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Reduction of leaf lettuce tipburn using an indicator cultivar

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A B S T R A C T

Tipburn is a physiological disorder caused by a calcium (Ca) deficiency that occurs mainly in leafy vegetables, such as lettuce, resulting in a reduced commercial value. To prevent tipburn injury, a sensitive cultivar was tested as an indicator for early symptom detection. An indicator cultivar began to develop tipburn two days earlier than the target cultivar. This allowed for the rescue of the target cultivar by the addition of extra Ca. The yield rate was improved from 4% to 70% with the use of an indicator cultivar in the hydroponic cultures. The top fresh weight of the target cultivar when using an indicator cultivar decreased compared with control plants, but increased compared with negative control plants lacking Ca in the culture. Water contents and root lengths were not affected by the use of an indicator cultivar and additional Ca. These results were consistent with other target cultivars under excess ammonium conditions. This system may be used against tipburn incidence without additional costs and equipment in plant factories.

1. Introduction

Leaf lettuce is grown in almost all of the plant factories in Japan because it can be harvested in a short time without the formation of fruits or heads. Additionally, it does not need a great deal of light, unlike other vegetables. It is essential to reduce the electricity costs associated with both lighting and reducing the heat generated from lights in plant factories. Operators optimize environmental conditions for rapid growth. However, the more rapidly plants grow in appropriate environmental conditions, the higher the risk of physiological disorders, especially the incidence of tipburn, that reduce the market prices of lettuce due to the rotting leaf edges, which are perceived by consumers as a sign of pathogen activity. In an extreme case, 50% of the harvested lettuce had tipburn symptoms (Benoit and Ceustermans, 1986). Many packing companies reject entire fields of lettuce with a tipburn incidence greater than 5% (Jenni and Hayes, 2010).

Saure (1998) reviewed the mechanism of tipburn, which results from the plant's inability to supply sufficient calcium (Ca) to rapidly developing leaves. Ca is important for cell membrane integrity and cell wall strength.

Attempts at the cultivation and breeding level have been undertaken to overcome tipburn. However, some methods are not practical because they require new equipment and additional costs, such as providing an air supply to inner leaves (Goto and Takakura, 1992) and the use of a far-red absorbent film (Kleemann, 2004). Through breeding strategies, a resistant cultivar is required to overcome tipburn. A crossbreeding program, which took more than 20 years, developed 'Tiber', which is a crisphead lettuce cultivar resistant to tipburn (Ryder and Waycott, 1998). However,'Tiber' is susceptible to verticillium wilt and still requires further improvement (Hayes et al., 2007). The search for the genes responsible for tipburn is still in progress due to the complexity of the physiological disorder, which is under the control of several genes. One quantitative trait locus (QTL) was detected as a useful candidate gene for breeding tipburn resistance using marker-assisted selection (Jenni et al., 2013). Other studies also revealed some candidate genes, such as cabbage *cation exchanger* (Lee et al., 2013) and tomato *pectin methylesterases* (*PME*; de Freitas et al., 2012). These findings indicate that many genes might be associated with the tipburn phenomenon and the difficulty in breeding resistant cultivars. Additionally, there is a trade-off between tipburn and growth speed, with rapid growth increasing the risk of developing tipburn (Wissemeier and Zühlke, 2002). This suggests that there may be difficulty in breeding rapidly growing cultivar with tipburn resistance. However, farmers in plant factories need cultivars that grow

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rapidly. In our previous study (Koyama et al., 2012), lettuce cultivars were classified by their susceptibility to tipburn injury. Resistant cultivars are candidates for cultivation, however, they are not necessarily target cultivars due to other characteristics, such as bolting and taste. Tipburn-sensitive cultivars might be useful as indicator plants used to monitor environmental conditions by growing them alongside target plants. In the present study, we tested this hypothesis in several experiments under different cultivation conditions.

2. Materials and methods

2.1. Plant material

Two leaf lettuces, 'Hollywood' (Yokohama Nursery Co., Japan), and 'Frillice' (Snow Brand Seed Co., Ltd.), were used for deep flow hydroponics. A crisphead lettuce 'Casper' (Yokohama Nursery Co., Japan) was used for soil hydroponics. 'Marino' (Yokohama Nursery Co., Japan) was used as an indicator plant to monitor environmental conditions by growing it alongside the target cultivated plants because it was evaluated as a tipburn-susceptible cultivar (Koyama et al., 2012).

