PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.3969

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RESEARCH ARTICLE

# Effect of different NaCl concentrations on secondary metabolites and antioxidant activity in three radish (*Raphanus sativus* L.) varieties

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OPEN ACCESS

**ARTICLE HISTORY**

Received: 24 May 2024
Accepted: 06 August 2024

Available online
Version 1.0 : 22 November 2024

Check for updates

**Additional information**

**Peer review**: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Bungala LTC, Lim J, Kim K, Sathasivam R, Subramani P, Kim JK, Park SU. Effect of different NaCl concentrations on secondary metabolites and antioxidant activity in three radish (*Raphanus sativus* L.) varieties. Plant Science Today (Early Access). https://doi.org/10.14719/pst.3969

## Abstract

Salinity, a critical environmental stressor, imposes constraints on field crops' anatomical structure, growth, and physiology. This study has uniquely evaluated the effect of 0 (control), 100, and 200 mM NaCl on phenolic acids (PAs), total anthocyanin content (TAC), chlorophyll, and antioxidant activity in three radish varieties and contributes novel insights to the scientific co community. The salt concentration significantly influenced shoot length (SL), root length (RL), and fresh weight (FW) in radish sprouts. The varying salt concentrations did not affect *chlorophyll a*, but *chlorophyll b* and total chlorophyll content (TCC) increased the red and super red varieties. The total phenolic content (TPC) and total flavonoid content (TFC) registered a slight increase in some varieties under 100 mM salt concentration, with the highest accumulation of these secondary metabolites found in the super red variety. Five individual PAs were identified using high-performance liquid chromatography (HPLC) analysis, with salt treatment significantly affecting ferulic acid and *trans*-cinnamic acid concentrations. The lowest level of anthocyanin was found in all green varieties. Salinity stress of 100 and 200 mM affected the TAC in the red and super red varieties compared to the control. Furthermore, among the three varieties treated with NaCl, the super red $IC_{50}$ values displayed the highest 2,2,1-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic Acid (ABTS) free radical scavenging activity compared to the other treatments. These results suggest that a 100 mM NaCl concentration can be used as an inducer to improve the accumulation of phytochemicals in the radish seedlings 12 days after sowing (DAS).

## Keywords

Radish cultivars; salt stress; concentration; phytochemicals

## Introduction

Radish (*Raphanus sativus* L.) is an important root-grown vegetable consumed worldwide because of its nutritional content and benefits to human health and well-being (1). Recent studies on the nutritional properties of radish sprouts show that consumers are becoming more inclined towards consuming this type of food due to their high content of phytochemicals (2). However, the crop faces losses due to the various abiotic stresses.

Salt stress, among other abiotic stressors, has a tremendous impact on agricultural net productivity in various ways (Fig. 1) (3). A wide array of salt-inducing conditions causes the world's crop plants to suffer from poor growth and yield. Globally, salinity makes 25-30 % of the irrigated lands unproductive and affects over 2 billion acres of land through salt accumulation (4, 5). This impairs nutrient availability by increasing soil osmotic pressure, thus affecting nutrition and negatively impacting plant growth (6). There are claims that the plant's primary and secondary metabolites may be modified to face abiotic stresses (7, 8). However, plant type and salt concentration determine these physiological and phytochemical changes (9, 10). Plants can be categorized into two primary groups depending on their tolerance to salinity stress: halophytic and glycophytic (11). A glycophytic plant can allow a low salt concentration (about 50~250 mM) in the soil. However, a halophytic plant is tailored to tolerate a higher salt concentration (about 500~1000 mM) (12).

