Concentrations of Nitrate, Organic Acids, Free Amino Acids, Cations and Sugars in Komatsuna (*Brassica campestris* var. *perviridis*) Grown with Carbonate, Sulfate and Chloride Application

Shoji Nakagawa*, Kazuhiro Kikegawa and Mihoko Nomura

Institute for Agro-Microbiology, Nishiatami-cho, Atami 413–0038, Japan

This study compared the concentrations of components such as nitrate, organic acids, free amino acids, cations and sugars in komatsuna (*Brassica campestris* var. *perviridis*) grown with carbonate, sulfate and chloride application (CO$_3$-TK, SO$_4$-TK and Cl-TK, respectively). The komatsuna was cultivated in 1/2000-a Wagner pots (6 hills per pot) filled with light-colored Andosol in a glasshouse for 37 days at soil water potential (SWP) right before harvesting of $-6.2$ and $-62$ kPa. And 26.5 mmol$_e$ of carbonate, sulfate or chloride in the form of potassium salts was applied to each pot. Chloride application to the komatsuna induced relatively high concentrations of potassium, calcium and magnesium ions, and the low concentrations of malate, glucose and fructose on a dry weight basis (DWB). Carbonate application induced relatively high concentrations of malate and low concentrations of nitrate. Sulfate was almost between chloride and carbonate. Concentration variation was not substantial in free amino acids. Those tendencies were almost the same in both SWPs. Most of the variations in component concentrations were attributed to the regulation of ionic and osmotic balance to respond to chloride, nitrate and sulfate absorption, as judged from quantitative relationships among the components and water in the komatsuna. It seems that the absorption of nitrate is influenced by the pH of the soil. There were clear differences in glucose, fructose and malate concentrations among CO$_3$-TK, SO$_4$-TK and Cl-TK on a fresh weight basis just as on a DWB.

Key Words: counter anion, ionic balance, leafy vegetable, osmotic balance, quality.

Introduction

Many types of fertilizers contain carbonate, sulfate and chloride as counter anions for ammonium, potassium, calcium and magnesium; therefore, these counter anions have frequently been applied to plants in amounts that substantially exceed the required quantity. If counter anions affect the quality of vegetables, fertilizers that contain these counter anions should be selected carefully.

The effect of each counter anion on the quality of vegetables has been reported as follows: depression in the uptake and accumulation of nitrate, which may be hazardous to human health (Maynard et al., 1976), in several types of vegetables due to chloride application (Katoh et al., 2006; Liu and Shelp, 1996; Maynard et al., 1976); increase in the concentrations of glucosinolate or isothiocyanate, which are indicators of the degree of pungency and bitterness in radish and turnip, due to sulfate application (Ishii and Saijo, 1987; Kim et al., 2002); and increase in the concentration of pyruvate, which is an estimator of the degree of pungency in onions, due to sulfate application (Bakr and Gawish, 1998; Randle and Bussard, 1993).

However, to the best of our knowledge, few studies have compared the quality of vegetables to which carbonate, sulfate and chloride were applied. The sensory evaluation of carrots (Nakagawa et al., 2003) and the nitrate concentration of leaf vegetables (Katoh et al., 2006) were addressed in those studies.

The purpose of the present study was to compare the concentrations of certain components in leafy vegetables that were grown with carbonate, sulfate and chloride application. These components included nitrate, organic acids (organic anions), free amino acids, cations and sugars. The rationale behind this aim is that during the absorption and accumulation of carbonate (mostly in the form of bicarbonate) (Bialczyk and Lechowski, 1992), sulfate (Lass and Ullrich-Eberius, 1984) and chloride (Jacoby and Rudich, 1980) by plants, the ionic and
osmotic balance is altered, thereby influencing the absorption and/or accumulation of these components. It is known that these components influence the ionic (Kirkby and Knight, 1977) and/or osmotic (Crecelius et al., 2003; Sagisaka et al., 1988) balance of plants and, in turn, the quality of vegetables (Maynard et al., 1976; Mengel, 1979; Yano and Hayami, 1978). Furthermore, a leafy vegetable was cultivated under 2 levels of water stress for several days before harvesting in the present study, because it should be checked whether the result changes depending on irrigation management before harvesting.

