Article

Accumulation of Phenylpropanoids in Tartary Buckwheat (*Fagopyrum tataricum*) under Salt Stress

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Abstract: Salinity stress affects plants by reducing the water potential and causing ion imbalance or disturbances in ion homeostasis and toxicity. Salinity stress frequently causes both osmotic and ionic stress in plants, resulting in the increase or decrease of certain secondary metabolites in plants. In this study, the effect of NaCl treatment on the nutritional quality of tartary buckwheat plants was studied by conducting an HPLC analysis of phenylpropanoid and anthocyanin content. It was observed that there was no significant change of color in tartary buckwheat during salt treatment. The accumulation of most phenylpropanoid compounds increased slightly in response to the NaCl concentration. The total phenylpropanoid content in tartary buckwheat was the highest at 100 mM NaCl treatment. Seven-day-old wheat plantlets treated with 100 mM NaCl for 2, 4, 6, and 8 days showed the highest accumulation of total phenylpropanoids at day 8 after treatment, while the content of most phenylpropanoids was higher than that in the control during this period. Although the development of tartary buckwheat slightly decreased with NaCl treatment and the accumulation of anthocyanin compounds did not change in plants with a diffident NaCl concentration and time treatment, the results suggest that the salinity treatment of tartary buckwheat causes antioxidant activity improvement by inducing an accumulation of flavonoid and phenolic compounds. However, since the anthocyanin content did not increase, the antioxidant effect of the treatment is not expected to be significant.

Keywords: tartary buckwheat; phenylpropanoid; anthocyanin; salinity stress; NaCl

1. Introduction

High salinity stress induces osmotic stress and removes water from the cytoplasm, causing cell dehydration, which reduces the vacuole volume and cytoplasm. It is required for plants to cope with two main kinds of stress, namely, ionic stress and osmotic stress against high salinity. Osmotic stress indicates that salinity levels rise outside the roots and immediately reach the plant by inhibiting water ingestion, cell swelling, and subsequent bud development [1]. Ionic stress results from an excess of toxic ions, such as Na$^+$, which leads to an increase in the chlorophyll content and necrosis of leaves and a decrease in essential cell metabolic activities, including photosynthesis [1,2]. Salinity stress frequently causes both osmotic and ionic stress in plants, resulting in an increase or decrease of certain secondary

metabolites in plants [3]. Anthocyanins have been reported to accumulate under salinity stress [4]. In contrast, salinity stress decreases the accumulation of anthocyanin in salinity-sensitive plants [5]. For example, proline accumulates doubly fast in the roots of alfalfa, a halophyte, when compared with the situation in glycophytes [6]. In tomato (*Solanum lycopersicum*) cultivars under salinity stress, the accumulation of endogenous jasmonic acid was increased [7]. The accumulation and synthesis of phenolic compounds usually increase the levels of physiological or nervous activity in response to plant stress [8,9]. It has been reported that the accumulation of phenolic compounds in other tissues increased under an increasing salt concentration in many plants [10]. It has also been reported that red peppers under moderate salinity show an increased accumulation of total phenolic compounds [11].