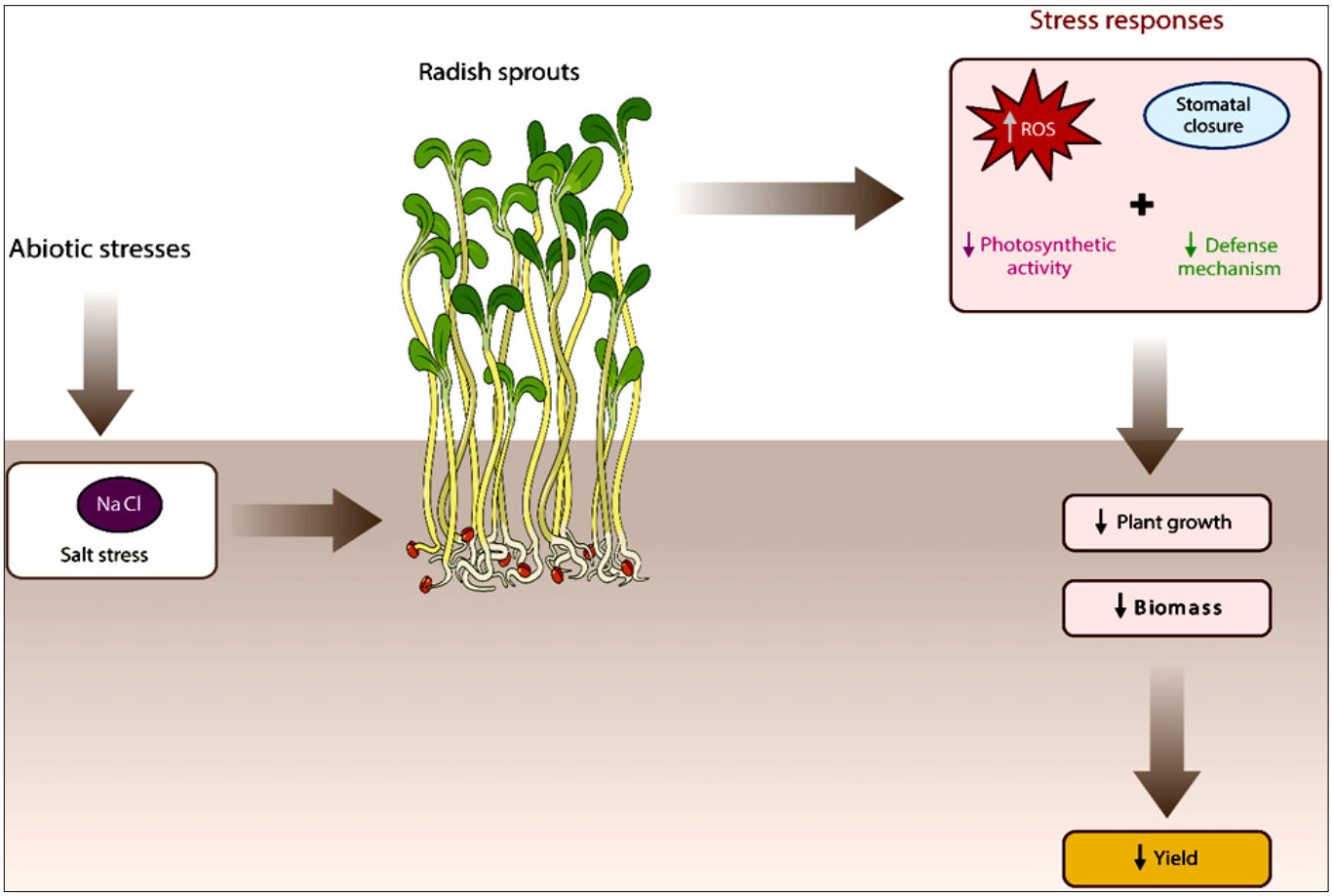


**Fig. 1.** A brief overview of the effect of salt stress on plants. Abbreviations: NaCl, sodium chloride; ROS, reactive oxygen species.

Secondary metabolites are crucial in various ecological interactions and serve multiple plant functions (13). Due to their distinct biological properties, these metabolites have a wide range of benefits to humanity, such as antimicrobial, antioxidant, or anticancer properties (14, 15). Yang et al. (16) used only one variety of radish to determine the effect of salt stress on phenolic compounds, glucosinolates, and antioxidant activity. A relationship exists between the production of secondary metabolites in radish sprouts and salt concentration. Therefore, this study analyzed the effect of different amounts of salt on phenolic, compounds, anthocyanin, chlorophylls, and antioxidant activity in three radish varieties.

## Materials and Methods

### *Plant material*

Seeds of three radish varieties, green, red, and super red, were obtained from Asia Seeds, Seoul, South Korea. The experiment was conducted in a controlled room. With five replications, the experiment was designed with a completely randomized design (CRD). Around 30 seeds were sowed per plastic pot containing substrate. In one LED plant growth chamber (Sejong Scientific Co., Sejong, South Korea), under controlled conditions (fluorescent light photon flux of 700 lux, 60–70% humidity, 24.8–26.8°C, and photoperiod of 16/8 h), the radish sprouts were grown. There were three treatments with different salt concentrations. Watering was carried out from the day of sowing with 200 mL of 0, 100, and 200 mM NaCl and every day with 100 mL for 12 days with the same NaCl concentrations. Twelve days after sowing (DAS), the radish sprouts 0.64cm

### *Growth measurements*

12 DAS, ten individual radish sprouts were selected randomly from each treatment for growth measurements. The shoot length (SL) and root length (RL) were determined using a meter ruler and expressed in cm. For fresh weight (FW), each radish sprout was weighed using balance and expressed in mg.

### Chlorophyll content

The leaf chlorophyll content was extracted and analyzed as described by Ritchie (17), with light changes. Fleetingly, leaf material was ground in liquid nitrogen, and 100 mg of powdered leaf material was mixed with 2 mL of cold ethanol (EtOH). The material was transferred to a 2 mL microcentrifuge tube and mixed well. Subsequently, 1 mL of the solution was transferred to a new microcentrifuge tube. Then, 200 µL of the sample was mixed well with 1 mL of cold EtOH. The mixture was incubated at 4°C for 1 h and centrifuged in a cooled microcentrifuge at 14,000 rpm for 5 min at 4°C. The collected supernatant was used to determine the total chlorophyll content (TCC) at 663.6 and 646.6 nm using a UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan). The chlorophyll content was estimated using the formula described previously by Porra and Scheer (18).

### Individual phenolic acid determination

Using the previous method described by Lee et al. (19), individual phenolic acids (PAs) were analyzed. 100 mg of the sample powder was added to 1.5 mL of aqueous methanol (MeOH). The samples were sonicated at 25°C for 1 h. After centrifugation at 10,000 rpm for 15 min, the collected supernatant was filtered through a 0.45 µm PTFE syringe filter. The high-performance liquid chromatography (HPLC) analysis system, gradient program, and protocols were according to a study conducted by Lee et al. (19). Calibration curves were used to identify and quantify PAs using retention times and spiking tests. The results were expressed in micrograms per gram of dry weight (µg/g dw).

