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Effects of Night-break Treatment Performed Three Times per Week and Exposure to Cold Air on Spinach Growth and Quality in Winter Season

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Abstract

We investigated the combined effects of night-break treatment performed three times per week and subsequent exposure to cold air by opening the greenhouse windows on the growth and quality of December-sown spinach (*Spinacia oleracea* L.). Plant height and fresh weight increased as a result of the night-break treatment, but plant elongation decreased because of the exposure to cold air. On comparing similar-sized harvests, the sugar concentration (Brix value) and L-ascorbic acid level were significantly higher but the nitrate (NO_3^-) level was lower in the combined treatment than in the control plants without the night break and exposure to cold air. These results suggest that the combined method of the night break and subsequent exposure to cold air would be effective to produce high-quality spinach without delaying the harvest.

Discipline: Horticulture
Additional key words: Brix, L-ascorbic acid, nitrate, opening greenhouse windows

Introduction

Night-break treatment for winter spinach (*Spinacia oleracea* L.) cultivation has been shown to promote both the growth and bolting of spinach before harvest because of its reproductive response to long day-length conditions[12]. It is difficult to use night break to promote spinach growth due to the risk of premature bolting. However, Hamamoto et al.[5-7] found that a night break every second or third night promotes spinach growth without promoting excessive bolting, and introduced night-break treatment in a new method for promoting spinach growth under short day-length conditions.

Opening the side cover of a greenhouse (i.e., exposing plants to ambient cold air) for a week or more before harvest increases the sugar and ascorbic acid content and decreases the nitrate content of leaf vegetables, including spinach, grown in a greenhouse in winter[2,10]. Ascorbic acid is an essential vitamin as an antioxidant in the human body[11,14], whereas nitrate is a material for nitrous acid, which is harmful to human health[8]. Therefore, this treatment is useful to increase plants' nutritive value and is sometimes adopted in the northern regions or highlands of Japan as a method for the greenhouse production of high-quality winter leaf vegetables. Japanese growers and researchers call the treatment Kanjime (tightening plants in the cold)[1].

In the present study, we attempted to combine two cultivation methods in winter spinach cultivation: a night break every second or third night, and exposure to cold air before harvest. The former method is expected to accelerate plant growth, and the latter is expected to increase the quality of the harvested plants.

Materials and methods

Alrite spinach seeds (Takii Seed Co., Ltd., Kyoto, Japan) were sown on cell trays (16-mL cells, 171 per tray) filled with growing media (Tanemakibaido; Takii Seed Co., Ltd., Kyoto, Japan) on 4 Dec. and 12 Dec., 2003. The germinated seedlings were grown in a greenhouse. The greenhouse had side and roof windows (opening temperature was set at 25 °C) and a hot water heating system

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(heating temperature was set at 5 °C). When the plants had produced their first true leaves, 24 plants per treatment were selected and transplanted into two 13-L planters sized 61 x 19 x 14 cm with one plant approximately every 10 cm (12 plants per planter) on 22 Dec. (for plants sown on 4 Dec.) and 29 Dec. (for plants sown on 12 Dec.). The planters were filled with fertilized soil (0.4 N – 0.7 P – 0.3 K g·L^{-1}). After transplant, the plants were grown uninterruptedly in the greenhouse.

A one-hour night break around midnight was applied from 40-W incandescent lamps (Matsushita Electric Industrial Co., Ltd., Osaka, Japan) at a photosynthetic photon flux of about 1 μmol·m^{-2}·s^{-1}. For plants sown on 4 Dec., three night-break treatments were applied from 24 Dec.: (1) a night break every Monday, Wednesday, and Friday (NB), (2) NB treatment until 30 Jan., 2004, when the plant height was about 21 cm, followed by exposure to ambient cold air without night-break treatment (NBCA), (3) no night break and no exposure to cold air (control), and for plants sown on 12 Dec., NB treatment was applied from 31 Dec. (NBlate). The exposure to cold air was achieved by moving the planter into a similar neighboring greenhouse and opening all its windows all day without heating. The air temperatures in the greenhouses for NBCA and for the other treatments were recorded with temperature data loggers (Thermo recorder TR-71S, T&D Corp., Matsumoto, Japan) during the exposure to cold air.

Plant height was measured informally in all treatments, and 12 plants per treatment were sampled for the measurement of plant height, fresh weight and dry weight of above-ground part, and stem length of harvest on 6 Feb., 2004, for NB and on 12 Feb. for other treatments. In the three treatments harvested on the same day: NBlate, NBCA, and control treatments, 10 samples of plants' above-ground parts per treatment were sampled for Brix, L-ascorbic acid, and nitrate (NO_3^-) analysis on 13 Feb. Each sample was minced, and 10 g and 5 g were used for analysis of L-ascorbic acid and nitrate, respectively. The rest of the minced sample was pressed and the Brix value, which is a measurement of the dissolved sugar-to-water mass ratio of a liquid, was measured with a refractometer (N1, Atago Co., Ltd., Tokyo, Japan).

