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Effect of salinity stress on phenolic compounds and carotenoids in buckwheat (*Fagopyrum esculentum* M.) sprout

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ABSTRACT

The effect of salinity stress on the nutritional quality of buckwheat sprouts cultivated for 1, 3, 5, and 7 d was investigated by analysis of the antioxidant activity and levels of phenolic compounds and carotenoids. Treatment with various concentrations of NaCl (10, 50, 100, and 200 mM) resulted in an increase in the amount of phenolic compounds and carotenoids in the sprouts compared with the control (0 mM). The phenolic contents of sprouts treated with 10, 50, and 100 mM after 7 d of cultivation were 57%, 121%, and 153%, respectively, higher than that of the control (0 mM NaCl). Moreover, the accumulation of phenolic compounds was primarily caused by an increase in the levels of 4 compounds: isoorientin, orientin, rutin, and vitexin. The carotenoid content of sprouts treated with 50 and 100 mM NaCl was twice higher than that of the control. In addition, the antioxidant activity of ethanol extracts of the sprouts was increased by NaCl treatment. Although the growth rate of sprouts decreased with >50 mM NaCl, these results suggest that treatment of an appropriate concentration of NaCl improves the nutritional quality of sprouts, including the level of phenolic compounds, carotenoids, and antioxidant activity.

1. Introduction

Clinical evidence has shown that the consumption of plants and plant-derived foods is positively associated with the prevention of various chronic diseases and improvement of general health. Thus, interest in the use of plant-derived foods for health benefits is growing (Foley & Kratz, 2000). In particular, seed sprouts have attracted attention as a phytonutrient-rich food despite health concerns over their association with foodborne illnesses and antinutritional factors (Taormina, Beuchat, & Slutsker, 1999). Buckwheat sprouts—as well as broccoli, alfalfa, and bean sprouts—have received considerable attention from the sprout market due to their rich content of phytonutrients, including phenolic compounds, proteins, vitamins, and minerals (Kim, Kim, & Park, 2004). Buckwheat is an important source of rutin having many interesting pharmacological effects such as anti-platelet aggregation and antiasthmatic activity (Jung, Lee, Cho, & Kim, 2007; Sheu, Hsiao, Chou, Shen, & Chou, 2004), and stabilization effect on high blood pressure (Havsteen, 1983). Furthermore, buckwheat also contains proteins with hypercholesterolemia and hypotensive properties (Chen, Jiao, & Ma, 2008). The level of phytonutrients is higher in sprouts than in seeds or seed products (Lintschinger et al., 1997). Recently, the effect of abiotic stress on the level of phytonutrients in buckwheat and its sprouts has been investigated (Kim, Park, & Lim, 2011); however, the effect of salinity stress on the content of phytonutrients in buckwheat sprouts is unknown.

Under conditions of high salinity concentration in soil and irrigation water, many plants are affected by nutritional imbalance, osmotic stress, water deficiency, and oxidative stress (Parida & Das, 2005; Shalhevet & Hsiao, 1986), which result in plant growth, leaf surface expansion, and primary carbon metabolism. Moreover, many previous studies have indicated that changes in the level of secondary metabolites in plants, including phenolic compounds and terpenoids, enhance plant defense mechanisms against stress, particularly oxidative stress induced by high salinity concentration (Wahid & Ghazanfar, 2006). Germination—generally considered as the most salt-sensitive stage of the plant life cycle (Ashraf & Wahid, 2000)—leads to a series of metabolic changes, including changes in enzyme activities, an imbalance in the levels of plant growth regulators, and a reduction in hydrolysis and utilization of food reserves (Ahmad & Bano, 1994; Khan & Rizvi, 1994).

Recently, several studies have shown that salinity stress affects the quality and nutritional value of the pea (Noreen & Ashraf, 2009), romaine lettuce (Kim, Fonseca, Choi, Kubota, & Kwon, 2008), and maize (Hichema, Mounir, & Naceurc, 2009). However, the effect of salinity stress on secondary metabolites in buckwheat sprouts has not been investigated. Therefore, we aimed to determine the effect of salinity stress on the levels of phenolic compounds and carotenoids and free radical scavenging ability in

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buckwheat sprouts by using various concentrations of NaCl. In addition, the yield and appearance of the sprouts were also investigated for each harvest period.

2. Materials and methods

2.1. Buckwheat sprout cultivation and NaCl treatment

Buckwheat (*Fagopyrum esculentum* Moench) seeds (40 g) were washed and soaked in distilled water at 25 °C for 4 h, and the seeds were subsequently placed in a tray (32 × 6 × 2.8 cm) with a cheesecloth. Four separate trays were placed in a commercial sprout cultivator (MikroFarm; EasyGreen) with an automatic water spray system. The sprouts were cultivated in the dark at 18 ± 2 °C for 7 d. Water was automatically sprayed for 30 min every 12 h. For NaCl treatment, 100 mL of various concentrations of NaCl (0, 10, 50, 100, and 200 mM) were sprayed onto the sprouts daily. The sprouts were harvested following different growth periods (1, 3, 5, and 7 d), immediately lyophilized, ground in a blender, and individually stored in a closed plastic bottle in a desiccator before being subjected to analysis.