### Measurement of the total anthocyanin content by spectrophotometry

For total anthocyanin content (TAC) extraction, 2 mL of 70% EtOH was mixed with 100 mg of a dried sample in a 5 mL tube and then sonicated for 1 h. After sonication, the sample was centrifuged at 12,000 rpm for 20 min at 4°C, and the supernatant was transferred to a new tube using a 0.45 µm PTFE hydrophilic syringe filter. The TAC was quantified using a pH differential method described by Febriany et al. (20). The absorbances of each mixture were assessed using a UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan) at 510 and 700 nm. The TAC was measured in triplicate for each sample extract. The TAC was estimated as cyanidin-3-glucoside equivalents:

$$\text{Total Anthocyanin content (mg/L)} = \frac{\text{A x MW x DF x CF}}{\varepsilon \text{ x } 1}$$

Where A: absorbance; MW: molecular weight of cyanidin-3-glucoside (449.2 g/mol); DF: dilution factor; CF: conversion factor (1,000), and ε: extinction molar coefficient (26,900 L/cm*mol). The TAC was expressed in milligrams in one gram of dry weight (mg/g dw).

### Quantification of total phenolics

The Folin–Ciocalteau method was employed with slight modifications to determine the total phenolic content (TPC) using spectrophotometry (21). The sample extracts were mixed with 70% MeOH, and 0.1 mL of diluted extricates were added with 0.5 mL of 2N Folin and Ciocalteu's phenol reagent, followed by incubation for 3 min at room temperature (RT). Afterwards, 4 mL of 10% Sodium carbonate was added, and for 90 min, the samples were incubated in darkness. The TPC was quantified using a calibration curve of gallic acid, and the results were expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g dw).

### Quantification of total flavonoids in extracts

The total flavonoid content (TFC) was assessed using a modified Saeed et al. (22) technique. All sample extracts were initially diluted to the same concentration as the TPC assay to quantify the TFC. In a reaction tube, 0.5 mL of diluted extracts, 2 mL of distilled water, and 0.15 mL of 5% Sodium nitrite were mixed, followed by incubation for 5 min at RT. The absorbances of each sample were determined at 415 nm using a UV-Vis spectrophotometer. The TFC was quantified using a quercetin calibration curve, and the results were stated as milligrams of quercetin equivalent per gram of dry weight (mg QE/g dw).

### DPPH radical scavenging activity

The DPPH (2,2,1-diphenyl-1-picrylhydrazyl) scavenging activity was determined following previous reports by De Menezes et al. (23). A solution of 2 mM DPPH was dissolved in 99.9% MeOH and successively diluting 100 µL of each extracted sample in a 96-well plate with concentrations of 31.25, 62.5, 125, 250, 500 and 1000 µg/mL. Before incubating the solution in darkness for 30 min. 100 µL of 2 mM DPPH solution was added to each well. The decrease in absorbance was quantified at 517 nm using a UV-Vis spectrophotometer. The below formula was employed to calculate the DPPH scavenging activity:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\left(A_{control} - A_{sample}\right)}{A_{control}} * 100$$

Where, $A_{control}$ is the absorbance in the well without the sample extract, and $A_{sample}$ is the absorbance of sample extracts with DPPH solution. In the plotted curve, the measure of antioxidants required to inhibit DPPH by 50% was stated in mg/ mL.

### ABTS radical scavenging activity

In this study, the method employed by Arnao et al. (24) was slightly modified to evaluate ABTS (2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic Acid) scavenging activity. The 7 mM ABTS powder was completely dissolved in a 2.5 mM Potassium persulfate solution. Following incubation, distilled water was utilized to adjust the absorbance of the ABTS buffer solution to 0.7±0.002 at 734 nm. Succeeding, 70% MeOH was used to dilute 50 µL of each sample gradually in a 96-well plate, ranging from 31.25 to 1000µg/mL. The absorbance was measured at 734 nm using a UV-Vis spectrophotometer, and 70% MeOH was added to the sample extracts for the control. The results were similar to those of the DPPH radical scavenging activity.

### *Reducing power assay*

Using the method outlined by Ferreira et al. (25) as a guideline, we determined the reducing power. A volume of 300 µL of sample extract was mixed with 300 µL of 0.2 M Phosphate buffer and 300 µL of 1% Tetrapotassium hexacyanoferrate and incubated for 20 min at 50°C. The solution was centrifuged at 10,000 rpm for 10 min. The absorbance was measured at 700 nm using a UV-Vis spectrophotometer by combining 500 µL of the supernatant with 500 µL of distilled water and 100 µL of 0.1% Ferric chloride. By transforming a Ferric cation ($Fe^{3+}$) to a Ferrous cation ($Fe^{2+}$), an increase in the absorbance value indicates the strength of the reducing force (26).

### *Data analysis*

The results are shown as mean values with standard deviations. Each experiment comprised three individual repetitions. The study employed analysis of variance (ANOVA) in SPSS 20 for statistical analysis, and Duncan's multiple range test was used to establish significance at the $p < 0.05$ level.

## Results

### *NaCl effect on plant growth parameters*

Plant growth declined after 12 days in NaCl-treated plants, but no symptoms of toxicity, such as chlorosis, leaf fall, or leaf necrosis, were observed. A decrease in plant development was observed in the 200 mM NaCl treated samples (Fig. 2). The application of different salt concentrations had significant effects on SL, RL, and FW in all treatments (Table 1). Plants under 100 and 200 mM NaCl treatments for the green variety showed a reduced SL by 25% and 49%, respectively, compared with the control. For RL and FW, plants treated with 200 mM NaCl exhibited 43% and 32% reductions, respectively, compared with the control. Plants under 100 and 200 mM NaCl treatments for the red variety had a reduced SL (24 and 30%, respectively). For RL, only plants under 200 mM NaCl treatment showed a reduction (37%) compared to the control. The FW of plants under 100 mM NaCl treatment increased by 26%, and that of plants treated with 200 mM NaCl was reduced by 30% compared to the control. Furthermore, the SL of the super red variety was decreased by 16% and 52% in plants under 100 and 200 mM NaCl treatments, respectively. For RL and FW, those treated with 200 mM NaCl were reduced by 40% and 55%, respectively, compared to the control.