### Materials and Methods

#### Pot experiment

We used komatsuna (Brassica campestris var. perviridis) ‘Osome’ (Takii Seed, Kyoto, Japan) in this experiment. The soil used was light-colored Andosol (virgin soil) collected from an experimental farm of the Institute for Agro-Microbiology in Izuokuni City, Shizuoka, Japan. After collection, the soil was sieved through 7-mm mesh, and 4.7 g of fused magnesium phosphate was added per kg of soil (dry weight). The soil was then used for the pot experiment. The properties of the soil are listed in Table 1.

Komatsuna was cultivated in 1/2000-a Wagner pots (6 hills per pot) filled with the soil (dry weight: 8.6 kg) in a glasshouse at the experimental farm. The seeds were sown in the pots on November 4, 2004. We applied 1.25 g nitrogen, 0.44 g phosphorus and 1.04 g potassium to each pot. The sources of nitrogen and phosphorus were NH₄NO₃ and NH₄H₂PO₄. The potassium source was K₂CO₃, K₂SO₄, or KCl. Then, 26.5 mmol of carbonate, sulfate or chloride was applied to each pot. Split applications of the fertilizers were conducted on November 9 and November 28. Treatment with each fertilizer was replicated 5 times.

Irrigation was performed as needed with deionized water, but after December 3, two methods of irrigation were used: the soil water potential (SWP) was regulated at −6.2 kPa until harvesting or no irrigation was performed until harvesting, and SWP reached −62 kPa at harvesting time. SWP in the pot was observed using a soil water meter (SPAD PF-33, Fujiwara Scientific, Tokyo, Japan). The above fertilization treatments were performed for both SWPs.

The tops of the komatsuna were harvested on December 11 between 11:00 and 12:00. Of 6 hills in each pot, 3 were sampled. The sampled komatsuna was transported from the experimental farm to the laboratory within 30 min and stored in a deep freezer at −84°C after measurement. For a period of 5 days before harvesting, the maximum, minimum and average temperatures in the glasshouse were maintained at 21.6°C, 5.3°C, and 11.6°C, respectively.

#### Measurement of component concentrations and nitrate reductase (NR) activity in the komatsuna

To measure the component concentrations and NR activity, the 3 frozen hills selected from each pot were freeze-dried; they were then ground and the sample was mixed thoroughly. The water content was measured during freeze-drying. All inorganic anions except orthophosphate were extracted by shaking with water for 30 min (1:400, w/v), and the extract was analyzed using an ion chromatography (HIC-6A, Shimadzu, Kyoto, Japan). Ion chromatography conditions were: column, Shimpack IC-A1 (Shimadzu); conductivity detector, CDD-6A (Shimadzu); mobile phase, 2.5 mmol·L⁻¹ phthalic acid containing 2.4 mmol·L⁻¹ tris(hydroxymethyl)aminomethane; flow rate, 1.5 mL·min⁻¹; and column temperature, 40°C. The orthophosphate was extracted by shaking with water for 1 h (1:250, w/v), and the concentration was measured by the molybdenum blue method. Cations were extracted by shaking with water for 1 h (1:250, w/v), and the extract was analyzed using ion chromatography (HIC-6A, Shimadzu). Ion chromatography conditions were: column, Shimpack IC-C2 (Shimadzu); conductivity detector, CDD-6A (Shimadzu); mobile phase, 5.0 mmol·L⁻¹ tartaric acid containing 1.0 mmol·L⁻¹ dipicolinic acid; flow rate, 1.0 mL·min⁻¹; and column temperature, 40°C. Free amino acids, sugars and all organic anions, with the exception of ascorbate, were extracted with 0.8 L·L⁻¹ hot ethanol (1:50, w/v) for 20 min. After removing the ethanol by using centrifugal concentrators and the pigments by using chloroform, the water-soluble extract was separated using ion-exchange resins (Dowex 1-X8 and Dowex 50W-X2, Dow Chemical, Michigan, USA) to yield fractions of organic anions, free amino acids and sugars. The fractions were then analyzed by HPLC (LC-9A, Shimadzu) as follows. HPLC conditions for organic anions were: column, Shim-pack SCR-102H×2 (Shimadzu); UV-VIS detector,
SPD-6AV (Shimadzu); wave length, 210 nm; mobile phase, 5.0 mmol·L$^{-1}$ HClO$_4$; flow rate, 0.8 mL·min$^{-1}$; and column temperature, 50°C. Free amino acid concentrations were measured by the method of Yamaya and Matsumoto (1988). HPLC conditions for sugars were: column, TSKgel-Amido80, 4.6 mm $\times$ 250 mm (TOSOH, Tokyo, Japan); RI detector, RID-6A (Shimadzu); mobile phase, CH$_3$CN:H$_2$O = 80:20; flow rate, 1.0 mL·min$^{-1}$; and column temperature, 80°C. Ascorbic acid was extracted by shaking with 20 g·L$^{-1}$ metaphosphoric acid (1:150, w/v) for 1 h, and the extract was analyzed by HPLC (Nakagawa et al., 2000). Nitrogen concentration was measured using an NC analyzer (SUMIGRAPH NC-22F, Sumika Chemical Analysis, Osaka, Japan). NR activity was measured by the method of Dan et al. (2005).

