Water temperatures during vegetative growth affect cold tolerance at the booting stage of rice under controlled environmental conditions

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Abstract

We tested the effect of water temperature \( (T_w) \) during vegetative growth on the cold tolerance of rice under fully controlled environmental conditions. We grew seedlings of the rice cultivar ‘Hayayuki’ in a growth chamber, and at the four-leaf stage exposed some of the seedlings to \( T_w \) of \( 18^\circ C \) (low \( T_w \) treatment) for 14 days, and the rest to \( T_w \) of \( 23^\circ C \) (control). At the booting stage, plants were subjected to low air temperature \( (12^\circ C \) for 4 or 5 days) in another growth chamber to induce spikelet sterility. Palea length, which is an indicator of the pollen developmental stage, increased linearly with increasing auricle distance, and there was no difference in this relationship between treatments. Spikelet sterility was significantly higher in plants exposed to low \( T_w \) during vegetative growth. These results suggest that \( T_w \) during vegetative growth affects the degree of physiological cold tolerance of rice during the booting stage.

Key words: Chilling injury, Cold tolerance, Grain yield, Rice, Spikelet sterility.

1. Introduction

Spikelet sterility of rice \( (Oryza sativa \, \, L.) \) can be induced by low temperatures during the booting stage, and especially during the young microspore stage (Satake, 1976). This can significantly decrease rice production in temperate areas. Although the cold tolerance of rice cultivars and lines at the booting stage is basically controlled by genes inherited from their parents, cold tolerance is known to be affected by the growth environment before the critical part of the booting stage, and water temperature \( (T_w) \) has an especially strong effect. For example, Satake et al. (1988) found that high \( T_w \) between the panicle initiation and booting stages can increase the temperature of the developing panicles, increase the number of developing microspores, and improve cold tolerance during the booting stage. Recently, Shimono et al. (2007) found that high \( T_w \) during vegetative growth before the panicle initiation stage can improve cold tolerance at the booting stage in a greenhouse experiment with \( T_w \) controlled during vegetative growth. This response was confirmed by a field trial at multiple locations with different climates (Shimono and Kanda, 2008) and using artificial heating of the water (Shimono et al., 2011).

However, these results (Shimono et al., 2007, 2011; Shimono and Kanda, 2008) were obtained under largely natural environmental conditions, with only \( T_w \) controlled and other environmental factors left uncontrolled. This makes the results difficult to interpret, since environmental factors such as air temperature (Koike and Satake, 1987; Shimono et al., 2005) and solar radiation (Wada et al., 1972) could have affected cold tolerance. Because the growth stages on a given date differ between plants exposed to different \( T_w \)s, this suggests that characteristics of the seedling environment other than \( T_w \) during various growth stages, including booting, might differ under natural environmental conditions.

In addition, these cold tolerance tests were conducted by inducing sterility using long-term irrigation with cool water \( (19^\circ C \) for more than 30 days with deep water, 30 cm) from panicle initiation to full heading, not only at the booting stage. This method has been
successfully used for screening of cold-tolerant cultivars in breeding programs (Nagano, 1999), and is commonly used for physiological and molecular research (Saito et al., 2001; Oda et al., 2010). However, short-term exposure to cool air during the booting stage (12°C for 4 to 5 days), excluding changes in other factors during panicle development (Satake, 1976), would be better for analyzing the physiological and molecular responses of rice plants (Oliver et al., 2005; Sato et al., 2011).

Here, we examined the effects of $T_w$ during vegetative growth on cold tolerance under fully controlled environmental conditions. We evaluated the degree of cold tolerance using short-term exposure to cool air precisely during the booting stage.

2. Materials and Methods

2.1 Plant materials and growth conditions

We used the rice cultivar ‘Hayayuki’, a cultivar from the Hokkaido region (Japan’s northern island) that has strong cold tolerance and early maturation. Germinated seeds were directly sown at 20 seeds per pot in a circular pattern in 4-L plastic pots (1/5000-a Wagner pot, Fujiwara Co., Tokyo, Japan) filled with non-fertilized commercial soil (Muhiryou-baido, Ai-Kei Co., Akita, Japan). We then added fertilizer as 1.2 g each of N, P$_2$O$_5$, and K$_2$O. To exclude errors caused by physiological differences between the tillers, we removed all tillers except the main stem as they appeared, following the method of Satake (1976). The plants were grown under flooded conditions, with a 5-cm water depth, throughout the growing season in a PGW36 growth chamber (Conviron Co., Winnipeg, Manitoba, Canada). The air temperature was controlled at an optimal growth condition of 26.5±0.2°C (day) and 20.8±0.4°C (night) (12 h each, average ± standard deviation), at a light intensity of 400 μmol PAR m$^{-2}$ s$^{-1}$ and 67.2±3.2% relative humidity. Initially, $T_w$ was not controlled, and the daily average $T_w$ was 22.7±0.3°C (23.7±0.6°C for day and 21.6±0.4°C for night).