### *Effect of NaCl on chlorophyll content*

In general, the NaCl concentration negatively affected the chlorophyll content in all three varieties (Table 2). In the green variety, at 100 and 200 mM NaCl concentrations, the *chlorophyll a* content was reduced by 45% and 47%, respectively, and TCC content by 38% and 41%, respectively. However, the *chlorophyll b* content showed statistically no difference. In the red variety, a reduction of 34% was found in 200 mM treated plants. A high *chlorophyll b* content was observed in plants treated with 100 mM NaCl (84%) compared to the control, and in plants under 200 mM treatment, there was a reduction of 73% compared to the control. The TCC showed a decrease only in plants treated with 200 mM NaCl. In the super red variety, *chlorophylls a* and *b* and TCC for the 100 and 200 mM NaCl treated plants

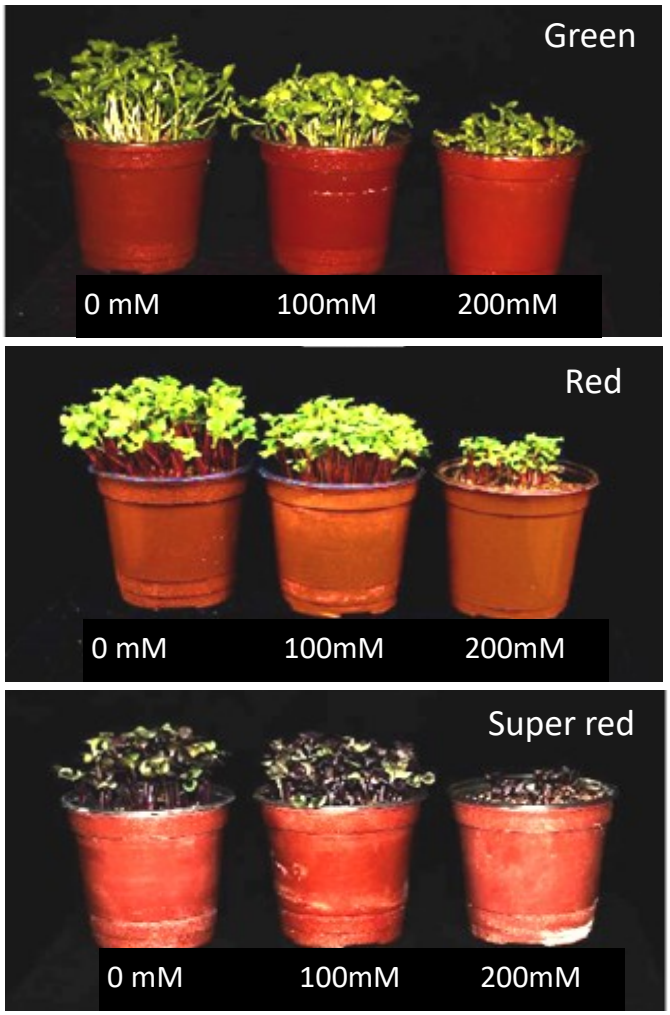


**Fig. 2.** Phenotypical growth of three radish varieties sprouts cultivated under different salt concentrations (0, 100, and 200 mM NaCl) for 12 DAS.

**Table 1.** Growth parameters of three radish varieties under different salt concentrations. Each value represents the mean of three replicates of 10 sprouts.

| Treatment/ Variety | SL (cm) | RL (cm) | FW (mg) |
|---|---|---|---|
| G0 | $5.33\pm0.87^{ab}$ | $12.18\pm1.54^{a}$ | $285.5\pm58.69^{ab}$ |
| G100 | $3.99\pm0.53^{d}$ | $12.83\pm1.05^{a}$ | $293.7\pm70.54^{ab}$ |
| G200 | $2.71\pm0.50^{e}$ | $7.0\pm1.26^{c}$ | $193\pm34.82^{c}$ |
| R0 | $5.75\pm0.91^{a}$ | $10.71\pm1.38^{b}$ | $194\pm33.27^{c}$ |
| R100 | $4.36\pm0.56^{cd}$ | $11.96\pm1.18^{ab}$ | $243.6\pm30.45^{b}$ |
| R200 | $4.02\pm0.59^{d}$ | $6.72\pm0.75^{c}$ | $136.3\pm26.28^{d}$ |
| SR0 | $4.78\pm1.15^{bc}$ | $10.8\pm1.66^{b}$ | $280.8\pm80.42^{ab}$ |
| SR100 | $4.02\pm0.71^{d}$ | $10.75\pm1.11^{b}$ | $310.2\pm41.5^{a}$ |
| SR200 | $2.29\pm0.44^{e}$ | $6.52\pm1.26^{c}$ | $126.3\pm33.79^{d}$ |

Same lowercase letters in the same column express a non-significant ($p<0.05$) difference, while different lowercase letters represent a significant difference ($p<0.05$) using Duncan's multiple range test. Lowercase letter a represents the highest SL, RL, and FW among the varieties in response to different salt stress concentrations, while lowercase letters e, c, and d represent the lowest SL, RL, and FW, respectively. The results are represented as the mean±SD of three independent replicates. Abbreviation: SL, shoot length; RL, root length, FW, fresh weight; G0, G100, G200, green variety under 0, 100, and 200 mM NaCl concentration; R0, R100, R200, red variety under 0, 100, and 200 mM NaCl concentration; SR0, SR100, SR200, super red variety under 0, 100, and 200 mM NaCl concentration.

