INTRODUCTION

Internal browning is a physiological disorder affecting tomatoes (Solanum lycopersicum L.), and presents a major problem to Japanese producers. Internal browning is characterized by the discoloration (browning) of the inside of the fruit while the external surface appears normal; consequently, affected fruits reach consumers without detection. Internal browning may be caused by Ca deficiency and may be suppressed by performing defoliation. We investigated the effect of defoliation on the occurrence of internal browning in Momotaro York tomatoes grown hydroponically in a glass greenhouse and in a controlled environment chamber. The occurrence of internal browning was significantly lower in the fruits of defoliated plants (50% of leaf area removed) than in the control plants (no defoliation). Moreover, the Ca content in the water fraction of young fruits obtained from defoliated plants was significantly higher than that in control plants. Overall, this study demonstrates that defoliation increases the Ca content in fruits and reduces the occurrence of internal browning in tomato plants.

Keywords: calcium level, physiological stress, hydroponics, Solanum lycopersicum L.

MATERIALS AND METHODS

Experimental tomato varieties
S. lycopersicum 'Momotaro York' (Takii Seed Co., Ltd., Kyoto, Japan) was used in all experiments. 'NDM0112' (Nippon Del Monte Co., Ltd., Gunma, Japan) and 'Summer Kiss' (Nippon Del Monte Co., Ltd., Gunma, Japan) was reduced from 30% to 16% and from 18% to 6%, respectively (Sato et al., 2004). Since internal browning and blossom-end rot may develop due to similar factors, we hypothesize that defoliation may also suppress internal browning.

To investigate the effects of defoliation on internal browning, we first investigated the effects of defoliation on the occurrence of internal browning in Momotaro York tomatoes grown hydroponically in a glass greenhouse and in a controlled environment chamber. In this experiment, we used a nutrient solution with a lower Ca concentration and higher NH4 concentration than that in the normal culture solution. This is because this type of nutrient solution has been associated with the occurrence of blossom-end rot (Ikeda and Osawa, 1988; Terabayashi et al., 1988). Second, we compared the effects of defoliation and lack of defoliation on the Ca concentration in tomato fruits.

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internal browning often occurs in the early growth stage of tomatoes planted in this month. Plants were cultivated in a rockwool cultivation system (Mizumac, Sanshu-Kogyo Co., Ltd., Chiba, Japan) that was placed in a glass greenhouse located at the Kanagawa Agricultural Technology Center, Hiratsuka, Japan (35°35′ N, 139°28′ E). The cultivation system allowed for the drainage of 20% of the nutrient solution waste. Fresh nutrient solution was supplied to the plants 3–7 times per day. The seedlings were temporarily planted in square rockwool pots (7.5 cm²). These seedlings were planted on rockwool slabs (width, 30 cm; thickness, 7.5 cm) in a rockwool cultivation system. Plants were sprayed with 4-chlorophenoxyacetic acid (Tomato tone; ISK Biosciences Co., Ltd., Tokyo, Japan) twice per week to induce fruit setting.

The tomato seeds were sown on July 22, 2012. On August 23, 2012, the seedlings were planted on a rockwool slab with intervals of 24 cm between plants and 175 cm between furrows. The nutrient solution contained the OAT-A formula (OAT Agrio Co., Ltd., Toko, Japan; NH₄⁻N = 23 ppm; NO₃⁻N = 233 ppm; P₂O₅ = 120 ppm; K₂O = 405 ppm; CaO = 230 ppm; MgO = 60 ppm; MnO = 1.5 ppm; B₂O₃ = 1.5 ppm; Fe = 2.7 ppm; Cu = 0.03 ppm; Zn = 0.09 ppm; Mo = 0.03 ppm), which was supplied until September 7, 2012 (time of first inflorescence). Thereafter, the nutrient solution was modified to contain 0.5 × Ca concentration and 3.4 × NH₄ concentration compared to those of the normal OAT-A formula. Supplementation of the nutrient solution resulted in an electrical conductivity (EC) value of 0.8 dS m⁻¹, which was maintained until the first inflorescence; thereafter, the EC was increased to 1.5 dS m⁻¹ by increasing the fertilizer concentration. After the third inflorescence, the concentrations of components in the nutrient solution were gradually increased to generate an EC of 2.0 dS m⁻¹. Plants were defoliated on September 7, 2012 (first inflorescence) by reducing the leaf area by 50% from the first to the second flower above the third flower cluster (Fig. 2). This was performed by removing the leaflets from one side of the plant. Internal browning was evaluated twice a week in fruits obtained from the first to third fruit trusses. The fruits were selected based on their color (redness) and ripeness at a suitable harvest time between October, 2012 and January, 2013. The selected fruits were cut several times in a direction parallel to the sepals, and internal heart rot symptoms were assessed. A total of 12 plants were examined; three replicate samples were collected from four plants, each of which had a defoliated and undefoliated (control) treatment. To examine the effects of defoliation, the rates of internal browning and blossom-end rot occurrence in the treatment groups were compared. The total of all fruits obtained from the first to the third trusses was determined for every 4 plants out of the 12 plants, and the incidence of each was calculated. The number of plants showing internal browning and blossom-end rot were summarized by calculating the averages of the defoliated and undefoliated treatments and then comparing these via t-tests after performing arcsine transformation.

