Effect of Nitrogen Fertilizer on Cell Size and Sugar Accumulation in the Leaves of Cabbage (*Brassica oleracea* L.)

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Abstract. Head and leaf weight of cabbage plants grown using half the nitrogen fertilizer applied to control plants (hereafter referred to as the half treatment) were markedly less than those obtained for control plants to which the standard amount of nitrogen fertilizer was applied. Sugar content 33 d after sowing (DAS) did not differ between treatments, but glucose and fructose content in the half treatment 82 DAS was higher than that of the control. Although the number of cell layers in cross-section for the leaves from both treatments was ≈ 20 , cells from the half treatment appeared smaller than those of the control. Therefore, it is suggested that the higher sugar content in leaves of cabbage plants grown on media containing less nitrogen fertilizer occurs in response to the smaller cells in the leaves.

Given that increasing the amount of nitrogen fertilizer is a relatively simple method for increasing the yields of leaf vegetables, growers are often inclined to apply more nitrogen fertilizer than is necessary. This increased application of nitrogen fertilizer can cause several problems in the quality of leaf vegetables. For example, oxalic acid, which promotes calculus formation, accumulates in spinach plants in response to increased nitrogen nutrients (Ota and Kagawa, 1996). Sugar content increases in many vegetables in response to nitrogen deficiency. In the leaves of cabbage, for example, nitrogen deficiency caused an increase in free sugar content, especially that of sucrose (Hara, 1989). Conversely, the sugar content in spinach has been observed to decrease in response to increased application of nitrogen fertilizer (Takebe et al., 1995; Watanabe et al., 1988). Kano illustrated that cell size in melon fruit is closely related to sugar accumulation (Kano, 2003, 2004, 2005). With these results in mind, it is reasonable to consider that sugar content in plant organs is directly related to the size of the cells in the respective plants. We therefore examined the effect of nitrogen fertilizer on cell size and sugar accumulation in the leaves of cabbage.

Materials and Methods

Plant materials. Standard nitrogen levels (hereafter referred to as the control), which

farmers in Ishikawa Prefecture generally used for cabbage culture, and half the amount of nitrogen used for the control (hereafter referred to as the half treatment) were used in this study. The experiments were carried out in the sandy soil field applied 2000 kg per 10a of barnyard manure in spring of the last year. Two fields 140 cm wide and 30 m long were used and each field was divided into two plots, control and the half treatment fields. In the control soil, 21 g·m⁻² of N, 29 g·m⁻² of P₂O₅, and 21 g·m⁻² of K₂O were applied as basal fertilizer, and 5 g·m⁻² of N, P₂O₅, and K₂O and 3 g·m⁻² of N and K₂O were applied as top dressing on 31 May and 16 June, respectively. In the half treatment soil, 14 g·m⁻² of N, 29 g·m⁻² of P₂O₅ and K₂O were applied as the basal fertilizer with no top dressing. Cabbage seeds (*Brassica oleracea* L. cv. 'Wakamine' Takii Seed Co. Ltd., Kyoto, Japan) were sown and grown in paper pots (4.7 cm length, 4.7 cm wide, and 5 cm depth) filled with the compost of Yosaku #1 (vermiculite with 500 mg nitrogen, 400 mg phosphorous, and 400 mg potassium; Chisso Asahi Fertilizer Co. Ltd., Tokyo, Japan) (50%) and soil (50%) under plastic film greenhouse. Uniform seedlings were set on staggered row planting on 19 May with a spacing of 35 cm between plants on the control and the half fields.

Five plants were collected from each of the respective fields 33 d after sowing (DAS) (10 June) and 82 DAS (29 July), respectively. Top weight of plants 33 DAS and head weight of plants 82 DAS were measured. We collected three average-sized leaves of each plant 33 DAS and the third, fourth, and fifth leaves from the most outside leaf of the heads 82 DAS, respectively.

Measurements of cell number and size in the leaf. Two quadrangular samples of ≈ 0.3 g were removed using a scalpel from the central portion of the leaf between the main vein and the leaf margin for measuring cell size and for sugar analysis. One quadrangle in each treatment was dehydrated using various concentrations of alcohol (70%, 80%, 90%, and 100%) before being embedded in paraffin. Seven 10- μ m-thick cross-sections were prepared from these paraffin blocks and the clearest section from each quadrangle for each treatment was then examined under a microscope. Cell size was measured at two locations between the veins in the cross-section of one quadrangle. As shown in Figure 1, the maximum diameters of all individual cells on the perpendicular line to the upper leaf surface were measured.