**Table 2.** Chlorophyll content in three varieties of radish sprouts under different salt treatments.

| Treatment/ Variety | *Chlorophyll a* (µg/ mL) | *Chlorophyll b* (µg/ mL) | TCC (µg/ mL) |
|---|---|---|---|
| G0 | 6.47±0.07$^{a}$ | 1.22±0.59$^{ab}$ | 7.69±0.66$^{a}$ |
| G100 | 3.56±0.30$^{c}$ | 1.23±0.35$^{ab}$ | 4.79±0.15$^{cd}$ |
| G200 | 3.41±0.05$^{cd}$ | 1.16±0.12$^{ab}$ | 4.56±0.08$^{d}$ |
| R0 | 4.83±0.79$^{b}$ | 0.83±0.05$^{bc}$ | 5.66±0.78$^{bc}$ |
| R100 | 4.72±0.44$^{b}$ | 1.53±0.10$^{a}$ | 6.25±0.52$^{b}$ |
| R200 | 3.17±0.21$^{cd}$ | 0.22±0.08$^{d}$ | 3.39±0.24$^{e}$ |
| SR0 | 4.82±0.09$^{b}$ | 0.81±0.17$^{bc}$ | 5.63±0.14$^{bc}$ |
| SR100 | 2.51±0.19$^{cd}$ | 0.34±0.21$^{cd}$ | 2.84±0.14$^{ef}$ |
| SR200 | 2.29±0.17$^{d}$ | 0.07±0.05$^{d}$ | 2.36±0.16$^{f}$ |

The means and standard errors are calculated based on three replicates per treatment. Same lowercase letters in the same column express a non-significant (p<0.05) difference, while different lowercase letters represent a significant difference (p<0.05) using Duncan's multiple range test. Lowercase letter a represents the highest *chlorophyll a, b,* and TCC among the varieties in response to different salt stress concentrations, while lowercase letters d and f, represent the lowest *chlorophyll a, b,* and TCC. Abbreviation: TCC, total chlorophyll content; G0, G100, G200, green variety under 0, 100, and 200 mM NaCl concentration; R0, R100, R200, red variety under 0, 100, and 200 mM NaCl concentration; SR0, SR100, SR200, super red variety under 0, 100, and 200 mM NaCl concentration.

**Table 3.** TPC and TFC in the sprouts of three radish varieties under different salt treatments 12 DAS.

| NaCl Treatment | TPC (mg GAE/g dw) | TFC (mg GAE/g dw) |
|---|---|---|
| G0 | 4.12±00$^{f}$ | 1.49±0.09$^{cd}$ |
| G100 | 4.35±0.08$^{f}$ | 1.37±0.19$^{cd}$ |
| G200 | 2.01±0.04$^{g}$ | 1.06±0.14$^{d}$ |
| R0 | 6.81±0.13$^{d}$ | 2.06±0.39$^{cd}$ |
| R100 | 6.47±0.04$^{e}$ | 2.11±0.38$^{cd}$ |
| R200 | 6.42±0.08$^{e}$ | 2.26±0.76$^{c}$ |
| SR0 | 19.53±0.31$^{a}$ | 9.46±0.97$^{a}$ |
| SR100 | 19.27±0.15$^{b}$ | 9.66±0.83$^{a}$ |
| SR200 | 16.50±0.15$^{c}$ | 6.81±0.59$^{b}$ |

The means and standard errors are calculated based on three replicates per treatment. Same lowercase letters in the same column express a non-significant (p<0.05) difference, while different lowercase letters represent a significant difference (p<0.05) using Duncan's multiple range test. Lowercase letters *a* represent the highest TPC and TFC among the varieties in response to different salt stress concentrations, while lowercase letters *f* and *d* represent the lowest TPC and TFC, respectively. Abbreviation: TPC, total phenolic content; TFC, total flavonoid content; G0, G100, G200, green variety under 0, 100, and 200 mM NaCl concentration; R0, R100, R200, red variety under 0, 100, and 200 mM NaCl concentration; SR0, SR100, SR200, super red variety under 0, 100, and 200 mM NaCl concentration.

reduced 48, 52, 34, 92, 50, and 58%, respectively.

### *Effect of NaCl on total phenolic and flavonoid content*

High amounts of TPC and TFC were obtained in the super red variety, followed by the red variety. However, all varieties treated with 100 mM NaCl showed no difference in TFC compared to the control. Although there was no statistical difference compared to the control, the red and super red varieties treated with 100 mM NaCl showed higher values than their controls. The differences were found among the TPC controls, red, and super red varieties under 100 mM NaCl. Our findings revealed a general tendency towards an increase in TPC and TFC under 100 mM NaCl stress treatments (Table 3). In contrast, the results indicated that 200 mM NaCl stress was associated with a decrease in TPC in all three varieties (51% in green, 6% in red, and 16% in super red variety). Although the super red variety showed a 28% decrease in the TFC, the other two varieties showed no difference.

Five individual PAs, comprising three hydroxycinnamic acids, one hydroxybenzoic acid, and one flavan-3-ol, were identified using HPLC analysis (Table 4). Although there were no statistical differences, salt stress decreased the epicatechin content in the green variety and increased it in the red and super red varieties compared with the control. Salt treatment had a significant effect on ferulic acid and *trans*-cinnamic acid concentrations. However, salt treatment did not affect the final sinapic content. Furthermore, salt treatment affected the benzoic acid concentration in the green and super red varieties but had no effect in the super red variety.