We measured the L-ascorbic acid and nitrate concentrations with reference to Fujiwara et al.[3] and Takebe and Yoneyama[13]. The 10-g minced samples for L-ascorbic acid analysis were homogenized with 50 mL of 10 % metaphosphoric acid and were diluted to 100 mL with distilled water. The 5-g minced samples for nitrate analysis were homogenized with 50 mL of distilled water and were also diluted to 100 mL with distilled water. The diluted solutions were used for measurements of L-ascorbic acid and nitrate concentrations with a RQ flex reflectometer (Merck KGaA, Darmstadt, Germany), but only for nitrate were diluted again three times with distilled water before measurement.

Results and discussion

NB treatment accelerated plant elongation. The mean plant height of the NB plants became harvestable (from 20 to 27 cm, quoted from Kagawa[8]) on 30 Jan. and reached 26 cm on 6 Feb. when the NB plants were sampled (Fig. 1). The NBCA plants elongated as well as the NB plants until exposure to cold air, when their growth in height slowed down. The mean plant height of the NBCA plants was 24 cm, which was also harvestable, on 12 Feb., when the NBlate, NBCA, and control plants were sampled. The height of the NBCA plants was reached by the control plants on the same day (Fig. 1). The NBlate plants were the latest to be sown in this experiment. However, their growth in height was accelerated by the NB treatment, and their height slightly passed that of the control plants and the NBCA plants on 10 Feb. and 12 Feb., respectively (Fig. 1).

The air temperatures in the greenhouses used in the present study from the first day of the CA treatment (i.e.,

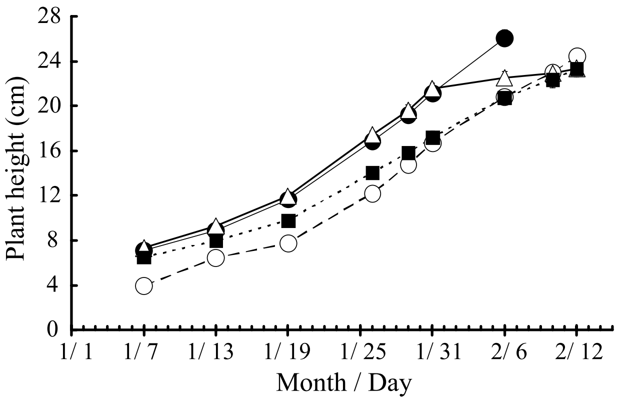

Fig. 1. Effects of night-break treatment three times a week and exposure to cold air on plant height of December-sown spinach

Plants were sown on 4 Dec., 2003 (plants in NBlate treatment were sown on 12 Dec.). Values show means of 24 plants ± standard errors. Data points without error bars indicate that the standard error was smaller than the symbol used. NB, NBlate: 1-h night break on Monday, Wednesday, and Friday nights, NBCA: NB treatment to 30 Jan. and then exposed to cold air without night break, Cont: no night break and no exposure to cold air.
—●— : NB, —○— : NBlate, —△— : NBCA,
---■--- : Cont.

exposure to ambient cold air) on the NBCA plants to the sampling day are shown in Fig. 2. In the greenhouse for the NBCA plants, the daily maximum temperature was 3 to 11°C, and the daily minimum and mean air temperatures were 2 to 8°C lower than those in the greenhouse for the NBlate and control plants. Delay to plant elongation in the NBCA plants during the CA treatment would be due to those low temperatures.

The growth of the NB plants sampled on 6 Feb. was similar to that of the NBlate plants sampled on 12 Feb. (Table 1). On comparing NBlate, NBCA, and control treatments, in which plants were sampled on the same day, the fresh weight of the plants was a little smaller in the NBlate plants but not significantly different. These results suggest that NB treatment promoted spinach growth in plant height and fresh weight. Dry matter percentage was larger in order of the NBCA, control, and NBlate plants. Stem length was small in all the treatments and there was no significant difference among the treatments. This indicates that NB treatment did not promote bolting (Table 1).

The Brix value of the sampled plants was significantly higher in order of the NBCA, control, and NBlate plants (Fig. 3). The concentration of L-ascorbic acid was significantly larger in the NBCA plants than in the NBlate and control plants (Fig. 4). There was no remarkable difference in the level of L-ascorbic acid between the NBlate plants and control plants. The concentration of nitrate (NO_3^-) was larger in order of the control, NBlate, and NBCA plants (Fig. 5). The nitrate concentration of the NBlate plants was not significantly different from those of the control plants and NBCA plants by Tukey's test at $p<0.05$ (Fig. 5) but was significantly different from only that of the control plants at $p<0.1$.