Unlike most cereals, common buckwheat (*Fagopyrum esculentum*), which belongs to the Polygonaceae family, is usually used as an alternative cereal. Buckwheat seed can be stored for a long time, without significant chemical alteration, because it contains antioxidant phytochemicals, such as phenolic acids, flavonoids, and tocopherols [12]. Rutin, which is a flavonol glycoside plant metabolite, has anti-inflammatory, anticarcinogenic, and antioxidant properties. It can also decrease the fragility of blood vessels associated with hypertension and hemorrhagic disease in humans [13]. Buckwheat has more rutin than most plants. Isovitexin and rutin are the only described flavonoids of buckwheat seed. Buckwheat hulls also have rutin, orientin, quercetin, isoorientin, vitexin, and isovitexin [12]. Differences in the antioxidant properties of buckwheat are mostly due to the cultivars and environmental effects [13]. Common buckwheat (*Fagopyrum esculentum*) and tartary buckwheat (*Fagopyrum tataricum*) are two major varieties of buckwheat. Common buckwheat and tartary buckwheat are used worldwide. Common buckwheat seeds have between 0.13 and 0.36 mg rutin/g dry weight (DW); in contrast, tartary buckwheat seeds have between 7 and 8 mg rutin/g DW [14,15]. Furthermore, tartary buckwheat sprouts contain between 50 and 60 mg rutin/g DW—2.2 folds higher than that of similarly cultivated common buckwheat sprouts [16].
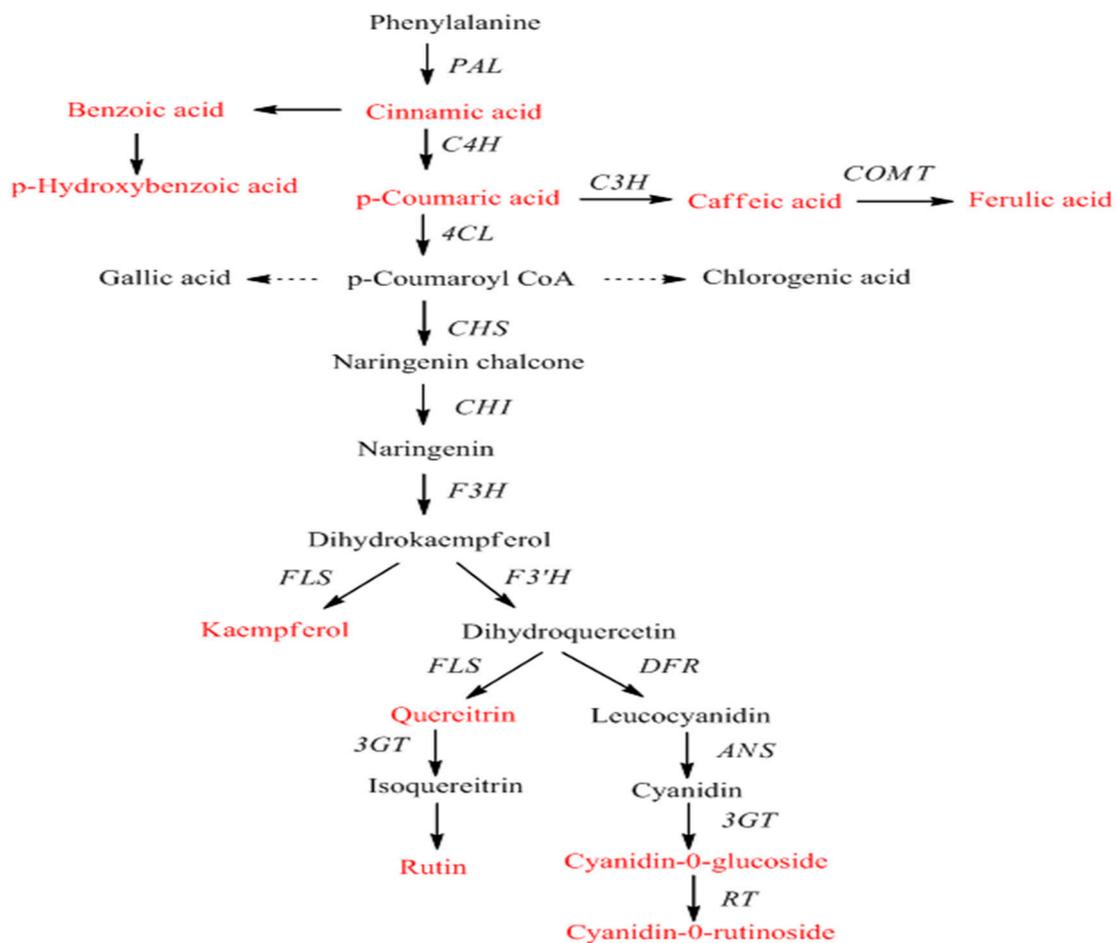
Phenylpropanoids are a group of organic compounds synthesized by plants from the amino acids phenylalanine and tyrosine [17]. This group's name indicates the presence of six carbons in the structure of the member compounds. The aromatic phenyl group and 3-carbon propene tail of coumaric acid are the main intermediates in phenylpropanoid biosynthesis. From 4-coumaroyl-CoA, numerous natural products are biosynthesized, such as lignols, isoflavonoids, aurones, catechin, flavonoids, stilbenes, coumarins, and phenylpropanoids (Figure 1) [18]. Phenylpropanoids, found in many plants, defend plants from pathogens and herbivores; protect them from ultraviolet light; and mediate plant–pollinator interactions, acting as scent compounds and floral pigments. The flavonoid synthesis pathway mainly includes derivatives such as flavonols, anthocyanins, and proanthocyanins [19–21]. Their antioxidant properties and structural variety play a physiological role when plants interact with biological and abiotic environments [19,22–26]. Furthermore, studies on the correlation between diverse hormones and flavonoids in the regulation of developmental processes, including seed size and fertility, are gaining attention [22,27,28]. These phenylpropanoids are responsible for the agricultural, nutritional, and industrial value of crops. Flavonoids affect the quality of food, fruit, and seed and the astringency of the plant product [26,27]. These phenylpropanoids are good models for an analysis of the extensive diversity of genetic, epigenetic, cellular, and biochemical processes, because of their variety and ease of isolation. It is supposed that they establish the well-studied metabolic and regulatory pathways in plants [29].