### *Effect of NaCl on anthocyanin content*

The lowest TAC was found in the green variety. Salinity stress with 100 and 200 mM reduced TAC pigment formation by 6% and 42% in the red variety compared to the control. However, in the super red variety, plants exposed to 100 mM NaCl increased TAC formation by 28%

**Table 4.** Individual PAs content in the sprouts of three radish varieties under different salt treatments 12 DAS.

| NaCl Treatment | PAs content (µg/g dw) | | | | |
|---|---|---|---|---|---|
| | (-) Epicatechin | Ferulic acid | Sinapic acid | Benzoic acid | *trans*-Cinnamic acid |
| G0 | 106.52±0.20$^{ab}$ | 11.27±0.69$^{d}$ | 291.51±25.77$^{b}$ | 86.41±9.85$^{d}$ | 27.03±0.21$^{bc}$ |
| G100 | 103.04±0.30$^{5b}$ | 11.94±0.31$^{cd}$ | 192.07±5.95$^{c}$ | 85.59±6.43$^{d}$ | 26.42±0.41$^{c}$ |
| G200 | 102.26±0.44$^{b}$ | 14.09±0.90$^{bcd}$ | 203.44±14.79$^{c}$ | 149.77±39.16$^{abc}$ | 28.21±1.02$^{ab}$ |
| R0 | 116.10±3.27$^{a}$ | 12.74±0.71$^{cd}$ | 274.42±37.56$^{b}$ | 165.86±30.74$^{ab}$ | 26.25±0.32$^{c}$ |
| R100 | 117.31±9.81$^{a}$ | 13.40±0.68$^{bcd}$ | 204.43±11.54$^{c}$ | 187.45±29.37$^{a}$ | 27.13±0.5b$^{c}$ |
| R200 | 108.29±3.23$^{ab}$ | 14.42±1.53$^{bcd}$ | 175.75±11.67$^{c}$ | 145.83±41$^{abc}$ | 29.02±1.48$^{a}$ |
| SR0 | 116.10±3.27$^{a}$ | 15.83±2.02$^{bc}$ | 471.13±37.53$^{a}$ | 124.94±10.39$^{bcd}$ | 25.79±0.08$^{c}$ |
| SR100 | 117.59±8.28$^{a}$ | 16.97±2.78$^{b}$ | 255.49±6.01$^{b}$ | 98.47±0.22$^{cd}$ | 25.95±0.11$^{c}$ |
| SR200 | 108.15±1.76$^{ab}$ | 27.26±3.38$^{a}$ | 209.36±8.98$^{c}$ | 75.92±2.23$^{d}$ | 27.23±0.69$^{bc}$ |

The means and standard errors are calculated based on three replicates per treatment. Same lowercase letters in the same row express a non-significant (p<0.05) difference, while different lowercase letters represent a significant difference (p<0.05) using Duncan's multiple range test. Lowercase letter *a* represents the highest PAs content within the variety in response to different salt stress concentrations, while the lowercase letter *d* represents the lowest PAs content. Abbreviation: PAs, phenolic acids; G0, G100, G200, green variety under 0, 100, and 200 mM NaCl concentration; R0, R100, R200, red variety under 0, 100, and 200 mM NaCl concentration; SR0, SR100, SR200, super red variety under 0, 100, and 200 mM NaCl concentration.

and showed a slight reduction by 3% when exposed to 200 mM NaCl stress (Fig. 3). The present findings reveal that plants with a high TAC respond to an adequate salt concentration by producing more TAC.

### *Effect of NaCl on antioxidant activity*

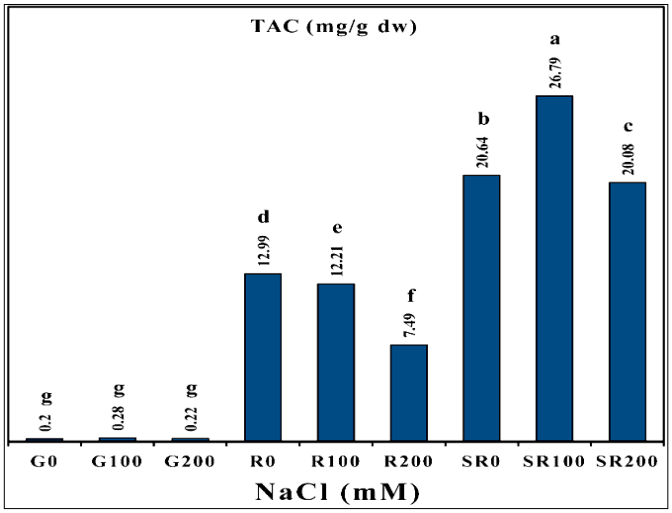


**Fig. 3.** Effect of salt concentrations (0, 100, and 200 mM NaCl) on TAC in the sprouts of the three radish varieties 12 DAS. The means and standard errors are calculated based on three replicates per treatment. Histogram bars with the same lowercase letters denote a non-significant ($p<0.05$) difference, while different lowercase letters specify a significant difference ($p<0.05$). The lowercase letter *a* represents the highest TAC among the varieties in response to different salt stress concentrations, while the lowercase letter *g* represents the lowest TAC. Abbreviation: TAC, total anthocyanin content; G0, G100, G200, green variety under 0, 100, and 200 mM NaCl concentration; R0, R100, R200, red variety under 0, 100, and 200 mM NaCl concentration; SR0, SR100, SR200, super red variety under 0, 100, and 200 mM NaCl concentration.

