Effects of the Application of Excessive Amounts of Sodium or Potassium on the Growth and Quality of Rapeseed (Brassica napus L.)

Takuma FUJII, Kazuyoshi NADA and Shin HIRATSUKA
Graduate School of Bioresources, Mie University, Tsu, Mie 514-8507, Japan

(Received November 19, 2018; Accepted December 26, 2018)

To investigate whether the application of excessive amounts of cations (Na\(^+\) or K\(^+\)) can promote plant growth and quality, hydroponically cultivated rapeseed plants were treated with high (100 mM), moderate (20 mM), or standard (6 mM) concentrations of NaCl or KCl. The treated plants were analyzed regarding their fresh weight, photosynthetic parameters, and abundance of selected phytochemicals with potential benefits for human health. Growth and photosynthesis were inhibited by the high cation concentration, while the effects of the moderate cation concentration were similar to those induced by the standard concentration. Additionally, the glucosinolate content increased following the application of 20 mM NaCl or KCl. Meanwhile, the chlorophyll and \(\beta\)-carotene contents increased significantly in response to 20 mM KCl, but not 20 mM NaCl.

In summary, rapeseed quality improved, while growth was maintained, following the application of 20 mM NaCl or KCl, but KCl had a greater effect than NaCl.

Keywords: \(\beta\)-carotene, chlorophyll, glucosinolates, quality enhancement

INTRODUCTION

Rapeseed is one of the most important oilseed crops worldwide. In Japan, rapeseed is cultivated for its oil on 1,980 ha per year (Ministry of Agriculture, Forestry and Fisheries, 2017). Additionally, rapeseed has been consumed as a nutritious green and yellow vegetable that is a rich source of minerals and vitamins, including \(\beta\)-carotene. For vegetable production, rapeseed is cultivated on 251 ha per year in Japan, and the young leaves and axillary bud stalks are harvested (Ministry of Agriculture, Forestry and Fisheries, 2014).

Rapeseed contains various types of glucosinolates, which are compounds that are characteristic of Brassicaceae species. When rapeseed tissue is damaged following an attack by herbivores, the glucosinolates are hydrolyzed by myrosinase into glucose, isothiocyanate, nitrite, and so on (Yan and Chen, 2007). Several types of rapeseed glucosinolates may have health benefits for humans because of their anticancer properties (Shapiro et al., 1998; Hounsome et al., 2008).

Crop growth is inhibited under severe salt stress, mainly because of the accumulation of the sodium salt in the plant body (Mato, 1987). However, in some plants, including Brassicaceae species, growth may be enhanced under low salt stress conditions (Osawa, 1965). For example, broccoli may benefit from an exposure to mild salinity stress, not only in terms of increased growth, but also regarding increased accumulation of glucosinolates in young leaves and florets (López-Berenguer et al., 2009). Therefore, treating plants with exogenous NaCl may be an effective cultivation practice to enhance the potential health benefits of rapeseed for humans, although the plants may be at risk of developing a salt stress disorder. Moreover, rapeseed is generally cultivated in soil, and salt treatments may increase the accumulation of salt within plants. Thus, there may be problems associated with NaCl treatments during rapeseed cultivation.

Like sodium, potassium is a monovalent cation, and it is the most absorbed element from the soil by plants. If enhanced plant growth and increased glucosinolate contents are due to the osmotic stress imposed by the accumulation of sodium, the application of excessive amounts of KCI may represent a viable alternative to NaCl treatments. Additionally, potassium is not an element considered to be a part of crop organic substances, but it has many important physiological functions. For example, within the crop body, potassium activates cellular metabolism, stabilizes the pH, and adjusts the cellular osmolarity. Thus, potassium not only promotes vegetative growth, it also influences crop yield and quality (Yamamoto, 1987). In fact, the lycopene content of tomato reportedly increases in response to increasing potassium concentrations in the applied nutrient solutions (Fanasca et al, 2006). Another study revealed that tomato fruit yield increases with increasing amounts of potassium applied to plants under field conditions (Taber et al., 2008). Although the positive effects of increasing exogenous potassium concentrations on carotenoid accumulation in fruits, such as tomato, have been confirmed, there are relatively few reports describing the effects of potassium treatments on leafy vegetables. Because rapeseed produces an abundance of \(\beta\)-carotene, which is a carotenoid, the application of excessive amounts
of potassium may further increase \( \beta \)-carotene contents in rapeseed plants.