Measurement of nitrate concentration and pH in the soils

After harvesting the komatsuna, the soil was air-dried and sieved through 2-mm mesh. Nitrate was extracted by shaking with water for 1 h, and the extract was analyzed as the inorganic anions in the komatsuna using ion chromatography. The pH was measured using an electrode pH meter.

Results and Discussion

Sizes of the harvested komatsuna

The length of the top of the harvested komatsuna plant was approximately 27 and 25 cm at SWP of $-6.2$ and $-62$ kPa, respectively. At $-6.2$ kPa, the fresh weight and the leaf number, except for the seed leaves, were approximately 15 g and 5.3 per hill, and at $-62$ kPa, these were approximately 12 g and 5.3, respectively. The differences in the length of the top and the fresh weight between both SWPs were due to irrigation treatment. Also, the effects of irrigation treatment were confirmed through the higher proline concentration and lower water content in the komatsuna at SWP of $-62$ kPa than that of $-6.2$ kPa, as shown later (Table 2 and Fig. 1). No statistical differences (ANOVA, $P < 0.05$) were observed in the plant length, fresh weight and leaf number among the carbonate-, sulfate- and chloride-treated komatsuna (CO$_3$-TK, SO$_4$-TK, and Cl-TK) at either SWP.

Table 2. Concentration of components in komatsuna grown with carbonate, sulfate and chloride application with a constant ionic equivalent amount at soil water potential of $-6.2$ and $-62$ kPa.

