to abiotic stress factors. Their rapid growth, uniform developmental stage, and ease of cultivation under controlled conditions make them suitable for evaluating physiological and biochemical stress responses within a relatively short time frame (Mallor et al., 2023; Laila et al., 2026). Recent research suggests that moderate levels of environmental stress may even enhance the accumulation of certain secondary metabolites in microgreens, thereby increasing their nutritional and functional value (Kaushal et al., 2026; Rasmi et al., 2026). However, salinity tolerance, as reflected in growth performance and biochemical responses, may vary considerably among different plant species.

Although numerous studies have investigated the nutritional properties of microgreens, comparative information regarding salinity tolerance and the associated physiological and biochemical responses of different microgreen species remains limited (Ebert, 2022; Escobar et al., 2025; Seth et al., 2025). In particular, systematic comparisons among *Brassicaceae* microgreens under controlled salinity conditions are still scarce in literature. Understanding these responses is essential not only for improving the sustainability of microgreen production under suboptimal growing conditions but also for identifying species that exhibit greater salinity tolerance and possess enhanced nutritional potential.

Accordingly, the present study tested the hypothesis that different microgreen species exhibit species-dependent salinity tolerance mechanisms under saline conditions, which are reflected not only in growth performance but also in the accumulation of antioxidant metabolites such as phenolics and flavonoids.

MATERIALS and METHODS

Plant Material and Microgreen Production

Four microgreen species belonging to the *Brassicaceae* family were used as plant material in this study: radish (*Raphanus sativus* L.), broccoli (*Brassica oleracea* var. *italica*), red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*), and rocket (*Eruca sativa* Mill.). Commercial seeds were obtained from a certified seed supplier and stored under dry and cool conditions until use. In order to prevent microbial contamination, the seeds were subjected to surface sterilization before sowing. The sterilization process involved immersion in a 1% sodium hypochlorite solution for 2 min, followed by three successive rinses with sterile distilled water. Microgreens were cultivated under controlled environmental conditions in a climate-controlled growth chamber (JSPC-300C, Jisico Co., Ltd., Seoul, Korea) with a total capacity of

300 L. The chamber provides precise environmental regulation with a temperature control range of 0–60°C under dark conditions and 10–60°C under illuminated conditions, with a temperature accuracy of $\pm 0.3^\circ\text{C}$ at 20°C and 60% relative humidity. Relative humidity within the chamber can be controlled within a range of 30–85% RH. Illumination was provided by fluorescent lamps (14 x 36 W FPL), with adjustable light intensity up to 30,000 lux. Seeds were evenly sown on plastic trays (30 x 20 cm) filled with a sterile peat-based growing medium. The substrate was pre-moistened with distilled water to ensure uniform germination. After sowing, the trays were placed inside the growth chamber and maintained under controlled conditions at $22 \pm 2^\circ\text{C}$ temperature, 65–70% relative humidity, and a 16 h light / 8 h dark photoperiod. Light intensity during the growth period was maintained at approximately $200 \mu\text{mol m}^{-2} \text{s}^{-1}$. Trays were irrigated with distilled water as required to maintain adequate moisture in the growing medium. Microgreens were harvested when the cotyledons were fully expanded and before the emergence of the first true leaves, approximately 10–12 days after sowing. At harvest, plants were carefully cut at the substrate level using sterile scissors and immediately used for subsequent growth and biochemical analyses. Temperature, relative humidity, and CO_2 concentration within the chamber were continuously monitored using integrated PID-controlled sensors.

Salinity Treatments and Experimental Design

Salinity treatments were applied using sodium chloride (NaCl) solutions prepared at different concentrations. The experiment included a control treatment without NaCl (0 mM) and three salinity levels of 50, 100, and 150 mM NaCl. Salinity treatments were initiated on the third day after germination to ensure that the seedlings had completed the initial germination phase and were sufficiently established before exposure to salinity. This timing was selected to minimize the direct effects of NaCl on germination and to specifically evaluate post-germination growth, physiological, and biochemical responses to salinity. The NaCl solutions were distributed evenly across the substrate to ensure uniform salt exposure among seedlings within each treatment. The experiment was conducted according to a completely randomized design (CRD). Each species $\times$ salinity treatment combination was represented by three biological replicates, with each replicate consisting of an individual cultivation tray maintained under the same environmental conditions.

Growth Measurements

Growth and morphological parameters of microgreens were evaluated at the harvest stage to determine the effects of salinity on plant development and biomass accumulation. In microgreen studies, plant growth is commonly assessed using parameters such as shoot length, hypocotyl diameter, root length, fresh biomass, and dry biomass. In the present study, shoot length, root length, hypocotyl diameter, fresh weight, and dry weight were evaluated as indicators of plant growth, seedling morphology, and biomass production. Shoot length was measured from the base of the hypocotyl to the tip of the cotyledons using a digital caliper (Mitutoyo Corporation, Kanagawa, Japan) with millimeter

precision. For each replicate, randomly selected seedlings were measured individually and the obtained values were recorded in centimeters. Subsequently, the mean shoot length for each treatment was calculated. Fresh biomass was determined immediately after harvest to minimize moisture loss. Microgreen samples were carefully cut at the substrate level using sterile scissors and weighed using an analytical balance with a precision of 0.001 g (Sartorius Entris II, Sartorius AG, Göttingen, Germany). The obtained values were recorded as fresh weight in grams per replicate. For dry biomass determination, harvested samples were first weighed to obtain their fresh weight and then placed into paper envelopes. The samples were dried in a forced-air drying oven (Memmert UF55, Memmert GmbH, Schwabach, Germany) at 70 °C for 48 h until a constant weight was reached. After drying, the samples were cooled in a desiccator to prevent moisture absorption and weighed again using the same analytical balance. The resulting values were expressed as dry weight in grams. Hypocotyl diameter was measured at the midpoint of the hypocotyl using a digital caliper with millimeter precision. Root length was measured from the root–shoot junction to the root tip using a ruler or digital caliper. These parameters have been used in some microgreen studies to provide a more detailed evaluation of seedling morphology and growth performance.

