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Effects of Light during Low Temperature Treatment and Water Stress on Freezing Tolerance and Sugar Contents in Cabbage Seedlings

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Abstract
Cabbage seedlings exposed to non-freezing low temperature (5°C) under a 12 h photoperiod acquired the freezing tolerance, while the plants exposed to non-freezing low temperature in the dark did not acquire the freezing tolerance. On the contrary, in the case of reversal from cold acclimation, the freezing tolerance of the plants was reduced by exposure to normal growth temperature (20/15°C) regardless of light conditions. These changes in freezing tolerance coincided with the changes in the sugar contents of cabbage leaves. The plants subjected to water stress by withholding water displayed a higher degree of freezing tolerance and increase of sugar contents compared with the watered plants. Thus, it was assumed that cabbage seedlings required light to acquire the freezing tolerance after exposure to low temperature, while de-acclimation was induced without light and it was confirmed that water stress increased to a certain extent the freezing tolerance in cabbage seedlings.

Discipline: Horticulture
Additional key words: cold acclimation, de-acclimation

Introduction

Cabbage is one of the most important vegetables in Japan and is often injured by cold or frost. Most of the overwintering plants acquire the freezing tolerance by exposure to non-freezing low temperature, and this process is referred to as cold acclimation. Freezing tolerance of plants is affected by various environmental factors[1], besides temperature. Furthermore, cold acclimation is also influenced by several environmental factors. Drought and desiccation stress increased the freezing tolerance in rye[2] and winter wheat[14]. On the other hand, it has been reported that cold acclimation is accompanied by biochemical changes affecting sugars[9,11], proteins[10] and plasma membrane lipids[15]. Sasaki et al. (1996)[8] showed that cabbage seedlings exposed to non-freezing low temperature (5°C) acquired a freezing tolerance. However, the role of light during cold acclimation and de-acclimation and the relationship between the freezing tolerance and water stress in cabbage seedlings remained to be elucidated.

The analysis of the mechanism of acquisition and loss of freezing tolerance in cabbage could contribute to the stable production of cabbage and other vegetables. This study was conducted to investigate the effects of light during cold acclimation and de-acclimation and water stress on freezing tolerance and sugar contents in cabbage seedlings.

Materials and methods

1) Plant materials and growth conditions

Seeds of cabbage (*Brassica oleracea* L. cv. Banchu-

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risou) were sown in plastic pots filled with a soil mixture (Pretty Soil Gold N-140, Otsukasangyo, Nagano, Japan). Plants (one plant per pot) were grown in a growth chamber at 20/15°C (day/night) and under a 12 h photoperiod in which illumination was supplied by metal halid lamps (MLBOC400C-U, Mitsubishi Electric OSRAM, Yokohama, Japan) using a photosynthetic photon flux density (PPFD) of 230 ± 10 μmol · m^{-2} ·s^{-1}.

2) Exp.1 Effects of light on cold acclimation and de-acclimation

Three weeks after sowing, the seedlings were exposed to 5°C for 7 days under a 0 (dark) or 12 h (light) photoperiod as cold acclimation treatments. For de-acclimation treatments, seedlings cold-acclimated by exposure to 5°C under a 12 h photoperiod for 7 days were transferred to a growth chamber at 20/15°C for 2 days under a 0 or 12 h photoperiod. In the plants subjected to the low temperature and de-acclimation treatments, the freezing tolerance and sugar contents were determined.

3) Exp.2 Effects of water stress on freezing tolerance and sugar contents

The seedlings which unfolded 2 leaves and were watered daily, or not watered for 3 days, were grown in a growth chamber at 20/15°C under a 12 h photoperiod. The amount of water supplied to the plants exceeded 40 mL · pot^{-1} · day^{-1}. The soil water contents (mean ± standard error) of the watered and non-watered treatments were 45.4 ± 1.6% and 22.3 ± 0.9%, respectively. The plants that were watered and not watered were used for measuring the freezing tolerance and sugar contents.