<table>
<thead>
<tr>
<th></th>
<th>$-6.2$ kPa CO$_3$-TK (mmol·kg$^{-1}$ DW)</th>
<th>$-6.2$ kPa SO$_4$-TK (mmol·kg$^{-1}$ DW)</th>
<th>$-6.2$ kPa Cl-TK (mmol·kg$^{-1}$ DW)</th>
<th>ANOVA $P$ values</th>
<th>$-62$ kPa CO$_3$-TK (mmol·kg$^{-1}$ DW)</th>
<th>$-62$ kPa SO$_4$-TK (mmol·kg$^{-1}$ DW)</th>
<th>$-62$ kPa Cl-TK (mmol·kg$^{-1}$ DW)</th>
<th>ANOVA $P$ values</th>
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<tr>
<td>Inorganic anions</td>
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<tr>
<td>Chloride</td>
<td>84 a$^z$</td>
<td>74 a</td>
<td>363 b</td>
<td>0.000</td>
<td>67 a</td>
<td>67 a</td>
<td>416 b</td>
<td>0.000</td>
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<tr>
<td>Sulfate</td>
<td>104 a</td>
<td>128 b</td>
<td>87 a</td>
<td>0.001</td>
<td>68 a</td>
<td>105 b</td>
<td>59 a</td>
<td>0.000</td>
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<tr>
<td>Nitrate</td>
<td>971 a</td>
<td>1075 b</td>
<td>1152 b</td>
<td>0.002</td>
<td>903 a</td>
<td>1036 b</td>
<td>1113 b</td>
<td>0.001</td>
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<td>Orthophosphate</td>
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<td>99</td>
<td>101</td>
<td>0.968</td>
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<td>Malate</td>
<td>93 a</td>
<td>77 b</td>
<td>50 c</td>
<td>0.000</td>
<td>111 a</td>
<td>80 b</td>
<td>50 c</td>
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<td>Pyroglutamate</td>
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<td>9</td>
<td>7</td>
<td>0.414</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>0.958</td>
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<td>Citrate</td>
<td>8</td>
<td>7</td>
<td>5</td>
<td>0.155</td>
<td>8</td>
<td>7</td>
<td>5</td>
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<td>Ascorbate</td>
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<td>0.689</td>
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<td>48</td>
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<td>0.956</td>
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<td>Free amino acids</td>
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<td>Glutamate</td>
<td>20</td>
<td>19</td>
<td>21</td>
<td>0.278</td>
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<td>21</td>
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<td>0.147</td>
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<td>8</td>
<td>0.869</td>
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<td>Glutamine</td>
<td>6</td>
<td>5</td>
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<td>0.571</td>
<td>9</td>
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<td>9</td>
<td>11</td>
<td>0.415</td>
<td>16</td>
<td>14</td>
<td>18</td>
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<td>Arginine</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>0.316</td>
<td>9</td>
<td>9</td>
<td>10</td>
<td>0.346</td>
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<tr>
<td>Proline</td>
<td>6 a</td>
<td>5 b</td>
<td>5 b</td>
<td>0.028</td>
<td>28</td>
<td>19</td>
<td>24</td>
<td>0.472</td>
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<td>Cations</td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Potassium</td>
<td>761 a</td>
<td>793 a</td>
<td>882 b</td>
<td>0.000</td>
<td>693 a</td>
<td>704 a</td>
<td>824 b</td>
<td>0.000</td>
</tr>
<tr>
<td>Calcium</td>
<td>484 a</td>
<td>484 a</td>
<td>551 b</td>
<td>0.004</td>
<td>419 a</td>
<td>444 a</td>
<td>494 b</td>
<td>0.002</td>
</tr>
<tr>
<td>Magnesium</td>
<td>216 a</td>
<td>215 a</td>
<td>235 b</td>
<td>0.037</td>
<td>194 a</td>
<td>205 ab</td>
<td>219 b</td>
<td>0.019</td>
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<tr>
<td>Sodium</td>
<td>227</td>
<td>207</td>
<td>204</td>
<td>0.145</td>
<td>224</td>
<td>212</td>
<td>227</td>
<td>0.513</td>
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<td>Ammonium</td>
<td>19</td>
<td>20</td>
<td>20</td>
<td>0.863</td>
<td>18</td>
<td>17</td>
<td>18</td>
<td>0.743</td>
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<tr>
<td>Sugars</td>
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<td></td>
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<td></td>
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<tr>
<td>Glucose</td>
<td>361 a</td>
<td>403 b</td>
<td>336 a</td>
<td>0.013</td>
<td>469 a</td>
<td>437 a</td>
<td>364 b</td>
<td>0.010</td>
</tr>
<tr>
<td>Fructose</td>
<td>135 a</td>
<td>147 a</td>
<td>96 b</td>
<td>0.003</td>
<td>214 a</td>
<td>196 a</td>
<td>140 b</td>
<td>0.015</td>
</tr>
<tr>
<td>Sucrose</td>
<td>36</td>
<td>32</td>
<td>31</td>
<td>0.470</td>
<td>40</td>
<td>38</td>
<td>35</td>
<td>0.612</td>
</tr>
</tbody>
</table>

Each value is the mean of 5 replicates.
- TK: -treated komatsuna.
$^z$ Values within a row with different letters are significantly different (Fisher’s PLSD, $P < 0.05$).
Concentration of the components in the komatsuna

Table 2 lists the concentration of nitrate, organic anions, free amino acids, cations and sugars in the komatsuna on a dry weight basis (DWB). Nitrate concentration was lower in CO$_3$-TK than in others. Malate concentration was highest in CO$_3$-TK and lowest in Cl-TK. Potassium and calcium ion concentrations were higher in Cl-TK than in CO$_3$-TK and SO$_4$-TK. Fructose concentration was lower in Cl-TK than in the others. The variation tendencies observed at SWP of −6.2 and −62 kPa were identical. On the other hand, magnesium concentration was higher in Cl-TK and glucose concentration was higher in SO$_4$-TK than in the others when SWP was −62 kPa, whereas the former was higher in Cl-TK than in CO$_3$-TK and the latter was lower in Cl-TK than in the others when SWP was −62 kPa. Proline concentration was slightly higher in CO$_3$-TK than in the others only at SWP of −6.2 kPa. Although the concentration of glucose, a precursor of ascorbate, varied by the fertilization and irrigation treatments, the concentration of ascorbate, a vital vitamin for humans, did not vary. Incidentally, the concentrations of sulfate and chloride applications were reflected in komatsuna with bright clarity.