Table 1. Effects of night-break treatment three times a week and exposure to cold air on growth of December-sown spinach

Treatment	Plant height (cm)	Fresh weight (g)	Dry matter percentage (%)	Stem length (cm)
Harvest on 6 Feb.				
NB	26.0[a]	19.7[a]	10[c]	0.4[a]
Harvest on 12 Feb.				
NBlate	24.7[a]	18.8[a]	10[c]	0.4[a]
NBCA	23.8[a]	21.5[a]	13[a]	0.4[a]
Cont	23.8[a]	22.3[a]	11[b]	0.3[a]

Plants were sown on 4 December 2003 (plants in NBlate treatment were sown on 12 Dec.). Values show means of 12 plants. Values followed by the same letter are not significantly different at $p<0.05$ by Tukey's test.
NB, NBlate: treated 1-h night break at Monday, Wednesday, and Friday nights, NBCA: treated as NB to 30 January and then exposed to cold air without night break, Cont: no night break and no exposure to cold air.

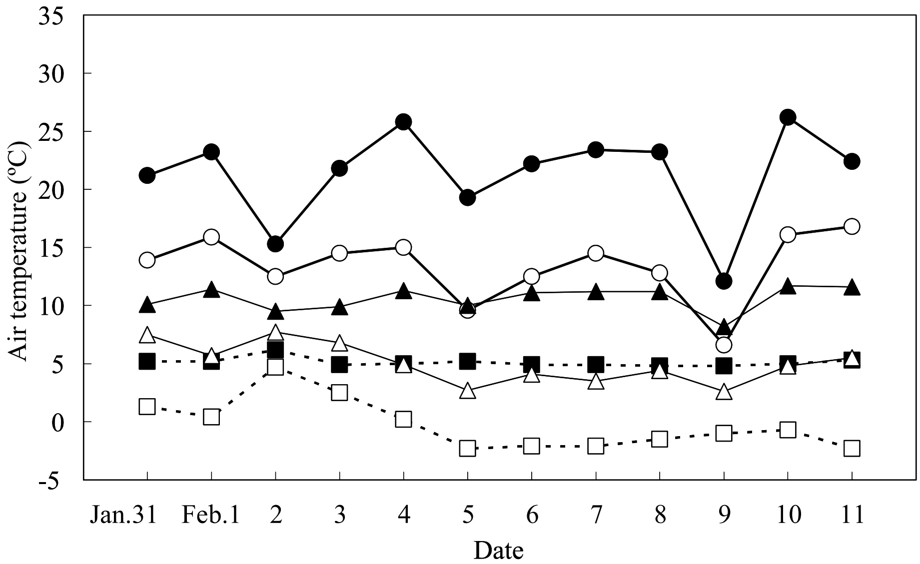

Fig. 2. Daily maximum, minimum, and average air temperatures in the greenhouse for the NBlate and control plants (Normal) and in the greenhouse for the NBCA plants (CA) during exposure to cold air for the NBCA plants
—●— : Daily max. Normal, —○— : Daily max. CA, ---■--- : Daily min. Normal, ---□--- : Daily min. CA,
—▲— : Daily mean Normal, —△— : Daily mean CA.

Relating to the effect of Kanjime on the nutritive quality of spinach crops, there have been some studies[2,10]. These studies described how Kanjime treatment increased ascorbic acid and sugar content. Aoki et al.[2] reported that Kanjime treatment also decreased water content in spinach. The present study also shows a decrease in plant water content as higher dry matter content in the NBCA plants. Those facts suggest that low plant water content caused by exposure to cold air probably relates to relatively higher L-ascorbic acid concentration and an index value related to the sugar content of liquid, Brix. Aoki et al.[2] also described that the sugar content of spinach was lower as the plant had a higher water content, and Aoki et al.[2] and Kato et al.[9] described how there was a negative correlation between the ascorbic acid content and water content in spinach.