In this study, we investigated the change in metabolomics of tartary buckwheat under salinity treatment using various concentrations of NaCl and different treatment times. Using an HPLC analysis system, the accumulation of phenylpropanoids and anthocyanins under NaCl treatment was studied. This study provides deeper insights into the change in metabolomics of tartary buckwheat under salinity stress.

2. Materials and Methods

2.1. Plant Materials

Daegwan No.3-3 seeds, representing one tartary buckwheat cultivar, which were provided by the Rural Development Administration (RDA), Korea, were used for this experiment. The soil used this experiment was horticultural soil and buckwheat seeds were sown in pots. NaCl aqueous solution was made with distilled water and sodium chloride. Buckwheat seedlings, which were 7 days old, were treated with different NaCl concentrations (0, 100, 200, and 300 mM) in a plant chamber under 16 h/8 h (light/dark) condition at 25 °C. After treatment for 4 weeks, buckwheat seedlings were harvested to investigate the response to salinity stress. For time treatment experiments, we sowed the seeds in pots and these seeds were grown in a plant chamber for 6 weeks at the temperature of 25 °C and a 16 h/8 h (light/dark) condition. Buckwheat, which was 6 weeks old, was treated with 100 mM NaCl at different times for finding the optimal treatment time. The samples were harvested on days 2, 4, 6, and 8 after treatment, and all samples were put in liquid nitrogen and then freeze-dried for an analysis of the phenylpropanoid and anthocyanin content.

Figure 1. Schematic representation of flavonoid biosynthesis in tartary buckwheat. PAL, phenylalanine ammonium lyase; C4H, cinnamic acid 4-hydroxylase; 4CL, 4-coumarate-CoA ligase; CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone-3-hydroxylase; F3′H, flavonoid-3′-hydroxylase; FLS, flavonol synthase; DFR, dihydroflavonol reductase; ANS, anthocyanin synthase; 3GT, flavonoid 3-O-glucosyltransferase; and RT, 3-Orhamnosyltransferase. Red colored words indicate that the compounds were detected in this study.

2.2. Extraction and HPLC Analysis of Anthocyanin

To analyze the anthocyanin content, we extracted 100 mg dried samples with 2 mL water:formic acid (95:5 *v/v*), and sonicated the solution for 20 min. After centrifuging the sample at 12,000× *g* for 10 min, the supernatant was filtered through a 0.45 µm hydrophilic PTFE syringe filter (Ø, 13 mm, Advantec, Tokyo, Japan) in a brown vial. Anthocyanins were detected by Agilent 1200 series HPLC (Santa Clara, CA, USA) at a wavelength of 520 nm equipped with a Security Guard Cartridges Kit AQ C18 column (Phenomenex, Torrance, CA, USA) and a Synergi 4 µm POLAR-RP 80A column (250 × 4.6 mm i.d., particle size 4 µm; Phenomenex, Torrance, CA, USA). The mobile phase consisted of a mixture of (A) water:formic acid (99.5:0.5, *v/v*) and (B) acetonitrile:formic acid (99.5:0.5, *v/v*). The mobile phase program was set as follows: the reaction was started with 5% solvent B; was increased to 10% solvent B until 8 min; was increased to 13% solvent B until 13 min and was maintained until 15 min; was increased to 15% solvent B until 18 min and maintained until 25 min; was increased to 18% solvent B until 30 min and maintained until 35 min; was increased to 21% solvent B until 40 min and maintained until 45 min; and was then decreased to 5% solvent B at 45.1 min and maintained until 50 min.