A factor that caused differences in the nitrate concentration in the komatsuna

Although the same amount of nitrogen was applied to each komatsuna, and there was no difference (ANOVA, $P<0.05$) in the nitrate concentration among soils in the 3 treatments (approximately 2.0 and 3.6 mmol·kg$^{-1}$ on DWB at SWP of −6.2 and −62 kPa, respectively), differences in the concentration of nitrate among CO$_3$-TK, SO$_4$-TK, and Cl-TK were observed. McClure et al. (1990) showed that nitrate uptake by the roots of nitrate-grown maize seedlings via the nitrate-proton cotransport system increases on decreasing the pH of the external solution. In our experiment, the increments in soil pH values due to carbonate, sulfate and chloride treatments were 0.3, −0.1, and −0.2 at SWP of −6.2 kPa and 0.2, −0.1 and −0.3 at SWP of −62 kPa, respectively, and clear relationships were observed between the decrease in nitrate concentrations in the komatsuna with increasing soil pH values (Fig. 1). On the other hand, Barber et al. (1989) showed that high ionic strength and chloride ions inhibit spinach NR activity. In our study, NR activity was observed to be highest in CO$_3$-TK and lowest in Cl-TK, but it was not considered a major factor responsible for the difference of the nitrate concentration among the 3 types of komatsuna because the non-nitrate nitrogen concentrations among the 3 komatsuna were almost equal (Table 3). Thus, we assume that differences in the nitrate concentration among the 3 types of komatsuna are predominantly induced by the variation in soil pH via the nitrate-proton cotransport system, which means at nitrate absorption at the root of the komatsuna was enhanced by increasing proton concentration in the soil in Cl treatment, and inverse in CO$_3$-TK.

It has been reported that chloride competitively inhibits the absorption or accumulation of nitrate (Katoh et al., 2006; Maynard et al., 1976) or enhances the nitrate reduction (Liu and Shelp, 1996). If such an inverse relationship was present in our experiment, some of the results might have been different. For example, Kirkby and Knight (1977) reported that the increase in organic anion accumulation is paralleled by an increase in cation concentration with relatively constant levels of total inorganic anion throughout tomato plants grown with a series of increasing levels of nitrate. Their result is different from ours in the relationship between cation and organic anion, and between cation and inorganic anion concentrations. Further investigations are necessary to clarify the conditions responsible for the inverse relationship between chlorine and nitrate.

### Table 3. Nitrate reductase (NR) activity and non-nitrate nitrogen concentration in komatsuna grown with carbonate, sulfate and chloride application with a constant ionic equivalent amount at soil water potential of −6.2 and −62 kPa.

<table>
<thead>
<tr>
<th>SWP (kPa)</th>
<th>CO$_3$-TK</th>
<th>SO$_4$-TK</th>
<th>Cl-TK</th>
</tr>
</thead>
<tbody>
<tr>
<td>−6.2</td>
<td>19 a$^\dagger$</td>
<td>7 b</td>
<td>3 b</td>
</tr>
<tr>
<td>−62</td>
<td>11 a</td>
<td>4 a</td>
<td>3 a</td>
</tr>
</tbody>
</table>

Each value is the mean of 5 replicates.

$^\dagger$ Measured after transporting within 30 min.

$^\ddagger$ Values within a column with different letters are significantly different (Fisher’s PLSD, $P<0.05$).

$^\times$ -TK: -treated komatsuna.
Quantitative relationships of the components in the komatsuna

We studied the relationships of the component concentrations of komatsuna from the viewpoint of ionic and osmotic balance to consider the causes of the differences among CO$_3$-TK, SO$_4$-TK, and Cl-TK as in Table 2. Components whose concentrations differed among CO$_3$-TK, SO$_4$-TK, and Cl-TK (ANOVA, $P < 0.05$ in Table 2) were used for the following analysis. Proline was omitted because its concentration difference was not substantial, and as a result, the types of components were the same in both SWPs. Incidentally, the analysis results barely changed even if all the components measured in our experiment were used.

In both SWPs, 15 data (3 treatments × 5 replicates) showed a negative correlation between inorganic anion (chloride + sulfate + nitrate) and organic anion (malate) equivalent concentrations (Fig. 2A, B), a positive correlation between the anion (inorganic + organic) and cation (potassium + calcium + magnesium) equivalent concentrations (Fig. 2C, D) and a negative correlation between the ion (anion + cation) and sugar (glucose + fructose) molar concentrations (Fig. 2E, F). These determination coefficient values ($R^2$) were very high (0.755–0.886), except for between the ion and sugar at SWP of −6.2 kPa (0.492).