4) Freezing tolerance test

The degree of freezing tolerance of the seedlings was expressed in terms of the electrolyte leakage determined by methods previously described[8]. Leaf discs excised from the 2nd leaves of the seedlings were placed in a test tube. Test tubes were transferred to a chamber where the temperature was lowered to –8°C at a rate of 0.25°C/min. When the temperature in the chamber was –2°C, test tubes were maintained at –2°C for 1 h and the leaf discs were sprayed with deionized water to initiate extracellular freezing. Test tubes were maintained at –2, –4, –6 or –8°C for 30 min and were allowed to thaw at room temperature for about 1 h. The warming rate was about 0.4°C/min. Test tubes in which deionized water (15 mL) was added were stored overnight at room temperature. Then the conductivity of the solution in the test tubes was measured. After heating in boiling water for 20 min, the conductivity was again measured. The degree of electrolyte leakage was calculated as a percentage of conductivity of the solution before heating to that after heating.

5) Determination of soluble sugars

Soluble sugar contents were measured by the method previously described[12]. The 2nd leaves were cut from the plants and their midribs were removed. The remainder of the leaf was extracted in 10 volumes of 80% (v/v) ethanol at approx. 75°C for 30 min. After cooling at room temperature, xylose (0.5% of the sample weight) was added as an internal standard, and the sample was homogenized. The homogenate was centrifuged at 1,500 × *g* for 10 min. The pellet was re-extracted twice in 5 volumes of 80% (v/v) ethanol, the three supernatants

Table 1. Effects of light during cold acclimation and de-acclimation treatments on freezing tolerance of cabbage seedlings

Treatment	Electrolyte leakage (%)			
	Freezing test			
	–2°C	–4°C	–6°C	–8°C
C[a]	10.3 ± 1.9[b]	85.7 ± 7.1	97.2 ± 2.8	99.9 ± 2.6
L-A	7.2 ± 1.7	27.4 ± 7.1	48.0 ± 8.1	90.1 ± 5.6
D-A	32.0 ± 3.3	98.3 ± 1.2	98.9 ± 3.7	100.0 ± 0.7
L-D	12.3 ± 1.8	70.4 ± 5.2	93.5 ± 3.9	100.0 ± 0.0
D-D	21.9 ± 1.3	83.7 ± 10.5	82.0 ± 12.1	98.6 ± 0.7

a): C, control; L-A, cold acclimation treatment (5°C) under 12 h photoperiod; D-A, cold acclimation treatment under 0 h photoperiod; L-D, cold acclimation under 12 h photoperiod followed by de-acclimation treatment (20/15°C) under 12 h photoperiod; D-D, cold acclimation under 12 h photoperiod followed by de-acclimation treatment under 0 h photoperiod.

b): Values are means ± standard errors (3 replications).

Freezing tolerance is expressed in terms of the percentage of electrolyte leakage from leaves after freezing test at –2, –4, –6 and –8°C.

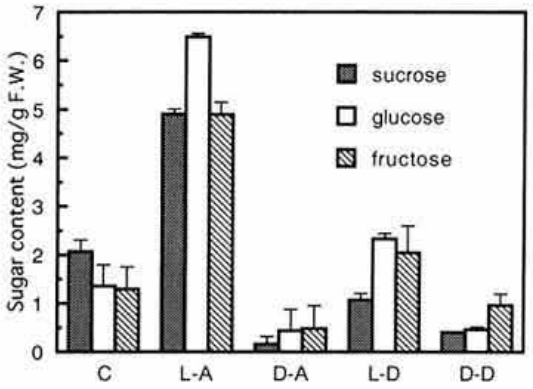

Fig. 1. Sugar content in cabbage leaves affected by light and dark conditions during cold acclimation and de-acclimation treatments

The symbols are the same as those in Table 1.
Vertical bars indicate standard errors (n=3).

were combined and dried in a vacuum centrifuge. The residue was dissolved in 1 mL of distilled water. The sample was passed through a SEP-PAK C18 cartridge (Millipore Corporation, Massachusetts, USA) which had been equilibrated with water, after which 2 mL of distilled water was eluted. An aliquot (20 µL) of the eluate was analyzed by HPLC using an 830-RI refractive index detector (JASCO, Tokyo, Japan) and a Shodex SUGAR SP0810 column (Showa Denko Co., Tokyo, Japan). The column temperature was 80°C, and the mobile phase consisted of distilled water at a flow rate of 0.8 mL min^{-1}.