Physiological and Biochemical Analyses

Fresh microgreen samples were used for the determination of photosynthetic pigments, chlorophyll fluorescence, total phenolic content, total flavonoid content, and antioxidant activity. For ion analysis, plant samples were dried and used for the determination of Na⁺ and K⁺ concentrations. All physiological and biochemical analyses were performed using three biological replicates for each treatment combination.

Determination of Na⁺, K⁺, and Na⁺/K⁺ Ratio

Sodium and potassium concentrations were determined in dried microgreen samples according to the method described by Wolf (1982), with minor modifications. Plant tissues were oven-dried at 70 °C until constant weight and then ground into a fine powder. Approximately 0.2 g of dried sample was digested using a nitric acid-based digestion procedure. After digestion, the final volume was adjusted with deionized water, and Na⁺ and K⁺ concentrations were measured using flame photometry or atomic absorption spectrophotometry. The Na⁺/K⁺ ratio was calculated by dividing the Na⁺ concentration by the K⁺ concentration for each sample. Results were expressed on a dry weight basis.

Determination of Chlorophyll Content

Chlorophyll content was determined using fresh microgreen tissue according to the method described by Arnon (1949), with minor modifications. Approximately 0.2 g of fresh sample was homogenized with 80% acetone under cold conditions. The homogenate was centrifuged at 10,000 rpm for 10 min, and the absorbance of the supernatant was measured at 663 and 645 nm using a UV–Vis spectrophotometer (Shimadzu UV-1800, Shimadzu Corporation, Kyoto, Japan). Chlorophyll a,

chlorophyll b, and total chlorophyll contents were calculated using standard equations and expressed as mg g^{-1} fresh weight.

Chlorophyll Fluorescence Measurement

Chlorophyll fluorescence was measured using a portable chlorophyll fluorometer according to the procedure described by Baker (2008), with minor modifications. Before measurement, microgreen samples were dark-adapted for 20–30 min. The maximum quantum efficiency of photosystem II was determined as F_v/F_m , where F_v represents variable fluorescence and F_m represents maximum fluorescence. Measurements were taken from fully expanded cotyledons, and the mean value was calculated for each replicate.

Preparation of Extracts for Antioxidant-Related Biochemical Analyses

Fresh microgreen samples were used for the determination of total phenolic content, total flavonoid content, and antioxidant activity. Approximately 0.5 g of fresh tissue from each treatment was homogenized under cold conditions using a mortar and pestle. The homogenized material was extracted with 10 mL of 80% methanol at a ratio of 1:20 (w/v) and vortexed for 1 min to enhance extraction efficiency. The extracts were subsequently centrifuged at 10,000 rpm for 10 min at 4 °C using a refrigerated centrifuge (Nüve NF-800R, Nüve, Ankara, Türkiye). The resulting supernatants were collected and used for the determination of total phenolic content, total flavonoid content, and antioxidant activity.

Determination of Total Phenolic Content

Total phenolic content (TPC) was determined using the Folin–Ciocalteu method according to Singleton and Rossi (1965). Briefly, 0.5 mL of extract was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent. After 5 min, 2 mL of 7.5% sodium carbonate (Na_2CO_3) solution was added, and the mixture was vortexed. The reaction mixture was incubated at room temperature in the dark for 30 min, and absorbance was recorded at 765 nm using a UV–Vis spectrophotometer (Shimadzu UV-1800, Shimadzu Corporation, Kyoto, Japan). Gallic acid was used for the calibration curve at concentrations of 0–200 mg L^{-1} , and the results were expressed as mg gallic acid equivalents per gram fresh weight (mg GAE g^{-1} FW).

Determination of Total Flavonoid Content

Total flavonoid content (TFC) was measured using the aluminum chloride colorimetric method described by Zhishen et al. (1999). Briefly, 0.5 mL of extract was mixed with 2 mL of distilled water and 0.15 mL of 5% sodium nitrite (NaNO_2). After 5 min, 0.15 mL of 10% aluminum chloride (AlCl_3) was added. Following a further 6 min incubation, 1 mL of 1 M sodium hydroxide (NaOH) was added, and the final volume was adjusted to 5 mL with distilled water. The mixture was vortexed, and absorbance was measured at 510 nm using a UV–Vis spectrophotometer (Shimadzu UV-1800).

Catechin was used as the standard at concentrations of 0–200 mg L⁻¹, and results were expressed as mg catechin equivalents per gram fresh weight (mg CE g⁻¹ FW).

Determination of Antioxidant Activity

Antioxidant activity was evaluated using the DPPH radical scavenging assay following Brand-Williams et al. (1999). In brief, 0.1 mL of extract was added to 3.9 mL of freshly prepared DPPH solution, prepared at 0.1 mM in methanol. The mixture was vortexed and incubated in the dark for 30 min at room temperature. The decrease in absorbance was measured at 517 nm using a UV–Vis spectrophotometer (Shimadzu UV-1800). Trolox was used as the reference antioxidant at concentrations of 0–200 µM, and antioxidant capacity was expressed as µmol Trolox equivalents per gram fresh weight (µmol TE g⁻¹ FW).

Experimental Design and Statistical Analysis

The study was conducted using a completely randomized design (CRD) with three biological replicates for each treatment combination. The experimental setup included two main factors: microgreen species (radish, broccoli, red cabbage, and rocket) and salinity treatments (0, 50, 100, and 150 mM NaCl). Each biological replicate corresponded to a single cultivation tray. Morphological parameters, including shoot length, fresh weight, and dry weight, as well as biochemical traits such as total phenolic content, total flavonoid content, and antioxidant activity, were determined using independent biological replicates. The results were presented as mean values accompanied by the standard error (mean ± SE). Statistical evaluation of the data was performed using two-way analysis of variance (ANOVA) to assess the individual and interactive effects of species and salinity levels on the measured variables. When statistically significant differences were detected, treatment means were separated using Tukey's multiple comparison test at a significance level of 0.05. All statistical computations were carried out using SAS software (Version 9.4, SAS Institute Inc., Cary, NC, USA). Statistical significance among treatments was accepted at $p < 0.05$. Graphical representations of the data were generated using Python (version 3.9) with the Matplotlib library.

