Effects of salt and low light intensity during the vegetative stage on susceptibility of rice to male sterility induced by chilling stress during the reproductive stage

Takemasa Koumotoa, Naoko Saitoa, Naohiro Aokib, Toshiki Iwasakic, Shigenao Kawaiia, Shuji Yokoiia and Hiroyuki Shimonoa

aFaculty of Agriculture, Iwate University, Ueda, Japan; bDepartment of Agricultural and Environmental Biology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan; cGraduate School of Science, Tohoku University, Sendai, Japan

ABSTRACT
We tested whether exposing rice plants to abiotic stress (salt or shade) during vegetative growth affects the chilling tolerance of reproductive organs, which is one of the most important traits for rice growing in a cool climate; we used two rice cultivars with different tolerance in two growing seasons. We divided the vegetative growth into three phases to clarify the most sensitive period: 7–22 days after transplanting (DAT), 23–38 DAT and 39–54 DAT. Chilling tolerance of the pre-stressed plants was based on the male sterility induced by low temperatures. Shade and salt stress during all three vegetative growth phases significantly reduced stomatal conductance. Shade decreased the specific leaf weight and the leaf sugar and starch contents, but salt had no significant effect, despite causing leaf damage. Low temperatures during the reproductive stage induced spikelet sterility in all plants, but the magnitude was greater in the salt- and shade-stressed plants of both cultivars, especially those stressed late during vegetative growth. The increased spikelet sterility caused by chilling was closely related to the reduction of the total spikelet number per panicle. This is the first study to show that salt and low light stress during vegetative growth increased the susceptibility of rice plants to chilling damage during panicle development.

1. Introduction
Rice (Oryza sativa L.), which originated in a tropical environment, is sensitive to suboptimal temperatures, especially during the reproductive stage, when temperatures lower than 20 °C can induce male sterility, leading to severe yield losses (Matsushima et al., 1964; Shimono et al., 2002). The ability of chilling to induce male sterility is determined by inherited characteristics, and quantitative trait loci (QTLs) have been detected on most chromosomes: chromosome 1 (Andaya & Mackill, 2003; Kuroki et al., 2011; Takeuchi et al., 2001; Xu et al., 2008), 2 (Andaya & Mackill, 2003), 3 (Andaya & Mackill, 2003; Dai et al., 2004; Mori et al., 2011; Shirasawa et al., 2012; Suh et al., 2010), 4 (Saito et al., 2001; Xu et al., 2008), 5 (Andaya & Mackill, 2003; Shimono et al., 2016; Xu et al., 2008), 7 (Suh et al., 2010; Takeuchi et al., 2001; Xu et al., 2008; Zhou et al., 2010), 8 (Kuroki et al., 2011), 9 (Suh et al., 2010), 10 (Dai et al., 2004; Ye et al., 2010), 11 (Oh et al., 2004; Takeuchi et al., 2001) and 12 (Andaya & Mackill, 2003; Li et al., 1997; Shimono et al., 2016). However, most QTLs have not been annotated in terms of their physiological or molecular roles in chilling tolerance and the complex nature of the regulatory mechanisms. The underlying mechanisms that control chilling tolerance are therefore not fully understood.

Recently, the inherited chilling tolerance of certain cultivars was reported to vary in response to the environmental conditions during vegetative growth. We found that the episode by low temperature at vegetative stage decreased the chilling tolerance (male sterility) (Shimono et al., 2007; Suzuki et al., 2015). This phenomena was commonly observed over nine rice cultivars at different conditions in a greenhouse (Shimono et al., 2007), in the field (Abe et al., 2013; Kanda et al., 2012; Shimono & Kanda, 2008; Shimono et al., 2011; Shimono et al., 2012), and under fully controlled-environment conditions (Matsumura et al., 2012; Suzuki et al., 2015). However, we found no studies that investigated whether the phenomena could be triggered by environmental cues other than temperature during vegetative growth.

In the field, additional environmental factors, such as solar radiation, which provides the energy used for...
photosynthesis, growth and stress responses (Yoshida, 1981), can fluctuate both seasonally and yearly. In the Tohoku region of northern Japan, one of the world’s northernmost rice-growing areas, the Yamase wind frequently carries fog and chilling air into rice-producing areas during the growing season (Kanno, 1997), leading to low radiation intensity and chilling. The combined effects of low light intensity and chilling during the reproductive stage can increase spikelet sterility, although this is not inevitable (reviewed by Wada, 1992). However, there has been no study that determined whether low light intensity during vegetative growth affects the chilling tolerance of rice during the reproductive stage.