2.3. Extraction and HPLC Analysis of Phenylpropanoid Compounds

To analyze the phenylpropanoid content, we extracted 100 mg dried powder with 2 mL 80% methanol, and sonicated the sample for 1 h at 37 °C. After centrifugation the sample at 12,000 rpm for 15 min, the supernatant was filtered through a 0.45 µm hydrophilic PTFE syringe filter (Ø, 13 mm, Advantec, Tokyo, Japan). Phenylpropanoids were detected using a Futecs model NS-4000 HPLC apparatus (Daejeon, Korea) equipped with a C18 column (250 × 4.6 mm, 5 µm; RStech, Daejeon, Korea) at a wavelength of 280 nm. The mobile phase consisted of a mixture of (A) water:acetic acid (99.85:0.15 *v/v*) and (B) methanol. The initial mobile phase composition was as follows: 5% solvent B, followed by a linear gradient from 0% to 80% solvent B over 93 min, which was then held at 5% solvent B for an additional 5 min. The column was maintained at 30 °C, the flow rate was 1.0 mL/min, and the injection volume was 20 µL. Different compounds were quantified based on peak areas, and the concentrations were calculated as equivalents of representative standard compounds.
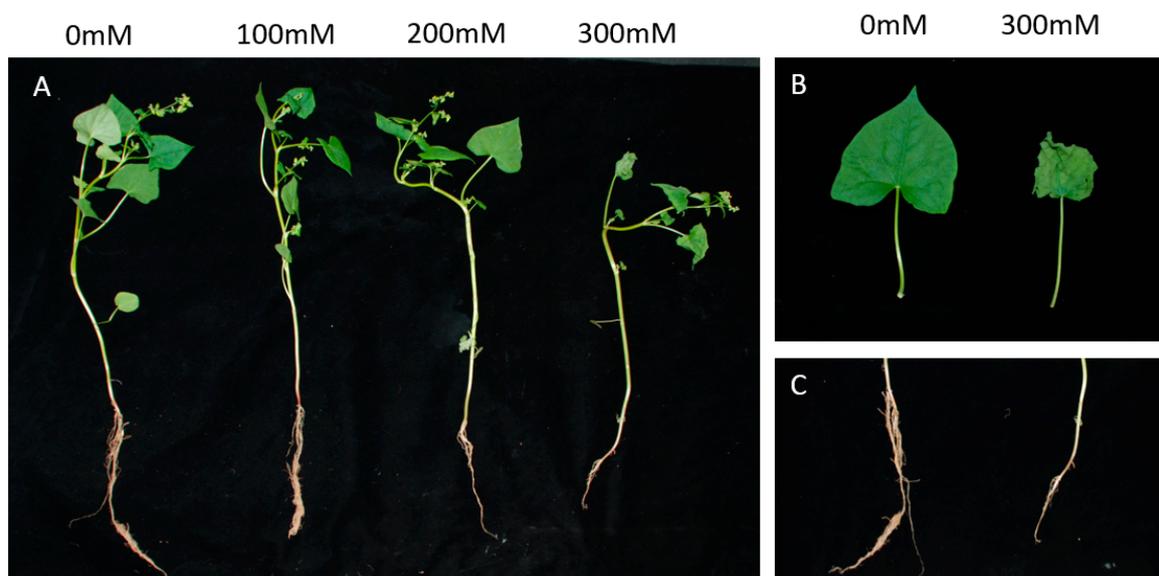
2.4. Statistical Analysis

Statistical analysis was performed with SPSS statistics 22 software (IBM Corp., Armonk, NY, USA) using Student's *t* test or an analysis of variance (ANOVA) with Tukey's honestly significant difference test. All data are given as the mean values and standard deviation or standard error of triplicate experiments.

3. Results

3.1. Phenotype of Tartary Buckwheat Plants under Salinity Stress

To find out whether tartary buckwheat plants are influenced by salinity stress, we examined the phenotype of tartary buckwheat after 4 weeks of treatment with different concentrations of NaCl (0, 100, 200, and 300 mM) (Figure 2). Sensory evaluation showed no overall color change in tartary buckwheat under control and salt treatments. However, the development of tartary buckwheat treated by NaCl was decreased with an increase in NaCl concentration from 0 to 300 mM. At a 300 mM NaCl concentration, the leaves of tartary buckwheat were dry and the roots were greatly inhibited (Figure 2).