RESULTS and DISCUSSION

Salinity treatments significantly affected the growth and morphological characteristics of the evaluated microgreen species, as presented in Table 1 and Table 2. Increasing NaCl concentrations resulted in a progressive decline in shoot length, hypocotyl diameter, root length, fresh biomass, and dry biomass across all species. However, the magnitude of these reductions varied among species, indicating differences in salinity tolerance at the early developmental stage. As shown in Table 1 and Figure 1, plants grown under control conditions (0 mM NaCl) exhibited the highest values for shoot length, root length, and hypocotyl diameter, whereas the highest salinity level (150 mM NaCl) caused the most pronounced reductions in these morphological parameters. Similarly, biomass production parameters presented in Table 2 and Figure 2 demonstrated that fresh weight and dry weight decreased progressively

with increasing salinity levels. Among the evaluated species, radish and broccoli microgreens exhibited relatively higher salinity tolerance, maintaining greater shoot length, hypocotyl diameter, root length, and biomass production under moderate salinity conditions compared with the other species. By comparison, rocket and red cabbage showed stronger growth restriction, particularly under higher NaCl concentrations. These reductions indicate that increasing salinity restricted both morphological development and biomass accumulation during the early growth stages of microgreens. The stronger restriction observed at 150 mM NaCl suggests that severe salinity exceeded the adaptive capacity of the seedlings. Under high salinity levels, reduced external water potential may limit water uptake and decrease cell turgor, thereby restricting cell expansion and elongation (Munns and Tester, 2008; Acosta-Motos et al., 2017). Furthermore, excessive Na⁺ and Cl⁻ accumulation may disturb ion homeostasis and interfere with metabolic processes required for active seedling growth (Munns and Tester, 2008). Thus, the reductions in shoot length, root length, hypocotyl diameter, and biomass at 150 mM NaCl can be interpreted as the combined consequence of osmotic limitation and ionic imbalance, both of which directly constrain early seedling development. Salinity levels significantly affected shoot length, root length, and hypocotyl diameter in the evaluated microgreen species (Table 1). Under control conditions (0 mM NaCl), the highest shoot length was observed in radish (6.85 cm), followed by broccoli (6.21 cm), red cabbage (5.94 cm), and rocket (5.48 cm). As salinity levels increased, a gradual decline in shoot length was observed in all species. At the highest salinity level (150 mM NaCl), shoot length decreased to 4.41 cm in radish, 3.98 cm in broccoli, 3.37 cm in red cabbage, and 3.08 cm in rocket. Compared with the control treatment, this corresponded to reductions of approximately 35.6%, 35.9%, 43.3%, and 43.8%, respectively. A similar trend was observed for root length.

Table 1. Effects of salinity levels on shoot length, root length, and hypocotyl diameter of different microgreen species

Parameter	NaCl (mM)	Radish	Broccoli	Red cabbage	Rocket
Shoot length (cm)	0	6.85±0.22 ^a	6.21±0.21 ^b	5.94±0.20 ^{bc}	5.48±0.19 ^c
	50	6.12±0.18 ^a	5.58±0.19 ^b	5.02±0.18 ^c	4.71±0.17 ^d
	100	5.34±0.20 ^a	4.87±0.17 ^b	4.26±0.16 ^{bc}	3.96±0.15 ^c
	150	4.41±0.17 ^a	3.98±0.16 ^b	3.37±0.15 ^c	3.08±0.14 ^d
Root length (cm)	0	7.42±0.26 ^a	7.05±0.24 ^{ab}	6.74±0.23 ^b	6.28±0.21 ^c
	50	6.58±0.23 ^a	6.24±0.22 ^{ab}	5.82±0.21 ^b	5.36±0.19 ^c
	100	5.73±0.21 ^a	5.38±0.20 ^b	4.94±0.19 ^c	4.48±0.17 ^d
	150	4.86±0.19 ^a	4.52±0.18 ^{ab}	4.05±0.17 ^b	3.62±0.15 ^c
Hypocotyl diameter (mm)	0	2.18±0.09 ^a	2.05±0.08 ^{ab}	1.98±0.08 ^b	1.86±0.07 ^c
	50	1.97±0.08 ^a	1.86±0.07 ^b	1.71±0.07 ^c	1.60±0.06 ^d
	100	1.74±0.07 ^a	1.63±0.06 ^{ab}	1.49±0.06 ^b	1.38±0.05 ^c
	150	1.52±0.06 ^a	1.42±0.05 ^b	1.26±0.05 ^c	1.14±0.04 ^d

Values are presented as mean ± standard error. Different lowercase letters within the same row indicate significant differences among species at the same NaCl concentration for the corresponding parameter ($p < 0.05$).

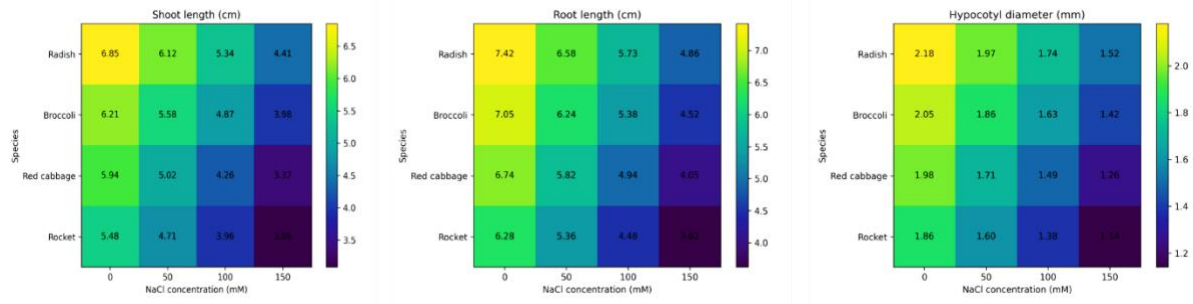


Figure 1. Heatmaps illustrating the effects of increasing NaCl concentrations (0–150 mM) on shoot length, root length, and hypocotyl diameter of different microgreen species. Color gradients represent the magnitude of the measured values