Sugar analysis. Another quadrangle was homogenized in 5 mL of water (Polytron, PT10/35; Kinematica, Littau-luzen, Switzerland) before being centrifuged at 8000 $\times$ g for 15 min (CFM-100; Iwaki, Tokyo, Japan) and filtered through a 0.45- μ m filter (0.45 μ m PTFE, hydrophilic; Millipore, Billerica, MA). Forty microliters of the filtrate was injected into a high-performance liquid chromatograph (HPLC) (LC-10Advp; Shimadzu, Kyoto, Japan) and fitted with a refractive index detector (RID-10A; Shimadzu) with a Shim-pack SCR-101C (Shimadzu) at 0.8 mL·min⁻¹ at 80 °C. To determine the presence and concentrations of each sugar, a 40- μ L solution of sucrose, glucose, and fructose, each at 20 g·L⁻¹, was injected into the HPLC before injection of the filtrates.

Results

Mean top weight 33 DAS and head weight 82 DAS for the half treatment were 144 g and 2283 g, which was 34 g and 523 g less than that of the control, respectively (Fig. 2). Leaf weight for both treatments 33 DAS was ≈ 20 g, but the weight of leaves from the half treatment 82 DAS was 45.5 g, which was 11 g less than that of the control.

The number of cell layers in the cross-section was ≈ 20 for each DAS in both treatments (data were not shown). The number of plants with cells larger than 45 μ m in maximum diameter 33 DAS was three and three for the control and the half treatments, respectively (Fig. 3). The number of plants with cells larger than 50 μ m 82 DAS was four from the control and two from the half treatments. The mean cell diameter of all the cells from the half treatment at 33 DAS was 43 μ m, which was 3 μ m less than that of the control. The mean cell diameter of the cells from the half treatment 82 DAS was 51 μ m, which was 4 μ m less than that of the control (Fig. 4).

Respective sugar contents in plants from each treatment 33 DAS was less than 3 mg and no sucrose was found in any plants 82 DAS (Fig. 5). At 82 DAS, the number of plants that had glucose contents greater than 10 mg·g⁻¹ fresh weight (FW) was only one in the control and five in the half treatment. The number of plants that had fructose contents

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Fig. 1. Photomicrograph shows size and number of cells sampled in the leaf of the cabbage cv. Wakamine. The black dots superimposed on cells indicate the actual cells measured.

Fig. 2. Effect of the amount of nitrogen fertilizer on cabbage growth. Control: experimental plots to which the standard amount of fertilizer was applied; Half: experimental plots to which half the standard amount of nitrogen fertilizer was applied. Vertical bars indicate sd ($n = 5$). P values above sd vertical bars are probabilities calculated by t test. DAS: days after sowing.

Fig. 3. Effects of the amount of nitrogen fertilizer on mean cell size of leaves per plant of cabbage cv. Wakamine. Half: a half amount of nitrogen fertilizer of the control was applied. Vertical bars are sd. The numerical values above sd vertical bars are actual number of cells tested. DAS: days after sowing; 1–5: number of plants.

Fig. 4. Effect of the amount of nitrogen fertilizer on cell size in leaf of cabbage. Control: experimental plots to which the standard amount of fertilizer was applied; Half: experimental plots to which half the standard amount of nitrogen fertilizer was applied. Vertical bars are sd [33 DAS (control) $n = 603$, (half) $n = 557$; 82 DAS (control) $n = 650$, (half) $n = 635$]. P values above sd vertical bars are probabilities calculated by t test. DAS: day after sowing.

greater than $10 \text{ mg} \cdot \text{g}^{-1} \text{ FW}$ at 82 DAS was three in the half treatment and zero in the control. The mean sucrose, glucose, and fructose contents in all of the plants 33 DAS was less than $3 \text{ mg} \cdot \text{g}^{-1} \text{ FW}$ (Fig. 6).