Thus, in the current study, we verified the quality enhancement induced by the application of excessive amounts of NaCl, and compared the rapeseed quality enhancement effects of KCl and NaCl.

MATERIALS AND METHODS

Plant material
Rapeseed (Brassica napus L.) seeds were added to a cell tray (128 holes, 8 \times 16) containing vermiculite. The seeds were germinated at 25°C for 2 days in darkness, and then vernalized at 4°C for 10 days. Seedlings were then transferred to a greenhouse and treated daily with half-strength Hoagland nutrient solution. After 32 days, plants were transferred to a hydroponic culturing system involving a nutrient solution (3 mM NO\(_3\), 3 mM NH\(_4\), 6 mM K\(_2\), 2 mM PO\(_4\), 2 mM SO\(_4\), 1 mM Mg\(^{2+}\), and 1 mM Ca\(^{2+}\)) for the subsequent treatments with 20 or 100 mM of NaCl or KCl. At 34 days after initiating treatments, photosynthetic parameters were measured. At 35 days after initiating treatments, SPAD values were determined with the SPAD-502 chlorophyll meter (Konica Minolta, Tokyo, Japan). The plants were then divided into the edible part (young leaves and stems) and the non-edible part (large leaves and stems), after which the fresh weights were determined. The edible and non-edible leaves were freeze-dried for analyses of the glucosinolate and mineral contents, respectively. Additionally, a portion of the edible part was stored at \(-30°C\) for analyses of the chlorophyll, \( \beta \)-carotene, and ascorbic acid contents.

Photosynthetic parameter measurements

The portable LCA-4 photosynthesis measuring device (Shimadzu, Kyoto, Japan) was used to measure the CO\(_2\) concentration and relative humidity in an open LPC-4 small assimilation chamber containing fully developed leaves (Shimadzu, Kyoto, Japan; 6.25 cm\(^2\) leaf area, 2.5 \times 18.0 \times 0.7 cm). The air flowing into the assimilation chamber was considered to be the atmosphere. Moreover, the relative humidity was adjusted to 30% using a steam adsorbent (Dry Lite), and the air inflow was set to 400 mL \( \text{min}^{-1} \). The leaf temperature was set at 25°C, while the photosynthetic photon flux density provided by an LS-180 metal halide lamp (SUMITA, Saitama, Japan) was 1,100 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). The net photosynthetic rate, respiration rate, stomatal conductance, and transpiration rate were calculated as described by von Caemmerer and Farquhar (1981). Additionally, the gross photosynthetic rate was calculated by adding the respiration rate to the net photosynthetic rate.

Mineral content analysis

The freeze-dried fully expanded leaves were ground to a powder, after which 100 mg leaf powder was heated at 75°C in a thermostatic bath for 5 minutes to inactivate myrosinase. Sinigrin was added to the leaf powder as an internal standard. The leaf sample was treated with 1 mL 70% MeOH and then incubated in a thermostatic bath at 75°C for 10 minutes. The sample was centrifuged (1,600 \( \times \)g, 10 minutes, and 4°C) to pellet the insoluble material, and the supernatant was collected. The sample preparation was repeated three times. The collected supernatants were added to an anion-exchange column, which adsorbed the glucosinolates. Sulfatase was added to the column, which was incubated at room temperature to enable the desulfurization. The desulfoglucosinolates were eluted with 3 mL ultrapure water, and then filtered through a 0.45- \( \mu \text{m} \) Millipore membrane. Each sample (20 \( \mu \text{L} \)) was analyzed in the SCL-10AVP HPLC system controller (Shimadzu, Kyoto, Japan) comprising the LC-10ATVP, liquid feeding unit (Shimadzu, Kyoto, Japan), a DGU-12A in-line degasser (Shimadzu, Kyoto, Japan), a CTO-10ASVP column oven (Shimadzu, Kyoto, Japan) set at 35°C, a UV-Vis Detector (227 nm), and a TSKgel ODS-100V column (250 \times 4.6 mm, 5 \( \mu \text{m} \) particle size; Tosoh, Japan). The mobile phase was ultrapure water (A) and 20% acetonitrile (B). A linear gradient was set (flow rate of 1 mL \( \text{min}^{-1} \)) starting at 1% B and reaching 99% B in 18 minutes, followed by 99% B for 11 minutes and then a decrease to 1% B at 32 minutes. Glucosinolates were identified by comparing the mass spectra of quasi-molecular ions (M+H\(^{+}\)) obtained by HPLC-mass spectrometry (LCMS-2020; Shimadzu, Kyoto, Japan) with the fragment ions reported by Ishida et al. (1997).