Experiment 2: Cultivation in a controlled environment chamber

Tomatoes were cultivated in a controlled environment chamber (KG-206SHL-D, Koito Electric Inc., Ltd., Shii-
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Fig. 3 Hydroponic apparatus used for experiments performed in a controlled environment chamber.

zuoka, Japan) and the effects of defoliation on the occurrence of internal browning of tomatoes and their Ca concentrations were investigated. The temperature of the controlled environment chamber was set at 25°C during the light period (12 h) and 20°C during the dark period (12 h).

The tomatoes were cultivated in a hydroponic device consisting of a tank (480 mm x 780 mm at the bottom; 840 mm x 880 mm at the top; 85 mm deep). A plastic mesh tray for supporting seedlings was installed above the tank, in which 12 tomato seedlings planted in rockwool cubes (75 mm³) were placed. A frame installed above the tank was used to fix the strings that supported the tomatoes (Fig. 3).

The tank was filled with nutrient solution (described later), and mulch (white and black mulch, Mikado Chemical M. F. G. Co., Ltd., Chiba, Japan) was applied to the surface of the tank to prevent light from directly reaching the nutrient solution. The temperature of the nutrient solution was maintained at 25°C using an electric heater. The nutrient solution was aerated during the cultivation period.

The OAT-A formula was used as the basis for the nutrient solution. The nutrient solution consisted of 0.5 units of the OAT-A formula until the first truss flowered, after which the nutrient solution was modified to contain 0.5 × Ca concentration and 3.4 × NH₄ concentration compared to those of the OAT-A formula. After sowing the seeds, the seedlings were grown in a closed seeding production system (Nae-Terrace; Mitsubishi Chemical Agri Dream Co., Ltd., Tokyo, Japan) for 14 d. At 50 d after sowing, 4-chlorophenoxyacetic acid was sprayed on all flowerets that bloomed within the first truss. In total, 6 of the 12 plants grown in the hydroponic devices were defoliated at the flowering stage of the first truss. The other six plants were cultivated without defoliation.

Defoliation was performed by removing all of the leaflets of all leaves on one side of the plant, thereby reducing the leaf area by 50%. All young fruits were collected 11 d after spraying 4-chlorophenoxyacetic acid and were cut open to examine the occurrence of heart rot and to determine the Ca concentration. The occurrence of internal browning was confirmed by cutting the fruit and observing the inside. The number of fruits showing internal browning was calculated for each plant, and analyzed via a t-test after performing arcsine transformation of the data.

**Determination of Ca concentration**

The Ca content in young tomato fruits was measured according to the method described by Terabayashi et al. (1998). Briefly, pieces of young fruits collected from tomato plants cultivated in the controlled environment chamber were removed and freeze-dried. Fruits of each plant were crushed using a mortar and pestle. Distilled water (50 mL) was added to 500 mg of the ground sample, and the mixture was placed in a 100 mL Erlenmeyer flask and shaken for 2 h. After shaking, the mixture was centrifuged at 1,500 ×g for 5 min, and the supernatant was collected. This step was repeated twice, and the supernatants were combined and used as the water fraction. Subsequently, 1 N sodium chloride (30 mL) was added to the precipitates, and the mixture was placed in a 100 mL Erlenmeyer flask and shaken for 2 h. After shaking, the mixture was centrifuged at 1,500 ×g for 5 min, and the supernatant was collected. This step was repeated twice, and the supernatants were combined with the 1 N sodium chloride fraction. Next, 2% acetic acid (30 mL) was added to the precipitates, and the mixture was shaken in a 100 mL Erlenmeyer flask for 12 h. The mixture was then centrifuged at 1,500 ×g for 5 min, and the supernatant was collected. This step was repeated twice, and the supernatants were combined with the 2% acetic acid fraction.

Similarly, 0.6 N hydrochloric acid (30 mL) was added to the precipitates, and the entire procedure was repeated, after which the supernatants were combined with the 0.6 N hydrochloric acid fraction. Ca was quantified using an inductively coupled plasma emission spectrophotometer (iCAP6300, Thermo Fisher Scientific Co., Ltd., Tokyo, Japan).