Fig. 5. Frequency distribution of sugar content in cabbage leaves from plants grown in plots containing different amount of nitrogen fertilizer. Control: experimental plots to which the standard amount of fertilizer was applied; Half: experimental plots to which half the standard amount of nitrogen fertilizer was applied. Vertical bars indicate sd ($n = 3$). DAS: days after sowing; 1–5: number of plants.

Although sucrose was not detected 82 DAS, the mean glucose and fructose contents of all plants from the half treatment 82 DAS were 16.2 mg and 9.9 mg , which was 2.6 mg and 2.0 mg greater than those measured in the control, respectively.

Discussion

Head weight and leaf weight of plants from the half treatment were markedly less than in the control. Plant weight and head yield of cabbage have been demonstrated to increase with an increase in the nitrogen component of culture solutions (Hara, 1989). The fresh weight of spinach plants grown in nutrient solution with a 1.5-fold decrease in the nitrate component was $\approx 10\%$ less than when the nitrate concentration was 1.3-fold or higher than normal (Zhang et al., 1990). Plant weight of spinach and komatsuna decreased in response to smaller application of nitrogen in Ando soil (Takebe et al., 1995).

Sugar content 33 DAS did not differ between treatments, but glucose and fructose content in the half treatment 82 DAS was higher than that of the control. No difference

Fig. 6. Effect of the amount of nitrogen fertilizer on sugar content in the leaf of cabbage. Control: experimental plots to which the standard amount of fertilizer was applied; Half: experimental plots to which half the standard amount of nitrogen fertilizer was applied. Vertical bars indicate SD ($n = 15$). P value above SD vertical bars are probabilities calculated by t test. DAS: days after sowing.

between two plots at 33 DAS seems attributable to the beginning of head formation. High nitrogen application to the soil caused a decrease in glucose and fructose in the leaves of cabbage (Yano et al., 1981). Conversely, the content of sucrose, glucose, and fructose in cabbage plants all increased in response to decreased nitrogen levels in culture solution (Hara, 1989), and in spinach and komatsuna leaves, sugar content also increased with decreased nitrogen application (Takebe et al., 1995). Sugar content in cabbage under conditions of intermittent nutrient solution supply increases to 60% of that of that observed when supply of nutrient solution is continuous (Watanabe et al., 1988). These findings indicate that sugar content in the leaves of cabbage appears to increase when the amount of nitrogen applied decreased below standard levels. Small values for sucrose content in Figures 5 and 6 might be incited by no heating for the inactivation of enzymes related with the decomposition of sucrose.

Most of the imported sugars then accumulate in the vacuoles of sink-tissue storage cells (Leigh et al., 1979; Yamaki and Ino, 1992). The small vacuoles of the meristematic cells increase in size and gradually

coalesce as the cell enlarges and ages (Esau, 1964). For example, the vacuoles in peach cv. Okubo are numerous and small at full bloom; however, they coalesced to form one large vacuole, which forced the cytoplasm to the outside of the mesocarp cell at the middle stage of fruit development (Ishida et al., 1973). Because moisture percentage of cabbage leaves is $\approx 85\%$ (Suzuki et al., 1999) or 92% (Kano, unpublished data), cell weight can be nearly regarded as vacuole one.

The smaller cells from the half treatment in this experiment points out the smaller vacuoles in cells, i.e., smaller water content from the half treatment than those of the control. The vacuoles of cabbage leaves treated with low temperature $\approx 1^\circ\text{C}$ becomes small and the contents of sucrose, glucose, and fructose increase (Suzuki et al., 1999). Leaves of Chinese cabbage showed slower growth at lower temperatures (Ootake, 1981), and the length of the parenchymatous cells in leaves from the plants is smaller than that from plants grown at higher temperatures (Ootake, 1982). Furthermore, the content of glucose and fructose is larger in the mechanically growth-restricted melon fruits, which have a greater number of smaller cells, than in untreated fruits (Kano, 2004).

By putting together these findings, the higher sugar content observed in the leaves of cabbage plants grown on media containing less nitrogen fertilizer can be considered as follows. Cells fail to increase in size in the leaves of the cabbage plants grown at less nitrogen fertilizer. Because cell weight is mostly attributed to vacuole weight, water content is smaller in the leaves with less nitrogen fertilizer. Consequently, sugar content per fresh weight of leaves results in higher in cabbage plants grown at less nitrogen fertilizer.

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