2.2 Low $T_w$ treatment during vegetative growth

At the four-leaf stage (18 days after sowing, DAS) (Fig. 1), rice plants were divided into two groups and exposed to two $T_w$ conditions: a control, with the normal $T_w$ condition (22.7±0.3°C, above mentioned), and the low $T_w$ condition, with a $T_w$ of 17.5±0.1°C of daily mean (17.9±0.1°C for day and 17.1±0.1°C for night) (this temperature range was utilized in the previous study (Shimono et al., 2007)) for 14 days; that is, the cold treatment used a temperature 5.2°C lower than the control. The low $T_w$ condition was produced by a closed-system bath circulator that supplied cool water (16.0°C) to the plants (NESLAB RTE 7 Digital One, Thermo Scientific Co., Massachusetts, USA) through silicone tubes. At the end of the low $T_w$ treatment, the mean number of leaves on the main stem was 5.8 (at 32 DAS), and we destructively sampled several plants to determine the panicle initiation status. We confirmed that no panicles had been initiated by inspection under a microscope. Panicle initiation was observed at the seven-leaf stage, and the final leaf (the flag leaf) in both the control and low $T_w$ conditions was the 9th leaf. This examination confirmed that we had exposed the plants to low $T_w$ during vegetative growth, before panicle initiation. Note that in the low $T_w$ treatment, some plants had their 10th leaf as the flag, but we used only plants with their 9th leaf as the

![Fig. 1. Diagram of experimental schedule for testing the impact of water temperature during vegetative growth on the cold tolerance in rice under fully controlled environmental conditions.](image-url)
flag leaf to avoid errors caused by this difference.

2.3 Cold tolerance during the booting stage

During the booting stage, around the young microspore stage (from 43 DAS at control $T_w$ and from 48 DAS at low $T_w$), plants were cooled in another growth chamber (PGW36, Conviron) with an air temperature of $12.3^\circ C \pm 0.2^\circ C$ (mean and standard deviation) (24 h per day) for 4 or 5 days, with all other environmental conditions identical to those in the chamber with optimal air temperature conditions except for a light intensity of 100 $\mu$mol PAR m$^{-2}$ s$^{-1}$ to uniformly induce sterility while excluding differences in surface temperature (Satake, 1976). After the cooling treatment, the pots were returned to the chamber with the normal air temperatures until maturation.

2.4 Measurements

We used the auricle distance method (Satake, 1976), in which the distance between the auricles of the flag leaf and the secondary leaf is used as an indicator of the developmental stage of the microspores. To identify the relationship between auricle distance and the actual microspore developmental stage, we sampled panicles at a range of auricle distances. To do so, we sampled the nine spikelets in each of the uppermost 3rd to 5th positions in the three uppermost primary branches per panicle, which Satake (1976) called the “specific spikelets”. The spikelets were stored in 50% ethanol at 4°C. They were then used to measure the palea length, and the developmental stages of the microspores were determined under a microscope at 400×. The definition of the microspore growth stage and most other experimental protocols followed the methods of Satake (1976).

At harvest, we sampled the specific spikelets and measured the total number of spikelets per panicle and the number of sterile spikelets at the specific spikelet positions, and calculated the spikelet sterility (%). Sterile spikelets were carefully identified by backlighting the heads using fluorescent lightbulbs; spikelets that showed no shadowy area (i.e., no developing embryo or grain) were considered to be sterile.

2.5 Statistical analysis

We tested for significant differences between the $T_w$ treatments using the $t$-test (Microsoft Excel 2003, Microsoft Co., Redmond, Washington, USA). We also used the software Excel 2003 to perform regressions of the relationships between auricle distance and the palea length, and used it to compare residual variations to test whether the individual regression lines for the two $T_w$ treatments differed significantly (Mead et al., 2003).

3. Results

3.1 Auricle distance, palea length, and pollen developmental stage

There was a significant linear correlation between

\[ y = 0.2521x + 6.6371 \]

\[ r = 0.946^{***} \]

![Graph](image)

Fig. 2. The relationship between auricle distance and palea length in the “specific spikelets” (see Materials and Methods for details) of rice plants at the booting stage exposed to different values of $T_w$ during vegetative growth. (A), (B), and (C) in figure indicates stages of microspore, tetrad phase, early microspore phase and middle microspore phase respectively. *** $P<0.001$. 