Figure 2. Morphology of whole plants (**A**), leaves (**B**), and roots (**C**) in response to salt stress.

3.2. Analysis of Phenylpropanoid Content under Different NaCl Concentrations

To investigate if the accumulation of phenolic compounds is influenced by salinity stress, we identified and quantified the phenolic compound contents under NaCl treatment by performing HPLC analysis. A total of eight phenolic compounds, namely, 4-hydroxybenzoic acid, chlorogenic acid, caffeic acid, ferulic acid, benzoic acid, rutin, trans-cinnamic acid, and quercetin, were identified and quantified (Table 1). Overall, 100 and 200 mM salt treatment showed slightly more phenylpropanoid accumulation. In particular, the contents of phenolic acid, 4-hydroxybenzoic acid, benzoic acid, and trans-cinnamic acid, and flavonoids, namely, rutin and quercetin, were increased. The quantity of phenolic acid (4-hydroxybenzoic acid) accumulated was 0.17 mg/g at 200 mM and was 1.17 folds higher than the control. Additionally, the quantity of benzoic acid accumulated was 0.16 mg/g and was 1.25 folds higher than the control. The quantity of flavonoid (rutin) accumulated was 14.98 mg/g at 100 mM and was 1.16 times higher than the control, and the quantity of quercetin accumulated was 0.15 mg/g and was 1.20 times higher than the control. On the other hand, chlorogenic acid, caffeic acid, and ferulic acid showed a tendency to decrease with salt treatment. The total phenylpropanoid content in tartary buckwheat was highest at a 100 mM NaCl concentration. This concentration can be of use for future time-treatment experiments. These results indicated that the accumulation of flavonoids in tartary buckwheat can be increased with NaCl treatment.

Table 1. The accumulation of phenolic compounds because of salinity stress in tartary buckwheat. (Average ± STDEV, mg/g dry wt.). The means and standard deviations were obtained from three independent experiments. Letters a–c indicate significant differences ($p < 0.05$).

Compound	0 mM	100 mM	200 mM	300 mM
4-hydroxy benzoic acid	0.145 ± 0.003 a	0.163 ± 0.011 bc	0.170 ± 0.011 c	0.153 ± 0.006 ab
Chlorogenic acid	0.679 ± 0.022 b	0.638 ± 0.034 b	0.545 ± 0.012 a	0.502 ± 0.023 a
Caffeic acid	0.091 ± 0.000 b	0.088 ± 0.011 b	0.086 ± 0.002 b	0.059 ± 0.004 a
Ferulic acid	0.015 ± 0.000 b	0.013 ± 0.001 b	0.013 ± 0.001 b	0.008 ± 0.000 a
Benzoic acid	0.127 ± 0.005 a	0.149 ± 0.009 ab	0.159 ± 0.008 b	0.144 ± 0.006 ab
Rutin	12.812 ± 0.181 ab	14.981 ± 0.150 b	14.745 ± 0.871 b	11.108 ± 0.512 a
Trans-cinnamic acid	0.012 ± 0.001 a	0.014 ± 0.001 ab	0.015 ± 0.001 b	0.012 ± 0.000 a
Quercetin	0.129 ± 0.005 b	0.155 ± 0.007 c	0.138 ± 0.003 bc	0.108 ± 0.001 a
Kaempferol	0.043 ± 0.001 c	0.035 ± 0.003 b	0.034 ± 0.001 b	0.025 ± 0.002 a
Total	14.057 ± 1.906 b	16.232 ± 1.640 c	15.909 ± 0.919 c	12.122 ± 0.553 a

3.3. Analysis of Anthocyanin Content under Different NaCl Concentrations

To investigate the accumulation of anthocyanin in tartar buckwheat under treatment with NaCl, HPLC analysis was performed. Two anthocyanins, namely, cyanidin-3-O-glucoside and cyanidin-3-O-rutinoside, were identified and quantified (Table 2). The results in Table 2 show no significant change in anthocyanin content in tartar buckwheat under NaCl and control treatment. This result indicated that salt treatment does not affect the synthesis of anthocyanins in tartary buckwheat.

Table 2. The accumulation of anthocyanin under salinity stress in tartary buckwheat plants. (Average ± STDEV, mg/g dry wt.). The means and standard deviations were obtained from three independent experiments. Letters a indicate significant differences ($p < 0.05$).