Results and discussion

1) Exp.1 Effects of light on cold acclimation and de-acclimation

Control seedlings showed a high electrolyte leakage in the freezing tolerance test below –4°C (Table 1). The electrolyte leakage from the plants exposed to low temperature under a 12 h photoperiod (L-A) was lower than that of the control in all the freezing tolerance tests, indicating the acquisition of freezing tolerance as cold acclimation. The electrolyte leakage from the plants exposed to low temperature without light (D-A) in the freezing tolerance test at –4°C was 98.3%, implying the existence of injury by the freeze-thaw cycle owing to freezing intolerance. Thus, light was necessary to acquire the freezing tolerance by cold acclimation. On the other hand, the acquired freezing tolerance by cold acclimation was lost by returning the plants to normal growth temperature (20/15°C) under the dark (D-D) and light (L-D) conditions. Although cold hardiness induction in woody plants was accelerated by a short photoperiod[4], photoperiod alone did not induce a cold-hardening response in winter rye[3]. Griffith & McIntyre (1993)[3] reported that the development of frost tolerance in winter rye depended upon irradiance, which affected the amount of photoassimilates available. Therefore, the lack of acquisition of freezing tolerance of the cabbage seedlings exposed to low temperature in the dark was attributed to the shortage of photosynthates.

In the plants exposed to a low temperature under a 12 h photoperiod (L-A), the contents of sucrose, glucose and fructose markedly increased as compared with the controls (Fig. 1), while in the plants exposed to low temperature without light (D-A) the sugar contents decreased. During the de-acclimation treatments, the amounts of accumulated sugars in leaves were reduced (L-D and D-D) regardless of light. These changes in the freezing tolerance coincided with the fluctuations of the sugar contents.

2) Exp.2 Effects of water stress on freezing tolerance and sugar contents

The leaves of the NW plants (non-watered plants) wilted, indicating that withholding of water led to water stress in the plants. In all the freezing tolerance tests (Table 2), electrolyte leakage from NW was lower than that from the watered plants as controls. Water stress has been reported to increase the freezing tolerance in some plants, such as *Arabidopsis thaliana*[6] and rye[2]. The extent of the freezing tolerance achieved by exposure to

Table 2. Effects of water stress on freezing tolerance of cabbage seedlings

Treatment	Electrolyte leakage (%)			
	Freezing test			
	–2°C	–4°C	–6°C	–8°C
C[a]	43.5 ± 3.1[b]	86.3 ± 1.3	93.6 ± 0.3	84.2 ± 15.8
NW	23.0 ± 2.1	70.2 ± 5.7	84.6 ± 3.5	83.8 ± 10.5

a): C, watered (more than 40 mL pot^{-1} day^{-1}); NW, not watered.
b): Values are means ± standard errors (3 replications).
Freezing tolerance is expressed in terms of the percentage of electrolyte leakage from leaves after freezing test at –2, –4, –6 and –8°C.

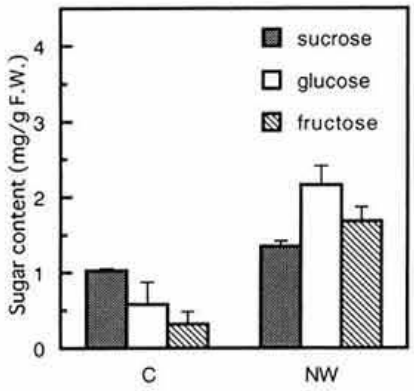

Fig. 2. Sugar content in cabbage leaves affected by water stress

The symbols are the same as those in Table 2.
Vertical bars indicate standard errors (n=3).

drought was of a similar magnitude to that observed in treated *Arabidopsis thaliana*[6] plants exposed to low temperature. However our results showed that the increase of the freezing tolerance induced by water stress was not appreciable. It is assumed that this discrepancy might be due to the drought tolerance of the plant species or difference in the methods of exposure to drought.

The contents of sucrose, glucose and fructose in the non-watered plants (NW) increased compared with those of the watered plants (Fig. 2). These findings are in agreement with the results obtained in cotton plants[13] and wheat[5]. Although sugars are the major constituents of osmoregulation under conditions of water deficit[7], it remains to be determined whether sugar accumulation properties (such as osmolyte systems and subcellular localization) are identical with the sugar accumulation induced by cold acclimation or not.

Conclusion

Cabbage seedlings required light to acquire freezing tolerance by exposure to non-freezing low temperature, while de-acclimation was induced without light. The increase of the freezing tolerance was associated with sugar accumulation in response to low temperature. Water stress induced the freezing tolerance and sugar accumulation at temperatures that did not induce cold acclimation. The results in this study suggest that the sugar metabolism is involved in the mechanism of acquisition and loss of freezing tolerance in cabbage seedlings.

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