In the control group, the longest root length was recorded in radish (7.42 cm), followed by broccoli (7.05 cm), red cabbage (6.74 cm), and rocket (6.28 cm). Increasing NaCl concentrations significantly restricted root development, and root length decreased to 4.86 cm in radish, 4.52 cm in broccoli, 4.05 cm in red cabbage, and 3.62 cm in rocket at 150 mM NaCl. Accordingly, the reductions in root length were approximately 34.5%, 35.9%, 39.9%, and 42.4%, respectively. Hypocotyl diameter was also markedly influenced by increasing salinity. In the control group, the highest hypocotyl diameter was recorded in radish (2.18 mm), followed by broccoli (2.05 mm), red cabbage (1.98 mm), and rocket (1.86 mm). Increasing salinity levels caused a consistent decline in hypocotyl thickness, and at 150 mM NaCl the values decreased to 1.52 mm in radish, 1.42 mm in broccoli, 1.26 mm in red cabbage, and 1.14 mm in rocket. These values correspond to reductions of approximately 30.3%, 30.7%, 36.4%, and 38.7%, respectively. The reductions in shoot length, root length, and hypocotyl diameter under increasing salinity are consistent with previous findings reported in literature. These responses are closely associated with mechanisms determining salinity tolerance, particularly osmotic and ionic regulation. High salinity reduces water uptake and cell expansion, while excessive Na^+ and Cl^- accumulation may cause ionic toxicity and disrupt metabolic processes. These physiological disturbances can restrict cell division and elongation during early seedling development (Shelden and Munns, 2023). Previous studies conducted on various vegetable crops have also reported that increasing salinity levels significantly reduce shoot and root growth during the early stages of plant development. For instance, Heydari et al. (2026) demonstrated that salinity significantly limits hypocotyl elongation and root development in *Brassicaceae* species.

Differences among species may be explained by variations in salinity tolerance mechanisms. Salinity tolerance in plants is associated with several physiological mechanisms, including ion homeostasis, osmotic adjustment capacity, and the efficiency of antioxidant defense systems (Liang et al., 2018). Species that are able to regulate these mechanisms more effectively can maintain higher growth performance under saline conditions. Radish and broccoli maintained relatively higher shoot and root growth than the other species, indicating comparatively greater salinity tolerance at the biochemical

level. By comparison, rocket and red cabbage showed stronger growth inhibition, particularly under higher NaCl concentrations, indicating lower salinity tolerance. Previous studies have also reported that salinity can reduce root meristem activity, thereby limiting root elongation and restricting water and nutrient uptake (Acosta-Motos et al., 2020). These effects indicate that high salinity impairs early seedling development by restricting both root and shoot growth in microgreens. The observed decreases in fresh and dry biomass under saline conditions are consistent with previous studies indicating that increasing salinity significantly limits plant growth and biomass accumulation. Salt stress primarily reduces plant growth by inducing osmotic stress, which restricts water uptake and lowers cell turgor, thereby inhibiting cell expansion and biomass formation (Yang et al., 2025). In addition, the excessive accumulation of Na^+ and Cl^- ions in plant tissues can lead to ionic imbalance and metabolic disturbances that negatively affect photosynthesis and overall plant growth. Similar reductions in biomass production under saline conditions have been reported in several studies on leafy vegetables and microgreens.

Table 2. Effects of salinity levels on fresh weight and dry weight of different microgreen species

Parameter (g)	NaCl (mM)	Radish	Broccoli	Red cabbage	Rocket
Fresh weight	0	0.91±0.04 ^a	0.86±0.03 ^b	0.80±0.03 ^{bc}	0.73±0.03 ^c
	50	0.79±0.03 ^a	0.74±0.03 ^{ab}	0.67±0.02 ^b	0.60±0.02 ^b
	100	0.66±0.03 ^a	0.61±0.02 ^{ab}	0.55±0.02 ^b	0.48±0.02 ^c
	150	0.53±0.02 ^a	0.49±0.02 ^b	0.43±0.02 ^{bc}	0.37±0.02 ^c
Dry weight	0	0.084±0.004 ^a	0.079±0.003 ^b	0.075±0.003 ^b	0.070±0.003 ^b
	50	0.072±0.003 ^a	0.068±0.003 ^{ab}	0.064±0.002 ^{bc}	0.059±0.002 ^c
	100	0.061±0.003 ^a	0.056±0.002 ^b	0.053±0.002 ^b	0.048±0.002 ^c
	150	0.050±0.002 ^a	0.046±0.002 ^{ab}	0.042±0.002 ^b	0.039±0.002 ^c

Values are presented as mean ± standard error. Different lowercase letters within the same row indicate significant differences among species at the same NaCl concentration for the corresponding parameter ($p < 0.05$).