Compound	0 mM	100 mM	200 mM	300 mM
Cyanidin 3-O-glucoside	0.010 ± 0.003 a	0.009 ± 0.001 a	0.010 ± 0.001 a	0.009 ± 0.001 a
Cyanidin 3-O-rutinoside	0.095 ± 0.012 a	0.091 ± 0.007 a	0.081 ± 0.004 a	0.094 ± 0.004 a

3.4. Analysis of Phenylpropanoid Content by Varying the Time of Treatment with Salt

To investigate effects of time of treatment on the accumulation of phenolic compounds in tartar buckwheat, in this experiment, we identified and quantified the phenolic compound contents by treatment with 100 mM NaCl for 2, 4, 6, and 8 days. Six phenolic acids were identified, including 4-hydroxybenzoic acid, chlorogenic acid, caffeic acid, ferulic acid, benzoic acid, and trans-cinnamic acid. Furthermore, three flavonoids were identified, namely, rutin, quercetin, and kaempferol (Table 3). The contents of phenolic compounds, except chlorogenic acid, caffeic acid, ferulic acid, and trans-cinnamic acid, increased under NaCl treatment. In particular, the contents of 4-hydroxybenzoic acid, benzoic acid, rutin, and quercetin were the highest on day 8, which were 0.165, 0.154, 15.045, and 0.155 mg/g, respectively; they were 1.1, 1.28, 1.18, and 1.22 times higher than the control group, respectively. The total quantity of phenolic compounds slightly increased above 1.17 folds from 13.893 mg/g DW at day 2 when untreated to 16.264 mg/g DW at day 8 with salt treatment. The accumulation of total phenylpropanoids was the highest at day 8 after treatment and most of the phenylpropanoid content was higher than that in the control during the same period. These results suggest that the salt treatment of tartary buckwheat causes antioxidant activity improvement by inducing the accumulation of flavonoids and phenolic compounds.

Table 3. The accumulation of phenolic compounds under salt stress in tartary buckwheat plants (Average ± STDEV, mg/g dry weight.) The means and standard deviations were obtained from three independent experiments. Letters a–c indicate significant differences ($p < 0.05$).

Compound	NaCl	2 Days	4 Days	6 Days	8 Days
4-hydroxy benzoic acid	0 mM	0.151 ± 0.007 b	0.150 ± 0.007 b	0.150 ± 0.05 b	0.150 ± 0.007 b
	100 mM	0.150 ± 0.006 b	0.156 ± 0.005 ab	0.161 ± 0.002 ab	0.165 ± 0.005 a
Chlorogenic acid	0 mM	0.675 ± 0.038 a	0.677 ± 0.014 a	0.678 ± 0.001 a	0.675 ± 0.016 a
	100 mM	0.674 ± 0.042 a	0.664 ± 0.014 ab	0.637 ± 0.003 b	0.631 ± 0.010 b
Caffeic acid	0 mM	0.090 ± 0.006 a	0.091 ± 0.005 a	0.090 ± 0.004 a	0.091 ± 0.006 a
	100 mM	0.091 ± 0.006 a	0.088 ± 0.002 a	0.086 ± 0.004 a	0.087 ± 0.005 a
Ferulic acid	0 mM	0.015 ± 0.004 a	0.016 ± 0.001 a	0.016 ± 0.001 a	0.015 ± 0.001 a
	100 mM	0.015 ± 0.000 a	0.015 ± 0.001 a	0.014 ± 0.000 a	0.013 ± 0.001 b
Benzoic acid	0 mM	0.120 ± 0.004 c	0.122 ± 0.003 c	0.122 ± 0.004 c	0.120 ± 0.007 c
	100 mM	0.120 ± 0.004 c	0.133 ± 0.001 b	0.151 ± 0.008 a	0.154 ± 0.006 a
Rutin	0 mM	12.702 ± 0.605 b	12.725 ± 0.601 b	12.721 ± 0.649 b	12.763 ± 0.573 b
	100 mM	12.702 ± 0.605 b	13.335 ± 0.213 b	14.432 ± 0.572 a	15.045 ± 0.740 a