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the auricle distance and the palea length of plants exposed to different $T_w$ during vegetative growth ($r=0.946, P<0.001$) in the range of -11 to 0 cm, and there was no significant difference between the regression lines of each treatment ($F_{2,15}=1.9, P=0.19$) (Fig. 2). This result indicates that the growth stage can be detected from the auricle distance in plants exposed to both $T_w$ control conditions without the need for destructive sampling. The microscopic observation also revealed that for a palea length ranging from 3.3 to 3.6 mm, tetrad microspore cells were visible (Fig. 2A), and for lengths ranging from 3.5 to 4.5 mm, early microspore cells were visible (Fig. 2B). When palea length $>5$ mm, microspores had reached the middle microspore phase (Fig. 2C). Our results suggest that microspores at the young microspore phase (i.e., at an auricle distance of -8 to -10 cm) were most sensitive to cold air, confirming the results of Satake (1976), and suggest that our cold tolerance test was conducted at an appropriate physiological stage of the plants.

3.2 Spikelet sterility

Spikelet sterility induced by cool air on the control was 14 percentage points higher after 5 days than after 4 days (Fig. 3). At both treatment durations, low $T_w$ during vegetative growth significantly increased spikelet sterility, with the increase amounting to 20 percentage points at 4 days ($P<0.01$) ($n=32$ and 15 at control $T_w$ and low $T_w$, respectively) and 28 percentage points at 5 days ($P<0.001$) ($n=20$ and 37, at control $T_w$ and low $T_w$, respectively). Note that without the cold air treatment, we saw no sterility in the plants (0% sterility, $n=18$ or 15 for control $T_w$ or low $T_w$, respectively).

4. Discussion

The results of our experiment, which was conducted under controlled environmental conditions, confirmed previous findings that $T_w$ during vegetative growth can affect the cold tolerance of rice. Our method, based on that of Satake (1976), is a standard method used in physiological and molecular studies, and has been used intensively in cold tolerance experiments. Our results therefore provide an important basis for further physiological and molecular analyses of the rice plant’s responses to cold temperatures.

Our results were obtained using the cultivar...
‘Hayayuki’, which originated in Hokkaido. This is the first report involving a Hokkaido cultivar, which has a different origin from the Tohoku cultivars (from the northern part of Honshu island) used in previous studies (Shimono et al., 2007, 2011; Shimono and Kanda, 2008). Our confirmation of those previous results indicates that the response of cold tolerance to $T_w$ during vegetative growth might be general (i.e., might be true for many or all rice cultivars), although further studies would be required to confirm this (e.g., for southern or Chinese cultivars). The cultivar ‘Hayayuki’ matures very early; it requires only 43 to 48 days after sowing to reach the booting stage, in contrast with a cultivar such as ‘Hitomebore’, a Tohoku cultivar that requires about 100 days in the fields of Iwate Prefecture. This rapid maturation makes it possible to efficiently evaluate the physiological and molecular responses of rice in a short time.

A slight difference in the pollen developmental stages can cause a large difference in sterility because the sensitivity to low temperature at the booting stage is strongly dependent on the pollen developmental stage (Satake, 1976). In the present study, the relationship between auricle distance and palea length was identical for plants grown under different $T_w$ during vegetative growth over the range of $T_w$ (Fig. 2). The palea length is well known to be an indicator of the developmental stage of pollen (Satake, 1976). The present cold tolerance test, judged using auricle distance at the booting stage, exposed plants to cold temperatures at the pollen developmental stage when rice is most sensitive to low temperatures. Thus, the present study found that low $T_w$ during vegetative growth affected physiological status in developing spikelets such as chemical component and/or gene expression for the cold tolerance of spikelet fertilization.

The effects of low $T_w$ on spikelet sterility amounted to about 4~5 percentage points per 1°C decrease (Fig. 3). Although it is difficult to directly compare these results with results derived from different methods (including different cultivars, different treatments during vegetative growth, and a different cold tolerance test), the magnitude was close to the previous result of 5 percentage points per 1°C (Shimono et al., 2007, 2011).

In conclusion, $T_w$ during vegetative growth can affect the cold tolerance of rice under a fully controlled environment. We established an experimental protocol that can be used with confidence in future physiological and molecular research.

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