In the field, salt creates another important abiotic stress that can strongly affect plant growth by increasing water stress and by the direct effects of salt ions (Munns & Tester, 2008). This is likely to become an increasingly serious problem in paddy areas close to the sea, as sea levels rise under global warming (IPCC, 2007), although periodic disasters such as tsunamis can expose even inland fields to salt (Shimono et al., 2012). It is well known that salt stress during vegetative growth can damage the photosynthetic apparatus, cause stomatal closure and reduce growth (Munns & Tester, 2008). During the reproductive stage, salt can induce spikelet sterility (Tsuda, 2007). However, we found no studies of whether salt stress during the vegetative stage can affect chilling tolerance during the reproductive stage.

In the present study, our goal was to provide some of the missing data on impact of low light intensity and salt at vegetative stage on male sterility at the reproductive stage.

2. Materials and methods

2.1. Plant materials

We conducted pot experiments in 2012 and 2013 at Iwate University, Japan (39°42’N, 141°8’E). Seedlings of two rice cultivars with different chilling tolerances (‘Hitomebore’, with strong tolerance, and ‘Sasanishiki’, with weak tolerance) were transplanted on 16 May in both years. Plants with the water temperature controlled at 21.5 °C (2012) or 25.0 °C (2013). Temperatures were controlled with a 40-W heater and cooling coils connected to a CA-1115A recirculating pump (Eyela, Tokyo Rikakikai Co. Ltd., Tokyo, Japan) controlled by a CR-10 datalogger (Campbell Scientific, Inc., Logan, UT, USA), as shown in Supplemental Figure S1a. A Pt100 thermometer was used to measure water temperature ($T_w$). Water was circulated by a pump to minimize the spatial variation in $T_w$. Shimono et al. (2007, 2011) provide a detailed description of the set-up.

In 2012, we used 42 pots (= 714 plants) for the experiments: 21 pots for each cultivar, with 9 pots treated at low light intensity and 9 pots for the salt treatments (in each case, 3 $T_w$ conditions during reproductive growth × 3 treatment phases during vegetative growth × 1 pot per treatment combination) and 3 pots for the control (3 $T_w$ conditions during reproductive growth only × 1 pot per treatment). Figure 1 shows the timing and durations of the treatments during vegetative and reproductive growth; section 2.2 provides details of each treatment. In 2013, we used 36 pots (= 612 plants) for the experiments: 18 pots for each cultivar, with 6 pots at low light intensity and 6 pots for the salt treatment (in each case, 3 $T_w$ conditions during reproductive growth × 1 treatment phase during vegetative growth × 2 pots per treatment combination) and 6 pots for the control (3 $T_w$ conditions during reproductive growth only × 2 pots per treatment combination).

2.2. Pre-treatments during vegetative growth and treatments during the reproductive stage

Figure 1 illustrates the study design. During vegetative growth in 2012, the pre-treatments with low light intensity and salt were conducted during three ‘phases’, each of ca. 2 weeks; starting dates were 7–22 days after transplanting (DAT), 23–38 DAT and 39–54 DAT. The date of end of the treatments corresponded to periods between 40 and 8 days before panicle initiation (DBPI). In 2013, there was only one pre-treatment phase (from 29–42 DAT, ending at 11 DBPI) because of the most sensitive phase judging from the trial in 2012. Since we defined panicle initiation as the date when the young panicle reached a length of 1 mm (measured for 2–3 stems (=plants) at 2-d intervals), and it is known that panicle differentiation begins 7 days before panicle initiation (Yoshida, 1981), we assumed that our pre-treatments occurred before the panicle differentiation stage, even for the phase III treatments, which ended at 8 DBPI in 2012 and 11 DBPI in 2013.

In the shade treatment, black mesh sheets attached to frames (.7 m × 1.7 m × .9 m in height) reduced light intensity to 25% of the ambient photosynthetically active radiation, measured using a linear quantum
sensor (Li-191R, Li-COR, Lincoln, NE, USA), as shown in Supplemental Figure S1b. In the salt treatment, NaCl was added to the water to achieve an electrical conductivity (EC) of 9.0 mS cm⁻¹, adjusted daily. EC was measured with an HI9813–6 EC meter (Hanna Instruments, Woonsocket, RI, USA). After the treatments, the pots were soaked in several changes of fresh water until the EC did not change.