2.2. Determination of the growth of buckwheat sprouts irrigated with various concentrations of NaCl

The growth of sprouts was measured in terms of the fresh weight and germination ratio. Forty sprouts were randomly selected from each tray and the fresh weight of sprouts harvested on different days (1, 3, 5 and 7 d) was measured. In addition, 30 seeds were germinated at 25 °C for 24 h, and germinated seeds were counted. All experiments were repeated 4 times.

2.3. Extraction of phenolic compounds from buckwheat sprouts

A sample of ground buckwheat sprout (0.1 g) was mixed with 1 mL of 80% ethanol and the mixture was shaken at room temperature for 3 h. After centrifugation at 10,000 × *g* for 10 min, the supernatant was used for the determination of phenolic content and antioxidant activity.

2.4. Determination of total phenolic content

The total amount of phenolic compounds in buckwheat sprouts was determined by using Folin–Ciocalteu reagent according to the method of Singleton and Rossi (1965). The extract (0.1 mL) was diluted 100 times with ethanol and mixed with 0.2 N Folin–Ciocalteu reagent (1 mL). After incubation for 3 min at room temperature, the mixture was added to 1 mL of saturated Na_2CO_3. The resulting solution was incubated in the dark at room temperature for 1 h and then centrifuged at 5000 rpm for 10 min. The absorbance of the supernatant was measured at 735 nm. A standard curve was prepared by using gallic acid (GA), and the absorbance was converted to phenolic content in terms of mg of GA equivalent (GAE) per g of dry weight (DW).

2.5. Qualitative and quantitative analysis of major phenolic compounds

Major phenolic compounds in sprout extracts were analyzed by reverse-phase C18 ultra fast liquid chromatography (UFLC; Shimadzu, Kyoto, Japan). A Discovery C18 column (250 × 4.6 mm, 5 μm; Waters) was equilibrated with 2% aqueous acetic acid. Ten microliters of the ethanol extract were injected into the column and eluted at a flow rate of 1 mL/min with 2% aqueous acetic acid and 45% acetonitrile containing 2% acetic acid. The absorbance of the elutant was measured at 355 nm. Chemical reference standards were used to identify and measure the concentration of major phenolic compounds in the sample.

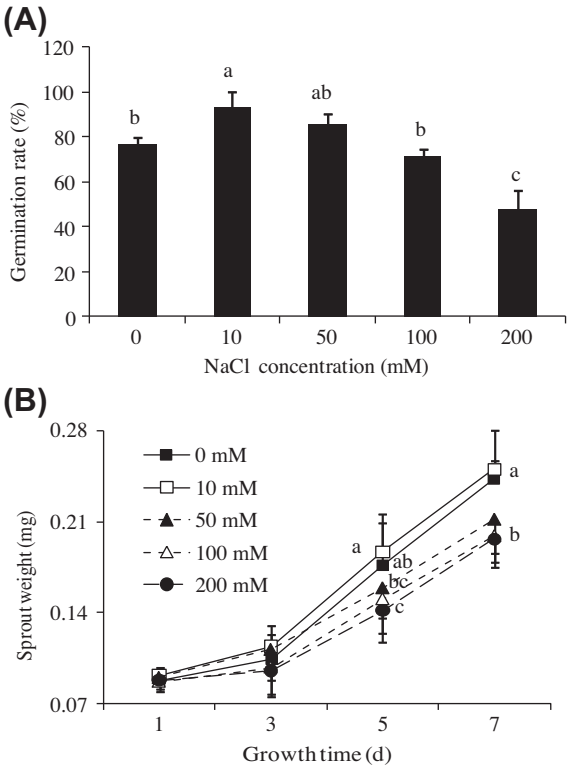

Fig. 1. Effect of various concentrations of NaCl on the germination rate of buckwheat seeds (A), or fresh weight of sprouts (B). Forty sprouts were randomly selected from each tray and the fresh weight of sprouts harvested on different days (1, 3, 5 or 7 d) was measured. To measure the germination rate, 30 seeds were germinated at 25 °C for 24 h, and germinated seeds were counted. All experiments were repeated 4 times. Different letters denote significant differences ($p < 0.05$).

2.6. Determination of the antioxidant activity of sprout ethanol extracts

The antioxidant capacity of ethanol extracts of the sprouts was measured by using a DPPH· free radical scavenging assay according to the method of Yamaguchi, Takamura, Matoba, and Terao (1998) with some modifications. The reaction mixture, containing 0.2 mL of diluted sample and 3 mL of 0.1 mM DPPH· solution, was shaken and incubated in the dark at room temperature for 30 min. After incubation, the absorbance of the reaction mixture was measured at 517 nm and the scavenging activity of DPPH· free radicals was calculated by using the following formula: scavenging activity (%) = [(1 − absorbance of sample at 517 nm/absorbance of control at 517 nm)] × 100.

2.7. Extraction of carotenoids from buckwheat sprouts and determination of total carotenid content

Carotenoids were extracted from the sprouts according to the procedure of Moros et al. (Liu, Hu, Li, & Lin, 2007) with some modifications. The dried sample powder (0.2 g) was mixed with 6 mL of ethanol containing 0.1% BHT, and the sealed mixture solution was preincubated at 85 °C for 5 min. For saponification, 120 μL of 80% KOH were added to the preheated mixture solution, and the reaction solution was incubated at the same temperature for 10 min. The reaction solution was then immediately placed on ice, and