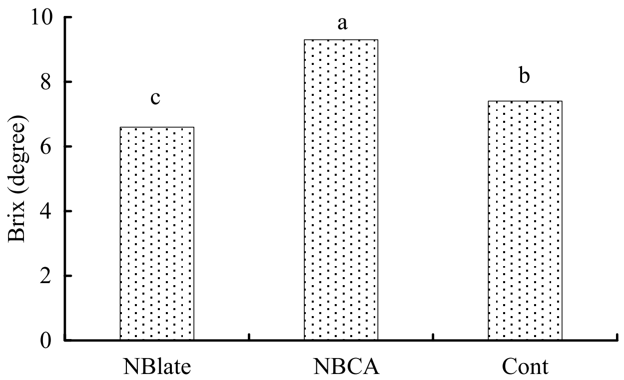

Fig. 3. Effects of night-break treatment three times a week and exposure to cold air on Brix of December-sown spinach

Values show means of 10 samples. Values followed by the same letter are not significantly different at $p<0.05$ by Tukey's test. Samples were collected on 13 Feb. 2004. NB, NBlate: 1-h night break on Monday, Wednesday, and Friday nights, NBCA: NB treatment to 30 Jan. and then exposed to cold air without night break, Cont: no night break and no exposure to cold air.

In the present study, CA treatment reduced the nitrate concentration to a lower level than that of the control plants. This result agrees with Aoki et al.[2], which reported that Kanjime treatment decreased the nitrate concentration of spinach. Aoki et al.[2] also described that nitrate concentration in plants did not depend on the plants' water content. Therefore, the decrease in nitrate concentration by CA treatment was probably caused by other factors than plant water content, e.g., a decrease in nitrate uptake.

Aoki[1] described how the Kanjime effect was achieved under conditions below 4 °C in mean air temperature. In the present study, the mean air temperature during CA treatment on the NBCA plants was 5.0 °C, a little higher than 4 °C, in the greenhouse for NBCA treatment. However, CA treatment in the present study indicated a similar effect to the Kanjime effect in the previous studies referenced here.

Considering the above, NB treatment would have the advantage in advancing the spinach harvest and decreasing nitrate concentration, but it has the disadvantage

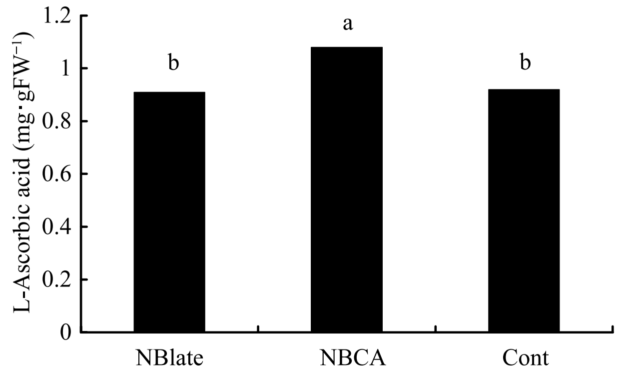

Fig. 4. Effects of night-break treatment three times a week and exposure to cold air on L-ascorbic acid concentration of December-sown spinach

Values show means of 10 samples. Values followed by the same letter are not significantly different at $p<0.05$ by Tukey's test. Samples were collected on 13 Feb., 2004. NB, NBlate: 1-h night break on Monday, Wednesday, and Friday nights, NBCA: NB treatment to 30 Jan. and then exposed to cold air without night break, Cont: no night break and no exposure to cold air.

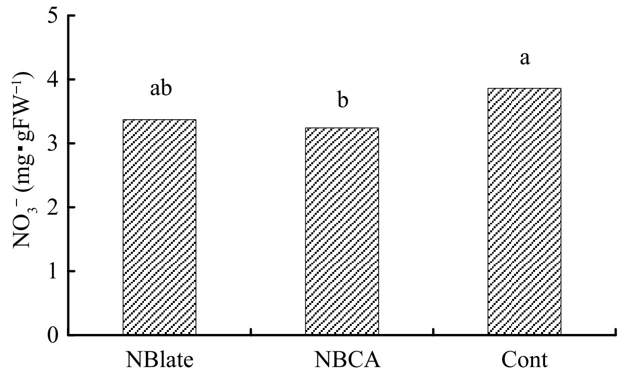

Fig. 5. Effects of night-break treatment three times a week and exposure to cold air on nitrate (NO_3^-) concentration of December-sown spinach

Values show means of 10 samples. Values followed by the same letter are not significantly different at $p<0.05$ by Tukey's test. Samples were collected on 13 Feb., 2004. NB, NBlate: 1-h night break on Monday, Wednesday, and Friday nights, NBCA: NB treatment to 30 Jan. and then exposed to cold air without night break, Cont: no night break and no exposure to cold air.

of decreasing Brix. On the other hand, NBCA treatment would bring high nutritive quality, high Brix value and L-ascorbic acid concentration, and low nitrate concentration, instead of the early harvest advantage. NBCA treatment, however, could be a method of harvesting spinach exposed to cold air earlier than the common Kanjime method because the exposure to cold air starts earlier because of the NB treatment.

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