Plant extracts' DPPH and ABTS free radical scavenging activities were evaluated at all salt concentrations to determine the antioxidant activity. Among the three varieties exposed to NaCl, the $IC_{50}$ values of the super red variety showed a high DPPH and ABTS free radical scavenging activity compared with other treatments (Table 5). These effects may be attributed to this variety's higher TPC and TFC. Individually, in the green and red varieties, the $IC_{50}$ values showed a high DPPH and ABTS free radical scavenging activity in plants submitted to 200 mM NaCl. However, in the super red variety, the differences in the $IC_{50}$ values for DPPH free radical scavenging were insignificant among the NaCl treatments. Plants treated with 200 mM NaCl presented a slightly high value. $IC_{50}$ values for ABTS free radical scavenging activity were lower in plants under 200 mM NaCl than in the control and plants under 100 mM NaCl.

**Table 5.** Effect of different salt concentrations on antioxidant activity ($IC_{50}$ values) in the sprouts of three radish varieties 12 DAS.

| Treatment/Variety | $IC_{50}$ of DPPH (mg/ mL) | $IC_{50}$ of ABTS (mg/ mL) |
|---|---|---|
| G0 | $2.20\pm0.19^{e}$ | $0.93\pm00^{f}$ |
| G100 | $2.28\pm0.01^{e}$ | $1.18\pm0.03^{g}$ |
| G200 | $1.55\pm0.06^{c}$ | $0.88\pm0.02^{e}$ |
| R0 | $1.45\pm0.08^{bc}$ | $0.71\pm0.01^{c}$ |
| R100 | $1.65\pm0.09^{d}$ | $0.77\pm0.01^{d}$ |
| R200 | $1.11\pm0.09^{b}$ | $0.70\pm0.01^{c}$ |
| SR0 | $0.53\pm0.02^{a}$ | $0.36\pm00^{a}$ |
| SR100 | $0.52\pm0.02^{a}$ | $0.37\pm00^{a}$ |
| SR200 | $0.55\pm0.02^{a}$ | $0.38\pm00^{b}$ |

The means and standard errors are calculated based on three replicates per treatment. Same lowercase letters in the same column denote a non-significant ($p<0.05$) difference, while different lowercase letters specify a significant difference ($p<0.05$) using Duncan's multiple range test. Lowercase letter *a* represents the best $IC_{50}$ values among the varieties in response to different salt stress concentrations, while lowercase letter *f* represents the worst $IC_{50}$ values. Abbreviation: G0, G100, G200, green variety under 0, 100, and 200 mM NaCl concentration; R0, R100, R200, red variety under 0, 100, and 200 mM NaCl concentration; SR0, SR100, SR200, super red variety under 0, 100, and 200 mM NaCl concentration.

## Discussion

### *Effect of NaCl on plant growth parameters*

The present study evaluated the effectiveness of treating three radish varieties with two different NaCl concentrations. Salt stress inhibits seed germination by decreasing enzyme activity (27). The growth of quinoa plants treated with NaCl decreased after 15 days compared to the control (28). Analogous outcomes were obtained in the current study, where after 12 days of NaCl treatment, a diminution in plant growth was observed. Fariduddin et al. (29) reported that salt stress reduced leaf area and SL by 34% and 47% in *Brassica napus*. These findings are similar to those found in the current study, where the two different concentrations of NaCl applied decreased SL in all three radish varieties. According to Yuan et al. (16), radish sprouts treated with NaCl had a markedly higher growth rate than controls after 5 and 7 days of treatment. These results comply with the outcomes presented in this study, where the plants treated with 100 mM NaCl showed higher values than the controls. However, the FW of 5- and 7-day-old buckwheat sprouts was not affected by low NaCl concentrations (10 mM) but reduced by 14 and 18%, respectively, under high NaCl concentrations (>100 mM) (30). Moreover, the FW of quinoa was not significantly affected by salt stress at 100 mM NaCl, but under 300 mM, there was a reduction (up to 47%) compared to the control (28). SL and RL decreased in *Glaux maritima* and *Spergularia marina* plants under high salinity conditions (300–500 mM) (31). Kaouther et al. (32) also showed that increasing salinity stress on chilli pepper cultivars had a negative impact on RL. Similar results were found in the current research, in which, in all three varieties, the size of the roots decreased when submitted to a high salt concentration.

### *Effect of NaCl on chlorophyll content*

Photosynthetic pigments are crucial for photosynthesis as they transform light into chemical energy; however, salt stress significantly impairs these pigments (33). The evaluation of two accessions of centipede grass showed that salinity treatment decreased the chlorophyll content in green-stemmed accessions, whereas the effect on purple-stemmed accessions was less noticeable (34). The present study found different results, with the chlorophyll content being less affected in the green variety than in the red and super red varieties. According to Tanaka et al. (35), salinity treatment did not affect the TCC in basil; it vaguely reduced the content in sage and considerably declined in thyme and oregano. These results are similar to those found in the present study. According to Taïbi et al. (36), when the NaCl concentration was increased in common beans, the

TCC was decreased by 52% and 57% in high- and low-yielding genotypes, respectively, compared to the control under high salinity.

Additionally, the *chlorophyll b* content was reduced by 33% and 43% in the high- and low-yielding genotypes, respectively. Salinity significantly reduced the TCC and considerably decreased the *chlorophyll b* after 10 days of treatment (37). The results presented in this study showed an opposite relationship between salinity and chlorophyll content (100 and 200 mM). Under moderate salinity (100 mM NaCl) in quinoa, no substantial variations in *chlorophylls a* and *b* and TCC were observed compared to the control (28). These findings do not agree with those presented in the current study. This assumes that not all plants respond similarly when exposed to the same salt concentration.