2.2. Deep flow hydroponics

Germination, seedling growth, transplanting and cultivation were carried out according to Koyama et al. (2012) with slight modifications. Briefly, from four to five seedlings were transferred to a hydroponic system using the Hoagland and Arnon nutrient solution (Hoagland and Arnon, 1950). Conditions for growth and treatments were as follows: 23 °C and a 16-h light/8-h dark photoperiod with light provided by cool-white fluorescent lamps (photosynthetic photon flux of 150 μmol m^{-2} s^{-1}). The number of tipburned leaves and the total number of leaves were counted every day, and the percentage of tipburned leaves was calculated. The yield rate was calculated using the following formula: (total number of plantlets−tipburned plantlets)/(total number of plantlets) × 100. After 20 days, at harvesting, fresh and dry weights and root lengths were measured. The water content was calculated using the following formula: water content = (fresh weight−dry weight)/(fresh weight) × 100. The experimental plot was set with, or without, Ca as a basal, or extra, fertilizer. When $Ca(NO_3)_2$ was not used as a basal fertilizer, $NaNO_3$ was used as an alternative. An indicator cultivar was used to monitor tipburn incidence in a ratio of one to five target cultivar. Extra Ca was provided in the form of a $CaCl_2$ solution at an equivalent molar concentration of 4 mM $Ca(NO_3)_2$ once when tipburn was observed. Foliar sprays of Ca were given daily using 500-fold diluted CAL-PLUS (Otsuka AgriTechno Co., Ltd., Tokyo), which included 1.1% Ca in the form of CaO. An excess nitrogen supply was achieved by doubling the amount of ammonium nitrate (NH_4NO_3).

2.3. Soil hydroponics

Pot experiments using soil hydroponics were carried out according to Koyama et al. (2012) with slight modifications. Briefly, seedlings with 5–10 mm fifth leaf growth were transplanted into planters with vermiculite and perlite (1:1, v/v) in a glasshouse. Four seedlings were grown in each planter using the Hoagland and Arnon nutrient solution (Hoagland and Arnon, 1950), with three replications. Three experimental plots were designed: without Ca as the extra fertilizer, and with Ca administered by solution or by spray. All of the plots were set without Ca as the basal fertilizer and with an indicator cultivar. The experiment was conducted from September to November and was terminated on the 53rd day.

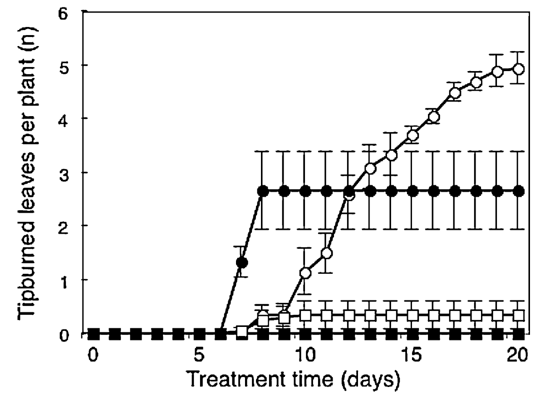

Fig. 1. Number of tipburned leaves, grown with or without the indicator cultivar, as a function of days in treatment. The cultivar 'Hollywood' was grown hydroponically in nutrient solution with Ca^{2+} (*black squares*), or without Ca^{2+} (*white circles*) during the whole term. Treatments initially lacking Ca^{2+} and then supplied a Ca^{2+} solution after tipburn in indicator cultivar 'Marino' are also shown with plots for 'Hollywood' (*white squares*) and 'Marino' (*black circles*). *Vertical bars* indicate the standard errors of 15–24 samples (n = 5–6, r = 3–4) for 'Hollywood' or 3–4 samples (n = 1, r = 3–4) for 'Marino'.

Top fresh weight, number of tip-burned leaves and percentage of tip-burned leaves were determined as described above.

2.4. Statistical analysis

The statistical analysis was performed using JMP (SAS Institute Japan Inc, Tokyo, Japan) software. Significant differences were determined by Tukey–Kramer's HSD test. The data are presented as the mean ± SE of more than five biological replicates (i.e. number of plantlets) and three technical replicates (i.e. number of batches, containers or planters for hydroponics) in each experiment. In case of the indicator cultivar, the biological replicates were not obtained (n = 1). Since the yield rate could only be calculated for every batch, which had less replicates (r = 3–4), the data was not distributed normally. In that case, the nonparametric Kruskal–Wallis test was used for analysis.

3. Results

3.1. Reduction in tipburn incidence by monitoring an indicator cultivar

A sensitive cultivar 'Marino' was selected as an indicator in an experiment assessing preventative measures against tipburn, based on our previous knowledge (Koyama et al., 2012). 'Hollywood' was selected as the target cultivar. At first, the timing and rate of tipburn occurrence were investigated. Fig. 1 shows a time course indicating the number of tipburned leaves in lettuce grown hydroponically. No tipburned leaves were detected when plants were grown in a normal medium including Ca (Fig. 1: black box). The number of tipburned leaves increased after 8 d and reached approximately five leaves per plant at 20 d after being transplanted in a Ca-free medium (Fig. 1: white circle). The indicator cultivar began to develop tipburn 2 d earlier than the target cultivar (Fig. 1:black circle). A $CaCl_2$ solution was added on the same day tipburn appeared, and the treatment reduced the number of tipburned leaves in the target (Fig. 1:white box). These results suggest that 'Marino' is more sensitive to Ca starvation than 'Hollywood' and that the extra Ca rescued the products from tipburn symptoms. A statistical analysis revealed the significant differences among measured parameters at harvest after various treatments (Table 1). The yield rate of the target cultivar was 4.2% when no indicator cultivar was grown, and it improved significantly to 70.0% with the use of