**Statistical analysis**

Mean values were analyzed using Student’s t-test for assessing the incidence rate of internal browning and blossom-end rot, calcium concentration after arcsine transformation, plant length, number of leaves, stem circumference, day of the harvest, yield number of fruits with disorders, and Brix value. The incidence rate of internal browning and blossom-end rot, calcium concentration, and Brix were analyzed after arcsine transformation. All analyses were conducted using the Excel add-in software Statcase2 (OMS Publishing Co., Ltd., Japan).
RESULTS

Effects of defoliation on internal browning

In Experiment 1, the incidence of internal browning in fruits harvested from defoliated plants was significantly lower than that in control plants when grown in the greenhouse on rockwool (Fig. 4) \((P < 0.01)\). Additionally, blossom-end rot was controlled by performing defoliation \((P < 0.05)\) (Fig. 4).

The occurrence of internal browning in tomatoes grown in the controlled environment chamber is shown in Fig. 5 (Experiment 2). Internal browning was not observed in young fruits collected from the defoliated plants. The difference between the defoliated and control plants was significant (Fig. 6) \((P < 0.05)\).

Effects of defoliation on Ca concentration in tomato fruits

The Ca content in young fruits collected from defoliated and control plants is shown in Fig. 7. The Ca content of the water fraction of young fruits collected from the defoliated plants was significantly higher than that of the fruits collected from the control plants \((P < 0.01)\). No significant differences were observed in the Ca content of other fractions and totals of the defoliated and undefoliated treatments \((P < 0.05); \) Fig. 7.

DISCUSSION

The present study investigated the correlation of defoliation and internal browning in tomatoes. The inhibitory effect of defoliation on internal browning was confirmed under cultivation conditions in a glass greenhouse and in a controlled environment chamber. Chemical analysis of...
young fruits collected from the controlled environment chamber showed that defoliation increased the concentration of water-soluble Ca in young fruits.

Internal browning occurs in tomatoes cultivated via hydroponics in solutions containing low Ca concentrations (Ishizuka et al., 2000; Suzuki et al., 2019). Furthermore, the frequency of internal browning increases in plants cultivated in nutrient solutions containing a low Ca concentration and a high NH₄ concentration (Terabayashi et al., 2010). The results of the present study support the fact that the cause of internal browning is Ca deficiency.

Ca absorbed by the roots is translocated at a slower rate through the plant compared to that of other elements. In the case of blossom-end rot, Ca deficiency in the fruit may be attributed to insufficient Ca absorption by the root, insufficient inflow of Ca into the fruit via the conduits, and insufficient distribution of Ca to the fruits (Ho et al., 1993; Ho and White, 2005). Therefore, in the case of internal browning, a large number of leaves may enable a relatively higher influx of Ca into the leaves instead of the fruits. We suspect that defoliation may increase the influx of water-soluble Ca to the fruits, which reduces the extent of internal browning.

The occurrence of internal browning differs among different varieties (Iwamoto et al., 1993). Indeche et al. compared the varieties ‘Momotaro Fight,’ ‘Tio,’ and ‘Pepe,’ and found that defoliation results in the highest influx of Ca to fruits of ‘Momotaro Fight,’ a variety that is highly susceptible to blossom-end rot (Indeche et al., 2020). In the present study, we tested the variety ‘Momotaro York,’ which is one of the major varieties cultivated in Japan. Similar to ‘Momotaro Fight,’ we hypothesized that defoliation of ‘Momotaro York’ plants would increase the transport rate of Ca to the fruits. Nakano et al. reported that the translocation of nutrients in ‘Momotaro York’ plants occurs at a slower rate than that of certain varieties, indicating that the effect of defoliation on reducing internal browning may be more potent in ‘Momotaro York’ compared to that in other varieties (Nakano et al., 2015). Future studies should investigate the mechanisms via which defoliation affects internal browning in different varieties.

Several aspects of internal browning remain unexplained. Internal browning may differ in the affected truss, which also depends on the period of planting (Iwamoto et al., 1993). Further investigation is needed to determine whether defoliation affects the growth and harvest of tomato plants.

Blossom-end rot can be detected on the basis of fruit appearance. However, internal browning does not create any external symptoms and thus goes undetected in distribution channels, resulting in consumer complaints. The present study demonstrated that internal browning in tomato plants can be reduced via defoliation. However, producers should aim to eliminate any occurrence of internal browning. This will require discovering the cause of internal browning and determining measures to prevent its occurrence.

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