### *Effect of NaCl on total and individual phenolic compounds*

Secondary metabolites, called phenolic compounds, are crucial for shielding plants from oxidative stress caused by salt stress (38). PAs have antioxidant action, elevate antioxidant levels, and enhance the detoxification of reactive oxygen species, which likely increases salinity resistance (39). The current outcomes showed improved TPC in plants under 100 mM NaCl treatment in all three varieties but without substantial differences compared to their controls. Yuan et al. (16) also found increased radish sprouts after treatment with 100 mM NaCl. The accumulation of phenolic compounds in plants caused by salt stress may vary depending on plant type; phenolic compounds did not accumulate in lettuce (40), broccoli (41) or *Spergularia marina* (31) after exposure to NaCl stress, while the phenolic content increased in red pepper (42), maize (43), *Salvia mirzayanii* (44), and *G. maritima* (31) after salt stress. Upon salt stress, two species of thyme plants showed a higher accumulation of phenolic compounds as part of antioxidant defense (45). In the present study, sinapic acid was found to be a significant PA, in addition to benzoic acid. A study by Linić et al. (46) found similar results, in which sinapic acid was a substantial component in three *Brassica* species (Chinese cabbage, white cabbage, and kale) under 50, 100, and 200 mM NaCl concentrations. It is thought that the accumulation of sinapic acid facilitates plants' adaptation to environmental stresses. Sarker and Oba (47) also found an increase in TPC, comprised of caffeic acid, sinapic acid, and trans-cinnamic, under saline conditions. However, in two cardoon genotypes submitted to short- and long-term saline stress, the major constituent of the leaves was chlorogenic acid (48). Salt stress triggered an improvement in TFC in the leaves of the *Aegilops cylindrica* genotype but decreased in two wheat cultivars (49). Additionally, a substantial rise in TFC was observed in three pea varieties under high salt stress levels (50). These results are in accordance with those found in the present study, specifically in the red and super red varieties. This shows that, within the same species, the accumulation of secondary compounds can respond differently in varieties when subjected to the same stressor.

### *Effect of NaCl on anthocyanin content*

Among the known mechanisms of plant resistance to abiotic stress, the increased accumulation of anthocyanins has been reported to improve plant resistance to salt stress (51-53). According to Liang et al. (54), anthocyanins are enhanced during the salt stress response; however, salt stress may also reduce the anthocyanin content in salt -sensitive plant species. Similar results were found in the current study, where we noticed that the radish varieties produced more TAC under adequate NaCl concentrations than the control. For instance, in *Hyssopus officinalis* L. plants, TAC was considerably augmented under salt stress treatment (55). Mbarki et al. (56) reported that in colored wheat genotypes, a higher TAC was retained than in non-colored genotypes. Eryılmaz (57) showed that TAC improved when NaCl concentrations were augmented in several tomato and red cabbage organs. These outcomes agree with those we observed in the current study, in which the super red variety showed high anthocyanin accumulation under 100 mM NaCl treatment. Salt stress-induced anthocyanins have also been reported in black glutinous rice, allowing plants to resist stress (58). These results show that plant species that naturally have a high anthocyanin content tend to respond with an increase in the production of this pigment when subjected to concentrations not exceeding 100 mM NaCl.

### *Effect of NaCl on antioxidant activity*

Salt boosted antioxidant activity, regardless of the methodology employed to evaluate it (DPPH or ABTS), possibly due to the increased production of compounds with antioxidant activity (35). These results agree with our findings, in which an increase in antioxidant activity was correlated with an increase in salt concentration. Antioxidant enzyme activity rose dramatically in barley roots exposed to salt stress (59). A study on *Catharanthus roseus* suspension cells found that salt stress impacted antioxidant enzyme activity (60). Similar findings were observed in potato seedlings (61) and common beans (62) exposed to NaCl stress. According to Valifard et al. (44), the effects of leaf extracts on DPPH free radical scavenging activity were highest at 6.8 dS $m^{-1}$ NaCl. Lim et al. (30) found that increasing NaCl concentrations increased buckwheat sprout DPPH radical scavenging activity. Similar results are shown in the present study. Salt stress significantly increased antioxidant activity in *Hyssopus officinalis* plants (55). In addition, basil plants treated with NaCl enhanced the content of important bioactive compounds, namely flavonoids, carotenoids, and phenolic acid derivatives, resulting in more significant antioxidant activity than untreated basil plants (63). Depending on the plant type and salt exposure concentration, plants can respond positively (decreasing antioxidant activity) and negatively (increasing antioxidant activity).

## Conclusion

Plants are constantly exposed to environmental conditions. The effects of salt stress on growth parameters,

phytochemical accumulation, and antioxidant activity in radish seedlings varied according to NaCl concentration and genetic background (variety). These findings suggested that salt stress (100 mM NaCl treatment) might improve the phytochemical content of radish sprouts and that sprout development under salt stress can be a beneficial technique for inducing improvement in phytochemicals, providing the ability to tolerate abiotic and biotic stressors. This can also help humans by providing healthier food for their daily diet.

## Acknowledgements

This research work was supported by Startup Pioneering in Research and Innovation (SPRINT) through the Commercialization Promotion Agency for R&D Outcomes (COMPA) grant funded by the Korean government (Ministry of Science and ICT) (2710001946) and this research was also supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MSIT) (No. RS-2024-00440478), Republic of Korea.

## Authors' contributions

JKK and SUP contributed to the study's conception and design. LTDCB, JL, KK, RS, and PS performed material preparation, data collection, and analysis. LTDCB wrote the first draft of the manuscript, and all authors commented on previous versions. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest**: Authors do not have any conflict of interests to declare.

**Ethical issues**: None.

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