Chlorophyll and \( \beta \)-carotene content analyses

\( \beta \)-carotene contents were analyzed based on a modified version of a method described by Nagata and Yamashita (1992). Specifically, 50 mg edible leaf added to a microtube was ground, after which the extraction solution (acetone/n-hexane; 4:6, v/v) was added and the sample was mixed with a 23M electric mixer (As-One, Osaka, Japan). The sample was centrifuged (5,200g, 1 minute) in room temperature with tabletop small centrifuge (Chibitan II, As-One, Osaka, Japan) and the supernatant was collected. The method described above was repeated until the extraction solution became colorless. The absorbance of the collected supernatants was immediately measured at various wavelengths (663, 645, 505, and 453 nm) with a UV-1200 spectrophotometer (Shimadzu, Kyoto, Japan). Chlorophyll \( a \) and \( b \) and \( \beta \)-carotene contents were calculated as described by Nagata and Yamashita (1992). The contents per fresh weight (mg 100 g\(^{-1}\) fresh weight) were determined as described by von Caemmerer and Farquhar (1981). The leaf sample was centrifuged (5,200g, 1 minute) in room temperature with tabletop small centrifuge (Chibitan II, As-One, Osaka, Japan) and the supernatant was collected. The method described above was repeated until the extraction solution became colorless. The absorbance of the collected supernatants was immediately measured at various wavelengths (663, 645, 505, and 453 nm) with a UV-1200 spectrophotometer (Shimadzu, Kyoto, Japan). Chlorophyll \( a \) and \( b \) and \( \beta \)-carotene contents were calculated as described by Nagata and Yamashita (1992). The contents per fresh weight (mg 100 g\(^{-1}\) fresh weight) were determined as described by von Caemmerer and Farquhar (1981).
calculated based on the concentrations (mg 100 mL⁻¹).

**Statistical analysis**

The data were analyzed with the Excel Statistics 2012 software package. Significant differences (P<0.05) among treatments were determined with the Tukey-Kramer test.

**RESULTS**

**Plant growth**

The 20 mM NaCl and KCl treatments had no significant effects on the fresh weights of the edible and non-edible rapeseed parts. In contrast, the 100 mM NaCl and KCl treatments significantly decreased the fresh weight (Table 1). Specifically, the fresh weight of shoots treated with 100 mM NaCl or KCl decreased by 47% and 44% of that of control, respectively.

**Photosynthetic parameters**

The gross photosynthetic rate, stomatal conductance, transpiration rate, and mesophyll conductance exhibited the same tendencies as the fresh weight. The application of 100 mM NaCl or KCl decreased the photosynthetic rate, stomatal conductance, transpiration rate, and mesophyll conductance relative to the control levels, while the application of 20 mM NaCl or KCl had no significant effect (Table 2).

**Cation content**

The Na content in fully expanded rapeseed leaves increased with increasing concentrations of the supplied NaCl (Fig. 1). Similarly, the K content in fully expanded rapeseed leaves increased with increasing concentrations of the supplied KCl. These results suggested that the supplied cations were absorbed and accumulated in the plants.

**Glucosinolate content**

Total glucosinolate content increased significantly relative to the control level in response to the 20 mM NaCl treatment (Table 3), which is consistent with the results of a previous study by López-Berenguer et al. (2009). The 20 mM NaCl treatment of rapeseed plants also significantly increased the abundance of functional glucosinolates with anticancer effects, which may enhance the health benefits of the rapeseed plant. Moreover, the total and functional glucosinolate contents following the 20 or 100 mM KCl treatments tended to increase relative to the control level, although the differences were not significant.

**Chlorophyll and β-carotene contents**

The effects of the NaCl and KCl treatments on chlorophyll and β-carotene contents in the edible rapeseed parts are presented in Fig. 2. The chlorophyll and β-carotene contents following the application of 20 mM KCl or 100 mM NaCl and KCl increased by 82%, 60%, and 45.5% relative to the control level, respectively. The 20 mM NaCl treatment had no significant effect.