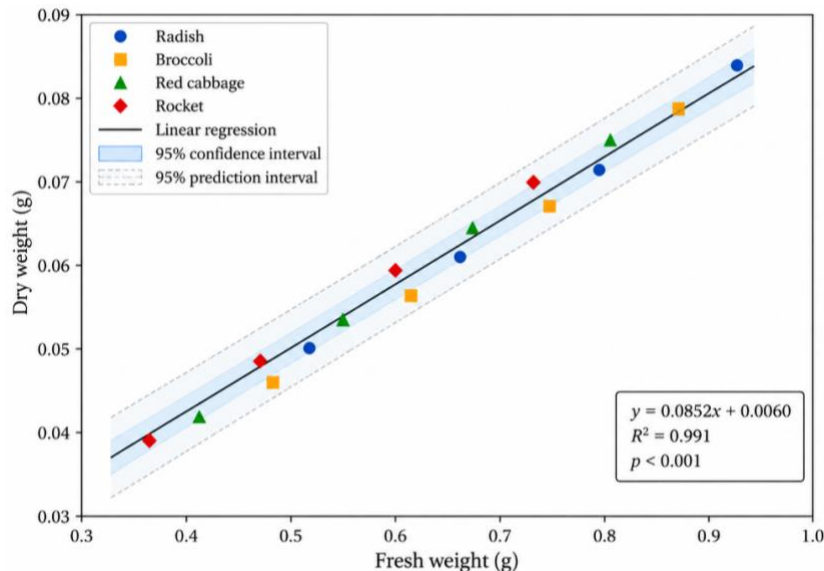


Figure 2. Relationship between fresh weight and dry weight of radish, broccoli, red cabbage, and rocket microgreens grown under different salinity levels (0, 50, 100 and 150 mM NaCl). Each color represents a different species, and the four data points of the same color correspond to the four salinity treatments. Each point represents the mean fresh and dry weight values of a species at a specific salinity level. The solid line represents the linear regression ($y = 0.0852x + 0.0060$; $R^2 = 0.991$).

For instance, Galieni et al. (2020) demonstrated that increasing NaCl concentrations significantly reduced fresh and dry biomass in microgreens due to osmotic constraints and reduced nutrient uptake efficiency. Likewise, Sarker and Oba (2020) reported that salinity caused a significant decline in biomass accumulation in leafy vegetable crops by impairing photosynthetic performance and reducing carbon assimilation. Differences in biomass reduction among species may reflect variation in physiological and biochemical mechanisms involved in salinity tolerance. Salinity tolerance in plants is often associated with the ability to maintain ionic balance, regulate osmotic potential, and activate antioxidant defense systems under stress conditions (Sohag et al., 2020). Species with stronger osmotic adjustment capacity are better able to maintain water status and metabolic activity under saline environments, which allows them to sustain higher biomass production. Radish and broccoli retained higher fresh and dry biomass than rocket and red cabbage as salinity increased, suggesting a greater capacity to sustain biomass production during early growth under saline conditions. Rocket and red cabbage, however, exhibited more pronounced reductions in biomass accumulation, suggesting greater sensitivity to elevated NaCl concentrations. Similar interspecific differences in salinity tolerance have been reported in leafy vegetable crops, where variations in osmotic adjustment, ion compartmentalization, and antioxidant activity determine the degree of growth inhibition under saline conditions (Kafeel, 2025). Salinity significantly influenced the biochemical composition of the evaluated microgreen species, particularly total phenolic content, total flavonoid content, and antioxidant activity (Table 3 and Figure 3).

Total phenolic content increased with increasing salinity levels up to 100 mM NaCl in all species, followed by a slight decline at 150 mM NaCl. Among the evaluated species, radish exhibited the highest phenolic accumulation under saline conditions, reaching 4.12 mg GAE g⁻¹ FW at 100 mM NaCl compared with 3.28 mg GAE g⁻¹ FW under control conditions. Broccoli also showed a marked increase from 3.05 mg GAE g⁻¹ FW in the control treatment to 3.89 mg GAE g⁻¹ FW at 100 mM NaCl. Lower phenolic levels were recorded in red cabbage and rocket, while both species exhibited a gradual increase under moderate salinity conditions. Moderate salinity appears to stimulate phenolic biosynthesis in microgreens.

Table 3. Effects of salinity levels on biochemical parameters of different microgreen species

Parameter	NaCl (mM)	Radish	Broccoli	Red cabbage	Rocket
Total phenolic content (mg GAE g ⁻¹ FW)	0	3.28±0.11 ^a	3.05±0.10 ^{ab}	2.84±0.09 ^b	2.63±0.08 ^c
	50	3.74±0.13 ^a	3.48±0.12 ^b	3.19±0.11 ^c	2.98±0.10 ^d
	100	4.12±0.15 ^a	3.89±0.13 ^b	3.55±0.12 ^c	3.21±0.11 ^d
	150	3.86±0.14 ^a	3.62±0.12 ^b	3.28±0.11 ^c	3.05±0.10 ^d
Total flavonoid content (mg CE g ⁻¹ FW)	0	1.42±0.05 ^a	1.31±0.05 ^b	1.21±0.04 ^c	1.10±0.04 ^d
	50	1.63±0.06 ^a	1.49±0.05 ^b	1.38±0.05 ^c	1.26±0.04 ^d
	100	1.79±0.07 ^a	1.65±0.06 ^b	1.54±0.05 ^c	1.39±0.05 ^d
	150	1.65±0.06 ^a	1.52±0.05 ^b	1.41±0.05 ^c	1.29±0.04 ^d
Antioxidant activity (μmol TE g ⁻¹ FW)	0	34.6±1.2 ^a	32.8±1.1 ^b	30.5±1.0 ^c	28.9±0.9 ^d
	50	39.2±1.4 ^a	36.7±1.3 ^b	34.2±1.2 ^c	31.7±1.1 ^d
	100	43.5±1.5 ^a	40.6±1.4 ^b	37.9±1.3 ^c	34.8±1.2 ^d
	150	40.8±1.4 ^a	38.2±1.3 ^b	35.6±1.2 ^c	33.1±1.1 ^d

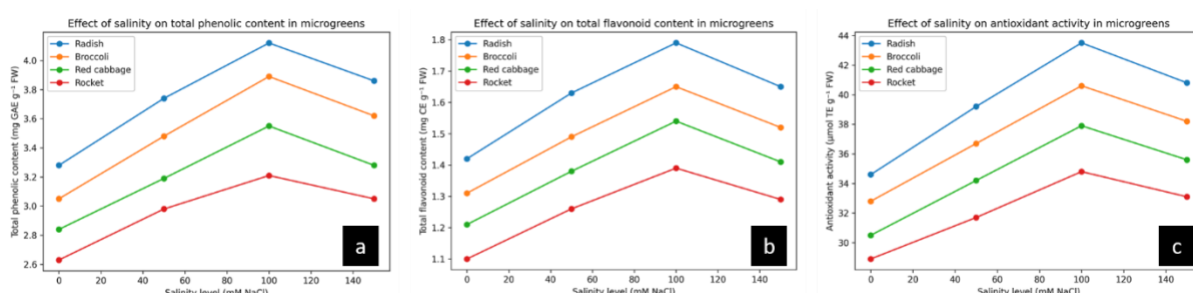


Figure 3. Effects of different salinity levels (0–150 mM NaCl) on (A) total phenolic content, (B) total flavonoid content, and (C) antioxidant activity in four microgreen species