Table 3. *Cont.*

Compound	NaCl	2 Days	4 Days	6 Days	8 Days
Trans-cinnamic acid	0 mM	0.012 ± 0.000 b	0.012 ± 0.001 b	0.012 ± 0.001 b	0.012 ± 0.007 b
	100 mM	0.012 ± 0.000 b	0.013 ± 0.001 b	0.014 ± 0.000 a	0.015 ± 0.001 a
Quercetin	0 mM	0.127 ± 0.005 c	0.127 ± 0.006 c	0.127 ± 0.006 c	0.128 ± 0.004 c
	100 mM	0.127 ± 0.005 c	0.141 ± 0.002 b	0.150 ± 0.007 ab	0.155 ± 0.001 a
Kaempferol	0 mM	0.043 ± 0.002 a	0.044 ± 0.002 a	0.045 ± 0.002 a	0.044 ± 0.003 a
	100 mM	0.043 ± 0.002 a	0.041 ± 0.002 ab	0.037 ± 0.004 bc	0.035 ± 0.003 c
Total	0 mM	13.893 ± 0.596 b	13.919 ± 0.605 b	13.915 ± 0.643 b	13.954 ± 0.571 b
	100 mM	13.891 ± 0.596 b	14.545 ± 0.214 b	15.645 ± 0.576 a	16.264 ± 0.754 a

3.5. Analysis of Anthocyanin Content by Varying the Time of Treatment with Salt

An analysis was conducted to investigate if the accumulation of anthocyanin is influenced by varying the time of treatment with 100 mM NaCl. HPLC analysis was used to identify and quantify the anthocyanin content in tartary buckwheat under 100 mM NaCl treatment for 2, 4, 6, and 8 days (Table 4). Similar to the previous experiment mentioned in this article on different concentrations of NaCl, here, both anthocyanins, namely, cyanidin-3-O-glucoside and cyanidin-3-O-rutinoside, were identified and quantified. However, the analysis showed that there was no significant change in the anthocyanin content. It can be seen that salt treatment does not affect the synthesis of anthocyanins.

Table 4. The accumulation of anthocyanins under salt stress in tartary buckwheat plants (Average ± STDEV, mg/g dry weight.). The means and standard deviations were obtained from three independent experiments. Letters a–b indicate significant differences ($p < 0.05$).

Compound	NaCl	2 Days	4 Days	6 Days	8 Days
Cyanidin 3-O-glucoside	0 mM	0.020 ± 0.000 b	0.017 ± 0.001 ab	0.014 ± 0.001 a	0.019 ± 0.004 ab
	100 mM	0.017 ± 0.000 ab	0.019 ± 0.002 ab	0.017 ± 0.000 ab	0.015 ± 0.001 a
Cyanidin 3-O-rutinoside	0 mM	0.110 ± 0.019 a	0.115 ± 0.024 a	0.117 ± 0.017 a	0.113 ± 0.029 a
	100 mM	0.112 ± 0.008 a	0.101 ± 0.007 a	0.120 ± 0.013 a	0.106 ± 0.013 a

4. Discussion

Salinity stress affects plants by reducing the water potential and causes ion imbalance or disturbances in ion homeostasis and toxicity. The detrimental effect is observed at the whole plant level as the death of plants or a decrease in productivity. Salt stress affects all the major processes, such as germination, growth, photosynthetic pigments and photosynthesis, water relation, nutrient imbalance, oxidative stress, and yield [30]. The salinity absorbed by the plant is concentrated in the old leaves. If the leaves continue to transport over a long time, the concentration of Na^+ and Cl^- will eventually increase, and the leaves will die (Figure 2B). Besides the direct impact of salinity on plants, an ordinary result of salinity stress is the derivation of an excessive accumulation of reactive oxygen species (ROS). ROS are highly reactive and may cause cellular damage through the oxidation of lipids, proteins, and nucleic acids [31–33]. Salinity stress frequently causes both osmotic and ionic stress in plants, resulting in an increase or decrease of certain secondary metabolites in plants [3]. Navarro, Josefa M and colleagues [11] revealed that red peppers (*Capsicum annuum* L.) under moderate salinity exhibited increased total phenolic contents, but green and turning peppers displayed a decreased accumulation of total phenolic compounds. Ben Abdallah et al. [34] reported that black nightshade (*Solanum nigrum* L.) under salinity stress demonstrated increased total phenolic contents and carotenoids using HPLC analysis. Additionally, flavonoid- and carotenoid-gene expression in salinity-treated plants were higher than in the non-treated control. Lim et al. [35] investigated the influence of salinity stress on carotenoids and phenolic compounds in common buckwheat (*Fagopyrum esculentum*) sprout, not tartary buckwheat (*Fagopyrum tataricum*). They also found no significant change of color in common