**DISCUSSION**

Highly saline rhizosphere soil generally inhibits plant growth. However, according to Osawa (1965), salinity tolerance varies among vegetable species. For example, NaCl concentrations required to decrease shoot growth by 50% are reportedly 188 mM for pak-choi, 154 mM for cabbage, 137 mM for Chinese cabbage, i.e. the salinity tolerant group, 51 mM for cucumber, 34 mM for lettuce, and 17 mM for strawberry i.e. the salinity sensitive group (Osawa, 1965). These results imply that Brassicaceae species are basically tolerant to salinity stress. Moreover, an earlier investigation revealed that the yield of saline-tolerant vegetables, such as pak-choi, cabbage, and Chinese cabba-
ge, increases following the application of 17 mM or 34 mM NaCl (Osawa, 1965). Similarly, Moreno et al. (2008) reported that the broccoli biomass increases when the plants are treated with 40 mM NaCl.

The shoot fresh weight of plants treated with 100 mM NaCl or KCl was only about 50% of the control shoot fresh weight (Table 1). Therefore, the salinity tolerance of rapeseed is likely similar to that of other Brassicaceae species. Additionally, the observed decrease in mesophyll conductance in response to the 100 mM NaCl or KCl treatments (Table 2) suggests that these salt concentrations decrease the gas exchange rates and enzyme activities (e.g., Rubisco) of rapeseed plants. These changes are not suitable for cultivating rapeseed. While the rapeseed shoot fresh weight did not decrease following the 20 mM NaCl treatment, there was also no increase, unlike in broccoli.

The effects of the application of 20 mM KCl were similar to those of the application of 20 mM NaCl. The application of NaCl did not increase rapeseed growth, the NaCl treatments were expected to enhance plant quality. López-Berenguer et al. (2009) reported that in broccoli, the total glucosinolate contents of young leaves and florets increase following the application of NaCl. In this study, the total glucosinolate content increased significantly in rapeseed plants treated with 20 mM NaCl, relative to control levels (Table 3). In contrast, although the total glucosinolate content of rapeseed plants supplied with 20 mM KCl tended to increase, the changes were not significant. The fact that KCl affected the total glucosinolate content less than NaCl may have been because the osmotic stress level of plants induced by the NaCl treatment was less than that induced by the NaCl treatment. This possibility is supported by the data indicating the Na content increased 2.5-fold following the application of 20 mM NaCl, while the K content increased only 1.3-fold in response to the application of 20 mM KCl (Fig. 1).

Chlorophyll and β-carotene contents increased in rapeseed plants treated with 100 mM NaCl or KCl (Fig. 2). However, the observed decrease in growth by about 50% suggests the increase in chlorophyll and β-carotene contents was likely a concentration effect rather than due to increased biosynthesis. Thus, we now discuss the effects of 20 mM cation treatments, which did not affect growth. The chlorophyll and β-carotene contents significantly increased following the KCl application, while they did not change significantly in response to the NaCl application. These results imply that potassium specifically influences chlorophyll and β-carotene production in rapeseed plants. While the increased carotenoid synthesis induced by the application of excessive amounts of potassium has been confirmed in tomato (Taber et al., 2008), there are few reports describing the effects of potassium treatments on the carotenoid synthesis in leafy vegetables. Therefore, the observed increase in the β-carotene content of rapeseed (i.e., leafy vegetable) following the 20 mM KCl treatment is interesting. Dean et al. (2004) reported that carotenoid and chlorophyll accumulations are highly correlated in 23 Brassica oleracea cultivars. Additionally, carotenoid and chlorophyll production requires geranylgeranyl diphosphate as a substrate. The biosynthesis of isopentenyl diphosphate, which is a precursor of geranylgeranyl diphosphate, occurs via the mevalonate pathway in the cytoplasm and the 2-C-methylerythritol 4-phosphate pathway (non-mevalonate pathway) in plastids. The non-mevalonate pathway is considerably involved in the production of chlorophylls and carotenoids.

The data presented herein suggest that the application of excessive amounts of potassium enhances the non-mevalonate pathway more than the carotenoid biosynthesis system, which contradicts what has been reported.
Table 3: Effect of the application of 20 or 100 mM NaCl or KCl on glucosinolate contents.

| Treatment         | Glucobrassicin content (μmol g⁻¹ DW) | Glucobrassicanapin content (μmol g⁻¹ DW) | Gluconasturtiin content (μmol g⁻¹ DW) | Functional glucosinolate content (μmol g⁻¹ DW) | Progoitrin content (μmol g⁻¹ DW) |
|-------------------|-------------------------------------|----------------------------------------|--------------------------------------|-----------------------------------------------|----------------------------------|
| Control           | 0.53                                | 4.9                                    | 0.32                                 | 0.32                                          | 22.3                             |
| 20 mM NaCl        | 1.8                                 | 2.0                                    | 2.2                                  | 2.2                                           | 2.2                              |
| 100 mM NaCl       | 5.1                                 | 1.0                                    | 2.4                                  | 2.4                                           | 2.4                              |
| 20 mM KCl         | 5.1                                 | 1.0                                    | 2.4                                  | 2.4                                           | 2.4                              |
| 100 mM KCl        | 5.1                                 | 1.0                                    | 2.4                                  | 2.4                                           | 2.4                              |

Different letters indicate significant differences among treatments at P < 0.05 as determined by the Tukey-Kramer test.