During the reproductive growth (i.e. after panicle initiation), chilling treatments were applied to induce sterility (Supplemental Figure S1c). After the young panicle had reached a length of 1 mm, rice plants were transferred into deep water baths (1 m × 1 m × 60 cm deep) that were controlled at a temperature of 18.5 °C (18.5 ± .8 °C, average ± standard deviation of observed data) or 19.0 °C (19.0 ± .2 °C) for both cultivars (in 2012) and of 18.5 °C (18.5 ± .3 °C) or 19.0 °C (18.7 ± .2 °C) for 'Sasanishiki' and 18.0 °C (17.8 ± .3 °C) or 18.5 °C (18.5 ± .3 °C) for 'Hitomebore' (in 2013) by supplying cool water at ca. 15 °C through an electromagnetically controlled valve to uniformly expose all panicles to low temperature. Water depth was kept at 30 cm from the shoot base for exposing the developing panicles which initiated at shoot base and then lifted upward. Water was circulated by a high blow air pump (C-5BN, Techno Takatsuki, Osaka, Japan) to minimize spatial variation in $T_w$. The treatments were maintained until the mid-ripening stage. This methodology is widely used in breeding of new cultivars (Matsunaga, 2005) and in physiology experiments (e.g. Sakata et al., 2014).

### 2.3. Measurements
During the pre-treatments that were applied during vegetative growth, we measured stomatal conductance...
(g_s) at the uppermost leaf at the lower surface in the morning between 09:00 h and 11:00 h with an SC-1 leaf porometer (Decagon Devices Co., Pullman, WA, USA) at 5~7 days before the end of the pre-treatment in fine weather. To measure the specific leaf weight (SLW; mg dry weight cm⁻²), we sampled the top three leaves at two days before the end of the pre-treatment; leaf area was measured with an AAM-9 leaf area meter (Hayashi-Denko, Tokyo, Japan); then, the leaves were dried at 80 °C for more than 72 h and weighed. The sugar and starch contents of the leaves were also measured following the method of Suzuki et al. (2015). At harvest, we counted the total numbers of spikelets, fertile spikelet and sterile spikelets on each panicle, and used this data to calculate the percentage of spikelet fertility. Sterile spikelets were carefully identified by backlighting the heads with fluorescent lightbulbs; spikelets that showed no shadowy area (i.e. no developing embryo or grain) were considered to be sterile, following the method of Shimono et al. (2007).

We tested for statistically significant differences using multiple comparisons with Bonferroni's correction.
Shade-II treatment in ‘Hitomebore’ and in Shade-II and Shade-III treatments in ‘Sasanishiki’ (Figure 2(e) and (f)). The leaf starch content was not significantly affected in either cultivar (Figure 2(g) and (h)). Panicle initiation stage was delayed by 4–7 days for ‘Hitomebore’ and for 3–5 days for ‘Sasanishiki’ (Figure 1). It is visually difficult to identify the differences in plant morphology between shaded and unshaded plants at this stage when is the timing of start of chilling tolerance test during the reproductive stage (Supplemental Figure S3).

In 2012, the spikelet number per panicle at harvest decreased in many of the chilled plants that had been exposed to shade during vegetative growth, but the difference compared with the unchilled plants (‘none’) in any given phase was significant only in ‘Hitomebore’ plants chilled to 18.5 °C in the Shade-I treatment; none of the differences were significant in the ‘Sasanishiki’ plants (Figure 3(a) and (b)). The later the shading was applied in 2012, the greater was the reduction in spikelet number, but the difference was not always significant compared with the unshaded control. In 2013, there was no significant difference in the unchilled plants in either Shade-II treatment in ‘Hitomebore’ and in the Shade-II and Shade-III treatments in ‘Sasanishiki’ (Figure 2(e) and (f)). The leaf starch content was not significantly affected in either cultivar (Figure 2(g) and (h)). Panicle initiation stage was delayed by 4–7 days for ‘Hitomebore’ and for 3–5 days for ‘Sasanishiki’ (Figure 1). It is visually difficult to identify the differences in plant morphology between shaded and unshaded plants at this stage when is the timing of start of chilling tolerance test during the reproductive stage (Supplemental Figure S3).