A similar trend was observed for total flavonoid content. Increasing salinity levels resulted in a progressive rise in flavonoid concentration, particularly up to 100 mM NaCl. Radish again showed the highest flavonoid accumulation, increasing from 1.42 mg CE g⁻¹ FW in the control treatment to 1.79 mg CE g⁻¹ FW at 100 mM NaCl. Broccoli, red cabbage, and rocket also exhibited gradual increases in flavonoid content under moderate salinity conditions. However, at the highest salinity level (150 mM NaCl), flavonoid content slightly decreased in most species, suggesting that excessive salt stress may inhibit secondary metabolite biosynthesis due to metabolic disruption.

Antioxidant activity measured by the DPPH assay followed a pattern similar to phenolic and flavonoid accumulation. In radish microgreens, antioxidant activity increased from 34.6 μmol TE g⁻¹ FW under control conditions to 43.5 μmol TE g⁻¹ FW at 100 mM NaCl, representing the highest antioxidant capacity observed in the study. Broccoli also exhibited a significant increase from 32.8 to 40.6 μmol TE g⁻¹ FW under the same salinity level. Red cabbage and rocket showed relatively lower antioxidant activity; however, both species still demonstrated a clear enhancement in antioxidant capacity under moderate salinity. At 150 mM NaCl, antioxidant activity slightly declined compared with 100 mM NaCl, indicating that excessive salinity may limit the efficiency of antioxidant defense systems.

The increase in total phenolic content, total flavonoid content, and antioxidant activity up to 100 mM NaCl suggests that moderate salinity may function as an eliciting factor for antioxidant-related secondary metabolism in microgreens. Under moderate salt stress, the controlled accumulation of ROS may act not only as a damaging factor but also as a signaling mechanism that activates defense-related metabolic pathways. Conversely, excessive ROS accumulation can damage cellular components if not effectively controlled (Ahmad et al., 2014; Bela et al., 2015; Tarfeen et al., 2022). Phenolic compounds and flavonoids are among the major non-enzymatic antioxidants involved in ROS scavenging, and their accumulation may contribute to the maintenance of cellular redox balance under saline conditions. The parallel increases in total phenolic content, total flavonoid content, and DPPH activity observed in this study indicate that the enhancement of antioxidant capacity was closely associated with the stimulation of secondary metabolite accumulation. Similar increases in phenolic accumulation and antioxidant activity under moderate salinity or abiotic stress conditions have also been reported in leafy vegetables and microgreens (Shivashankara et al., 2016; Varela et al., 2016). However, the slight decline in these biochemical traits at 150 mM NaCl suggests that severe salinity may impair metabolic activity and reduce the biosynthetic capacity of seedlings, limiting the accumulation of protective compounds despite the higher stress intensity. The capacity to synthesize antioxidant compounds varied among microgreen species, reflecting species-dependent differences in biochemical responses associated with salinity tolerance. Radish and broccoli exhibited higher phenolic and flavonoid accumulation as well as stronger antioxidant activity compared with red cabbage and rocket, indicating comparatively greater salinity tolerance at the biochemical level. Plants capable of producing higher levels of antioxidant metabolites are generally better equipped to mitigate oxidative damage and maintain metabolic stability under stress conditions (Perez and Brown, 2014). The comparatively better performance of radish and broccoli indicates that salinity tolerance within *Brassicaceae* microgreens is species-dependent rather than uniform across the family. These two species maintained higher biomass and morphological development under saline conditions while also showing stronger antioxidant-related biochemical responses. This combined response suggests that their relative tolerance may be associated with a greater capacity to balance growth maintenance and stress-induced secondary metabolism. Red cabbage and rocket, however, showed stronger growth inhibition, indicating a lower ability to sustain early seedling development under elevated NaCl concentrations. Taken together, these results suggest that radish and broccoli may be considered more promising *Brassicaceae* microgreens for production under moderate saline conditions, particularly where enhancement of antioxidant-related quality traits is desired.

Salinity treatments markedly affected Na^+ and K^+ accumulation as well as the Na^+/K^+ ratio in the evaluated microgreen species (Table 4). Na^+ content increased progressively with increasing NaCl concentration in all species, whereas K^+ content declined gradually. Consequently, the Na^+/K^+ ratio increased consistently with increasing salinity, with the highest values recorded at 150 mM NaCl. This response indicates that increasing external NaCl disturbed ionic homeostasis by promoting Na^+

accumulation and reducing K^+ retention. Such an ionic imbalance is a common feature of salt-stressed plants, as excessive Na^+ accumulation can interfere with K^+ uptake, enzyme activity, membrane function, and cellular metabolism (Munns and Tester, 2008; Assaha et al., 2017; Shabala and Pottosin, 2014). The magnitude of ionic disruption differed among microgreen species. Radish and broccoli maintained lower Na^+ accumulation and lower Na^+/K^+ ratios than red cabbage and rocket, particularly under 100 and 150 mM NaCl. This suggests that radish and broccoli had a greater capacity to maintain ionic balance under saline conditions. Similar responses have been reported in Brassica crops, where salt tolerance is associated with efficient Na^+ exclusion and better K^+ retention ability (Shahzad et al., 2022). In Brassica leafy vegetables, K^+/Na^+ ratio has also been used as a reliable indicator of salt stress status and species-dependent stress response. By comparison, the higher Na^+/K^+ ratios observed in red cabbage and rocket indicate stronger ionic imbalance, which may have contributed to their greater reductions in shoot growth, root growth, and biomass accumulation. Therefore, the relatively better growth performance of radish and broccoli may be partly associated with their ability to restrict Na^+ accumulation and maintain a more favorable Na^+/K^+ balance under saline conditions.