buckwheat sprouts. The carotenoid content of sprouts treated with 50 and 100 mM NaCl after 7 days of cultivation was two times higher than that of the control (0 mM NaCl). Moreover, the total phenolic content of sprouts increased in response to increasing NaCl concentrations, with the exception of 200 mM NaCl. In particular, 50 and 100 mM NaCl after 7 days was about two times higher than the control. Similar to the total phenolic content of sprouts by NaCl treatment, the radical scavenging activity of the sprouts increased in response to increasing NaCl concentrations

There are many studies on the relationship between abiotic stress and secondary metabolites in buckwheat. Jeon et al. [36] investigated the correlation between cold stress and secondary metabolism in tartary buckwheat. The contents of cyanidin 3-0-glucosid and cyanidin 3-0-rutinoside were increased under cold stress, and the contents of epicatechin and catechin were also increased. Additionally, the radical scavenging activity of the not-treated tartary buckwheat was not changed, but the radical scavenging activity was five times higher in cold-treated tartary buckwheat. Yoko Tsurunaga and colleagues [37] reported the relationship between various light conditions and secondary metabolites in common buckwheat. In particular, common buckwheat sprouts under irradiation with UV-B > 300 nm increased the levels of DPPH radical scavenging activity, rutin, and anthocyanin, but the growth of buckwheat sprouts under irradiation with UV-B 260–280 nm was detrimental, exhibiting yellowing or withering. Tatsuro Suzuki et al. [38] investigated the effect of UV-B radiation, cold, and desiccation in tartary buckwheat leaves. The content of rutin was increased by UV-B radiation and desiccation treatment, and rutin glucosidase activity was also increased by UV-B radiation, cold, and desiccation. These results suggested that rutin and rutin glucosidase activity may be related to the defense system against abiotic stress conditions.

In this study, we investigated the influence of salinity in tartary buckwheat plants by using HPLC analysis. The change of color was observed to not be significant in the salt treatment experiment in tartary buckwheat. Just as the color did not change in plants under NaCl treatment, the accumulation of anthocyanin was also not affected. However, the accumulation of phenolic compounds increased slightly in response to the NaCl concentration, except under 300 mM NaCl treatment. In particular, rutin, the most tartary buckwheat-containing flavonoid, increased the most. Furthermore, 4-hydroxy benzoic acid, benzoic acid, trans-cinnamic acid, and quercetin were increased, but chlorogenic acid, caffeic acid, ferulic acid, and kaempferol were decreased. Based on previously reported research and this experiment using salinity treatment in tartary buckwheat, we suggest that salinity plays a role in the acceleration of phenolic compound synthesis in tartary buckwheat to protect against salinity-induced oxidative damages. However, the antioxidant activity and mechanism of salt stress in tartary buckwheat have not yet been elucidated, so further research involving GC-TOFMS (gas chromatography time-of-flight mass spectrometry), DPPH scavenging assays, and an analysis of the carotenoid content is needed.

5. Conclusions

In this study, we investigated the phenylpropanoid content in tartary buckwheat in response to salinity stress. After NaCl treatment, there was no change in the accumulation of anthocyanin and the color of tartary buckwheat. However, the accumulation of phenolic compounds increased in response to the NaCl concentration. Rutin, 4-hydroxy benzoic acid, benzoic acid, trans-cinnamic acid, and quercetin were increased, but chlorogenic acid, caffeic acid, ferulic acid, and kaempferol were decreased. Previous studies have reported that phenylpropanoids are influenced by abiotic stress, such as cold and UV-irradiation, supporting the findings of the current study. These data provide functional information about secondary metabolites in tartary buckwheat under abiotic stress and may be useful for studying secondary metabolites in other plants.

Author Contributions: S.U.P. designed the experiments and analyzed the data. N.S.K., S.-J.K., D.M.C., J.J., and J.S.P. performed the experiments and analyzed the data. N.S.K., S.-J.K., and D.M.C. wrote the manuscript. All authors read and approved the final manuscript.

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