For tomato.

In this study, we revealed that the application of 20 mM NaCl increases the glucosinolate content in rapeseed (i.e., a quality improvement). Moreover, the application of 20 mM KCl increases the glucosinolate content as well as the chlorophyll and β-carotene contents in rapeseed (i.e., further quality improvements). However, the present study was conducted using a hydroculture system. Thus, additional studies are required to establish whether the application of excessive amounts of potassium can improve the cultivation and quality of Brassicaceae vegetable species under field conditions.

ACKNOWLEDGMENT

We thank Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

REFERENCES

Dean, A. K., Kopsell, D. E., Lefsrud, M.G., Curran-Celentano, J., Dukach, L.E. 2004. Variation in lutein, β-carotene, and chlorophyll concentrations among Brassica oleracea cultivars and seasons. HortScience 39: 361–364.

Fanasca, S., Colla, G., Maiani, G., Venneria, E., Rouphael, Y., Azzini, E., Saccardo, F. 2006. Changes in antioxidant content of tomato fruits in response to cultivar and nutrient solution composition. J. Agric. Food Chem. 54: 4319–4325.

Housonse, N., Houssorne, B., Tomos, D., Edwards-Jones, G. 2008. Plant metabolites and nutritional quality of vegetables. J. Food Sci. 73: 48–65.

Ishida, M., Chiba, I., Okuyama, Y., Takahata, Y., Kaizuma, N. 1997. Separation and identification of desulfoglucosinolates in Japanese rapeseed by LC/APCI-MS. JARQ 31: 73–80.

López-Berenguer, C., Martínez-Ballesta, M. C., Moreno, D.A., Carvajal, M., García-Viguera, C. 2009. Growing hardier crops for better health: salinity tolerance and the nutritional value of broccoli. J. Agric. Food Chem. 57: 572–578.

Mato, T. 1987. Stress tolerance, quality and crop nutrient: Potassium. In “Agriculture Out-Line of Technology. Soil and Application of Fertilizer. vol. 2”, Rural Culture Association Japan, Tokyo, p57–62.

Moreno, D.A., López-Berenguer, C., Martínez-Ballesta, M.C., Carvajal, M., García-Viguera, C. 2008. Basis for the new challenges of growing broccoli for health in hydroponics. J. Sci. Food Agric. 88: 1472–1481.

Nagata, M., Yamashita, I. 1992. Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. J. Jpn. Soc. Food Sci. Technol. 39: 925–928.

Osawa, T. 1965. Studies on the tolerance to mineral nutrition. Bull. Univ. Osaka Pref., Ser. B, 16: 13–57.

Shapiro, T. A., Fahey, J. W., Wade, K. L., Stephenson, K. K., Talalay, P. 1998. Human metabolism and excretion of cancer chemoprotective glucosinolates and isothiocyanates of cruciferous vegetables. Cancer Epidemiol. Biomark. Prev. 7: 1091–1100.

Statistics of Ministry of Agriculture, Forestry and Fisheries. 2014. http://www.maff.go.jp/j/tokei/kouhyou/sakumotsu/index.html

Statistics of Ministry of Agriculture, Forestry and Fisheries. 2017. http://www.maff.go.jp/j/tokei/kouhyou/sakumotsu/index.html

Taber, H., Perkins-Veazie, P., Lil, S., White, W., Rodermel, S., Xu, Y. 2008. Enhancement of tomato fruit lycopene by potassium is cultivar dependent. HortScience 43: 159–165.
von Caemmerer, S., Farquhar, G.D. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153: 376–387.

Yamamoto, T. 1987. Nutrient uptake and physiological effect of element: Potassium. In “Agriculture Out-Line of Technology, Soil and Application of Fertilizer. vol. 2”, Rural Culture Association Japan, Tokyo, p85–90.

Yan, X., Chen, S. 2007. Regulation of plant glucosinolate metabolism. Planta 226: 1343–1352.