Chlorophyll content and chlorophyll fluorescence were also affected by increasing salinity levels (Table 5). Chlorophyll a, chlorophyll b, and total chlorophyll contents declined progressively as NaCl concentration increased, with the greatest reductions observed at 150 mM NaCl. A similar pattern was recorded for Fv/Fm, indicating that severe salinity reduced the maximum quantum efficiency of photosystem II.

Table 4. Effects of salinity levels on Na^+ , K^+ , and Na^+/K^+ ratio of different microgreen species

Parameter	NaCl (mM)	Radish	Broccoli	Red cabbage	Rocket
Na^+ content (mg g ⁻¹ DW)	0	2.18±0.09 ^c	2.34±0.10 ^b	2.56±0.11 ^{ab}	2.73±0.12 ^a
	50	5.42±0.18 ^d	5.86±0.21 ^c	6.72±0.23 ^b	7.35±0.25 ^a
	100	8.76±0.26 ^d	9.42±0.29 ^c	10.85±0.32 ^b	12.18±0.36 ^a
	150	12.34±0.35 ^d	13.18±0.39 ^c	15.46±0.43 ^b	17.26±0.48 ^a
K^+ content (mg g ⁻¹ DW)	0	24.82±0.72 ^a	23.76±0.69 ^{ab}	22.48±0.65 ^b	21.86±0.63 ^c
	50	22.65±0.68 ^a	21.48±0.64 ^b	19.86±0.59 ^c	18.72±0.56 ^d
	100	20.14±0.61 ^a	18.86±0.57 ^b	16.94±0.52 ^c	15.48±0.49 ^d
	150	17.92±0.54 ^a	16.72±0.51 ^b	14.38±0.46 ^c	12.96±0.42 ^d
Na^+/K^+ ratio	0	0.09±0.01 ^b	0.10±0.01 ^{ab}	0.11±0.01 ^{ab}	0.12±0.01 ^a
	50	0.24±0.01 ^d	0.27±0.01 ^c	0.34±0.02 ^b	0.39±0.02 ^a
	100	0.44±0.02 ^d	0.50±0.02 ^c	0.64±0.03 ^b	0.79±0.03 ^a
	150	0.69±0.03 ^d	0.79±0.03 ^c	1.08±0.04 ^b	1.33±0.05 ^a

Values are presented as mean ± standard error. Different lowercase letters within the same row indicate significant differences among species at the same NaCl concentration for the corresponding parameter ($p < 0.05$).

These reductions suggest that high salinity impaired photosynthetic pigment stability and photochemical efficiency in microgreens. Salinity-induced decreases in chlorophyll content are commonly associated with inhibition of chlorophyll biosynthesis, enhanced pigment degradation, oxidative damage to chloroplast structures, and nutrient imbalance caused by excessive Na^+ accumulation. Similar reductions in photosynthetic pigments under saline conditions have been reported

in several plant species and are often linked to restricted photosynthetic capacity and reduced growth performance (Santos, 2004; Ashraf and Harris, 2013; Acosta-Motos et al., 2017). The decline in Fv/Fm further indicates that photosystem II functionality was adversely affected by increasing salinity, particularly at the highest NaCl concentration. Fv/Fm is widely used as a non-invasive indicator of the maximum photochemical efficiency of photosystem II and is sensitive to stress-induced photoinhibition or damage to the photosynthetic apparatus (Maxwell and Johnson, 2000; Baker, 2008). In the present experiment, radish and broccoli maintained higher chlorophyll content and Fv/Fm values than red cabbage and rocket under saline conditions, especially at 100 and 150 mM NaCl. This response suggests a greater ability of radish and broccoli to preserve photosynthetic pigment integrity and PSII efficiency under salt stress. By comparison, the stronger reductions observed in red cabbage and rocket indicate greater susceptibility of their photosynthetic apparatus to salinity-induced damage. The maintenance of higher chlorophyll content and Fv/Fm values in radish and broccoli may have contributed to their relatively better shoot growth, biomass accumulation, and overall salinity tolerance.

Table 5. Effects of salinity levels on chlorophyll content and chlorophyll fluorescence of different microgreen species

Parameter	NaCl (mM)	Radish	Broccoli	Red cabbage	Rocket
Chlorophyll a (mg g ⁻¹ FW)	0	1.42±0.05 ^a	1.34±0.04 ^b	1.26±0.04 ^c	1.18±0.04 ^d
	50	1.36±0.05 ^a	1.27±0.04 ^b	1.16±0.04 ^c	1.06±0.03 ^d
	100	1.25±0.04 ^a	1.15±0.04 ^b	1.02±0.03 ^c	0.91±0.03 ^d
	150	1.08±0.04 ^a	0.98±0.03 ^b	0.84±0.03 ^c	0.72±0.03 ^d
Chlorophyll b (mg g ⁻¹ FW)	0	0.58±0.02 ^a	0.54±0.02 ^{ab}	0.50±0.02 ^b	0.47±0.02 ^c
	50	0.55±0.02 ^a	0.51±0.02 ^{ab}	0.45±0.02 ^b	0.41±0.01 ^c
	100	0.50±0.02 ^a	0.46±0.02 ^b	0.39±0.01 ^c	0.35±0.01 ^d
	150	0.43±0.02 ^a	0.39±0.01 ^b	0.32±0.01 ^c	0.27±0.01 ^d
Total chlorophyll (mg g ⁻¹ FW)	0	2.00±0.07 ^a	1.88±0.06 ^{ab}	1.76±0.05 ^b	1.65±0.05 ^c
	50	1.91±0.06 ^a	1.78±0.06 ^b	1.61±0.05 ^c	1.47±0.04 ^d
	100	1.75±0.06 ^a	1.61±0.05 ^b	1.41±0.04 ^c	1.26±0.04 ^d
	150	1.51±0.05 ^a	1.37±0.04 ^b	1.16±0.04 ^c	0.99±0.03 ^d
Fv/Fm	0	0.817±0.006 ^a	0.809±0.006 ^{ab}	0.798±0.007 ^b	0.789±0.007 ^b
	50	0.801±0.006 ^a	0.790±0.007 ^{ab}	0.768±0.007 ^b	0.751±0.008 ^c
	100	0.774±0.007 ^a	0.759±0.007 ^{ab}	0.724±0.008 ^b	0.702±0.008 ^c
	150	0.731±0.008 ^a	0.711±0.008 ^b	0.671±0.009 ^c	0.642±0.010 ^d

Values are presented as mean ± standard error. Different lowercase letters within the same row indicate significant differences among species at the same NaCl concentration for the corresponding parameter (p < 0.05).