These findings imply the following: (1) Degradation of the organic anion due to decarboxylation is accelerated by increasing the inorganic anion concentration (Hiatt, 1967; Marschner, 1995) to regulate the anion concentration; however, the excessive inorganic anion concentration is not compensated only by decreasing the organic anion concentration; (2) The cation concentration is increased by increasing the anion concentration to regulate the anion-cation balance and pH. The phenomenon of the anion concentration exceeding that of the cation observed in our study is consistent with past findings (Cunningham, 1964; Haynes, 1990); (3) The sugar concentration is decreased by increasing the ion concentration required primarily to regulate the osmotic balance. Inverse relationships between the tissue concentrations of sugars, reducing sugars in particular, and ions, potassium in particular, have been reported (Pitman et al., 1971; Steingröver, 1983). Consequently, we consider that the absorption of a large amount of inorganic anions, chloride and nitrate in particular, induces the accumulation of a small amount of the organic anion and sugar, and of a large amount of the cation in Cl-TK, and an almost inverse result in CO$_3$-TK.

The cause of the relatively low coefficient between ion and sugar concentrations at an SWP of −6.2 kPa appears to be due to the relatively low concentration of sugar in spite of the low concentration of the ion in CO$_3$-TK, as judged from the scatter plot (not shown). The reason for this finding should be investigated in the future. In leafy vegetable cultivation, most growers suppress irrigation for several days before harvesting.

![Fig. 2](image_url)

Fig. 2. Relationships among ion and sugar concentrations in komatsuna grown with carbonate, sulfate and chloride applications with a constant ionic equivalent amount at soil water potential of −6.2 and −62 kPa. Components whose concentrations differed among the three types of komatsuna (ANOVA, $P < 0.05$ in Table 2) were applied in this analysis. A and B indicate CO$_3$-, SO$_4$- and Cl-treated komatsuna, respectively. Each plot and error bar indicates the mean and standard deviation of 5 replicates. A–F are classified as follows: A and B, inorganic anion × organic anion; C and D, anion × cation; E and F, ion × sugar. $R^2$ value was derived from 15 data (3 treatments × 5 replicates). ** and *** indicate $P < 0.01$ and $P < 0.001$, respectively. Charges of anions were defined as follows: NO$_3$, Cl-monovalent; SO$_4$ and malate, divalent. Charges of cations were defined as follows: Na and K, monovalent; Ca and Mg, divalent.

In this case, under greenhouse cultivation in particular, the relationship between the ion and sugar concentration is presumably closer to our result at SWP of −62 kPa than −6.2 kPa.

Quantitative relationships between the components and water in the komatsuna

There was an inverse relationship between the ion and sugar concentration to regulate the osmotic balance in the komatsuna, as mentioned above. Even so, there was a difference in the total osmotically-active component concentration among CO$_3$-TK, SO$_4$-TK and Cl-TK. We regarded the possibility that this difference was countered at some level by variations in water content. Figure 3 shows a high positive correlation between the
water content and total molar concentration of the components whose concentrations differed among CO$_3$-TK, SO$_4$-TK, and Cl-TK (ANOVA, $P < 0.05$ in Table 2) were applied in this analysis. ○, ▲, and ■ indicate CO$_3$-, SO$_4$-, and Cl-treated komatsuna, respectively. Each plot and error bar indicates the mean and standard deviation of 5 replicates, respectively. $R^2$ value was derived from 15 data (3 treatments $\times$ 5 replicates). *** indicates $P < 0.001$.

In conclusion, chloride application to the komatsuna induces relatively high concentrations of cations (potassium, calcium and magnesium), and low concentrations of an organic anion (malate) and sugars (glucose and fructose) in the komatsuna on DWB. Carbonate application induces relatively high concentrations of chloride and low concentrations of nitrate. Sulfate is almost between chloride and carbonate. These tendencies are hardly influenced by irrigation management before harvesting. Most of the variation in the component concentrations was attributed to the regulation of ionic and osmotic balance in the komatsuna. There are clear differences in glucose, fructose and malate concentrations among CO$_3$-TK, SO$_4$-TK, and Cl-TK on a FWB just as on a DWB. Sugars and malate are respiratory substrates, and the former expresses sweetness. Therefore, the avoidance of fertilizers that contain chloride in normal soil culture might be better for taste and to maintain the quality of leafy vegetables. It was reported that lettuce and cabbage cultivars maintaining good quality in storage were rich in sugar (Yano and Hayami, 1978).

**Literature Cited**


McClure, P. R., L. V. Kochian, R. M. Spanswick and J. E. Shaff. 1990. Evidence for cotransport of nitrate and protons in maize