To further explore the relationships among growth parameters and biochemical traits under different salinity levels, principal component analysis (PCA) was performed (Figure 4). The first two principal components explained most of the total variance, with PC1 accounting for 64.1% and PC2 explaining 35.7% of the variation. Growth-related parameters such as shoot length, root length, hypocotyl diameter, fresh weight, and dry weight were closely associated, forming a distinct cluster in the PCA space. Biochemical parameters, including total phenolics, flavonoids, and antioxidant activity, formed a separate cluster, indicating a strong positive correlation among these variables. The PCA also

revealed a clear separation of treatments along the salinity gradient, suggesting that increasing NaCl concentrations strongly influenced both growth performance and biochemical responses in microgreens. The opposite positioning of growth-related traits and antioxidant-related biochemical traits in the PCA space further supports the presence of a trade-off between biomass accumulation and stress-induced secondary metabolism under saline conditions. Treatments associated with higher salinity were more closely related to phenolic compounds, flavonoids, and antioxidant activity, whereas control and low-salinity treatments were more closely associated with growth and biomass traits. This pattern indicates that salinity shifted the physiological status of microgreens from growth-oriented development toward defense-oriented metabolism.

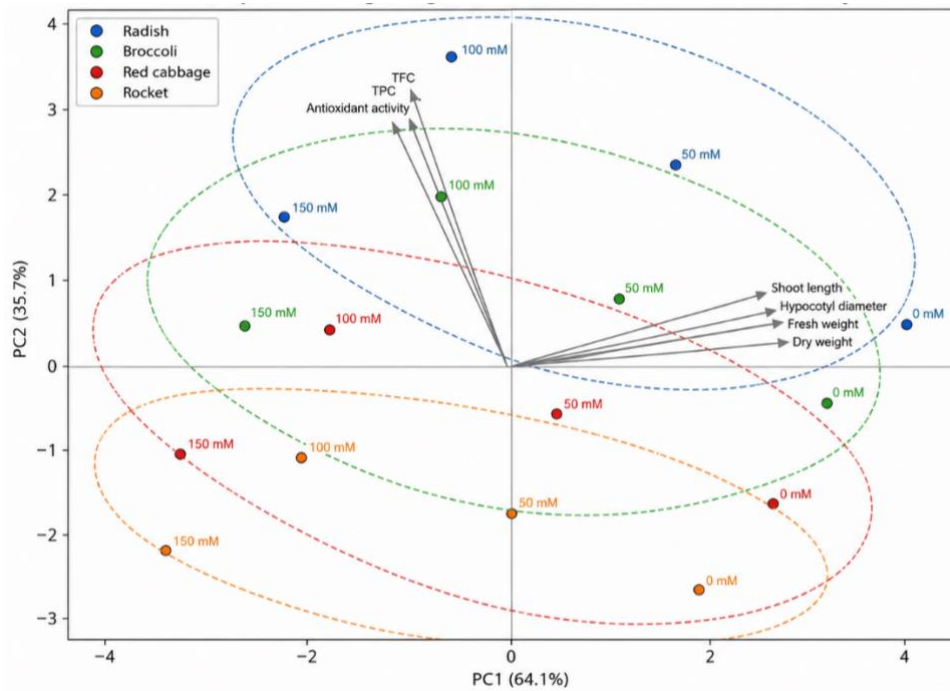


Figure 4. Principal component analysis (PCA) biplot showing the relationships among growth parameters (shoot length, root length, hypocotyl diameter, fresh weight, dry weight) and biochemical traits (total phenolic content, total flavonoid content, antioxidant activity) in microgreen species under different salinity levels

Conclusion

Salinity tolerance clearly affected both growth performance and antioxidant-related biochemical traits in the evaluated *Brassicaceae* microgreens. Increasing NaCl concentrations gradually reduced shoot length, root length, hypocotyl diameter, fresh weight, and dry weight, with the most pronounced growth inhibition observed at 150 mM NaCl. These reductions show that severe salinity limits early seedling development mainly through osmotic and ionic stress, which restrict water uptake, cell expansion, and biomass accumulation. In contrast, moderate salinity, particularly 100 mM NaCl, enhanced total phenolic content, total flavonoid content, and antioxidant activity in all species. This

response indicates that moderate salt stress may stimulate antioxidant-related secondary metabolism, whereas excessive salinity may disrupt metabolic activity and reduce the accumulation of protective compounds. The responses differed clearly among species. Radish and broccoli maintained relatively better growth and stronger antioxidant-related biochemical responses under saline conditions, while red cabbage and rocket showed greater growth inhibition. Based on these results, radish and broccoli may be suitable *Brassicaceae* microgreens for production under moderate saline conditions, especially when improvement of antioxidant-related quality traits is targeted. Further studies on osmolyte metabolism, lipid peroxidation, antioxidant enzyme activity, gene expression, and shelf-life performance would help clarify the mechanisms underlying salinity tolerance in microgreens.

Additional Declaration

Author Contributions

Ç.K. conceived and designed the study, conducted the experiments, analyzed and interpreted the data, and wrote the manuscript.

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Responsible Artificial Intelligence Statement

In this study, artificial intelligence tools were used only for basic language editing. I declare that I take full responsibility for any issues that may arise from the content generated by artificial intelligence.

Conflicts of Interest

The author declares that there is no conflict of interest regarding the publication of this article.

Ethics Approval

This study did not involve human participants or animals. Therefore, ethical approval and informed consent were not required.

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