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generally no significant effect on SLW, sugar content, or starch content (Figure 5(c)–(h)). However, the following differences were significant in ‘Sasanishiki’: compared with the unsalted plants, SLW was greater in Salt-III, sugar content was greater in Salt-I and starch content was greater in Salt II. Panicle initiation stage was delayed by salt treatments by 2~10 days for ‘Hitomebore’ and by 0~15 days for ‘Sasanishiki’, and the magnitude was greater for later treatments (Figure 1). We can identify that plants treated with salt at phase III only showed leaves that were still brown, and not plants that were treated with salt at early stages of this stage (Supplemental Figure S3).

The spikelet number per panicle generally decreased due the salt treatment in both years, but not all of the differences were significant (Figure 6). In ‘Hitomebore’, Salt-III significantly decreased spikelet number compared with the unsalted plants, especially for chilled plants in 2012; in ‘Sasanishiki’, Salt-III significantly decreased spikelet number compared with the unsalted plants in both years.

Spikelet fertility in plants that were not chilled during the reproductive stage (‘none’) was not significantly affected by the salt treatments, except for a significant decrease in the Shade-II treatment in ‘Hitomebore’ in 2012 (Figure 4). Chilling treatments during the reproductive stage significantly decreased spikelet fertility with greater magnitude for plants exposed to shade at the vegetative growth than control of not pre-treated plants. Thus, the susceptibility to chilling at the reproductive stage for inducing sterility was greater for the shaded plants than for unshaded plants.

3.2. Salt treatment

In 2012, the salt treatment decreased $g_s$ compared with the unsalted control in all vegetative growth phases, by up to 53%, but the difference was significant only in ‘Hitomebore’ in Salt-II (Figure 5(a) and (b)). Leaves became bleached or brown in the salt treatments, indicating tissue damage (Supplemental Figure S2), but there was generally no significant effect on SLW, sugar content, or starch content (Figure 5(c)–(h)). However, the following differences were significant in ‘Sasanishiki’: compared with the unsalted plants, SLW was greater in Salt-III, sugar content was greater in Salt-I and starch content was greater in Salt II. Panicle initiation stage was delayed by salt treatments by 2~10 days for ‘Hitomebore’ and by 0~15 days for ‘Sasanishiki’, and the magnitude was greater for later treatments (Figure 1). We can identify that plants treated with salt at phase III only showed leaves that were still brown, and not plants that were treated with salt at early stages of this stage (Supplemental Figure S3).

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Spikelet fertility in plants that were not chilled during the reproductive stage (‘none’) was not significantly affected by the salt treatments, except for a significant
sterility was greater for the salted plants than for unsalted plants.

4. Discussion

We found salt or shade treatment during vegetative growth decreased the chilling tolerance during the reproductive stage, thereby decreasing spikelet fertility in both years in both cultivars, even their cold tolerance at the reproductive stage is largely different (Figures 4 and 7). This decrease in ‘Sasanishiki’ in 2012 (Figure 7). Chilling treatments during the reproductive stage significantly decreased spikelet fertility with greater magnitude for plants exposed to salt at the vegetative growth period than plants of not pre-exposed, control. Even for ‘Sasanishiki’ in 2012, the reduction magnitude of spikelet fertility by chilling during the reproductive stage was greater for salt pre-treated plants during vegetative stage than control of non-pre-treated plants. Thus, the susceptibility to chilling at the reproductive stage for inducing sterility was greater for the salted plants than for unsalted plants.
Our results suggest that the reproductive growth of the plants was most sensitive to abiotic stress during phase III, which occurred between 39 and 54 DAT in 2012 (Figures 4 and 7). This phase was a period from 26–11 DBPI in ‘Sasanishiki’ and 23–8 DBPI in ‘Hitomebore’ for shade treatment, and from 29–14 DBPI in ‘Sasanishiki’ and 33-18 DBPI in ‘Hitomebore’ for salt treatment. Kanda and Shimono (2009) investigated the stage of vegetative growth when the pre-conditioning stress (low water temperature) produced the greatest effect on chilling tolerance of ‘Sasanishiki’ and ‘Hitomebore’ during the reproductive stage by dividing vegetative growth into five two-week intervals, and found that the plants were most sensitive between 16 and 11 DBPI, which overlapped with the present results, despite the use of different abiotic stresses. There are two possible explanations for this sensitivity: first is the magnitude of the damage since later growth stages generally experience more severe damage because of the higher transpirational demand by larger plants, combined with increased resistance to water uptake that is caused by...
pre-conditioning stress (low temperatures during vegetative growth) on chilling tolerance. A low temperature during vegetative growth can downregulate the expression of the stress response genes for ascorbate peroxidase (Os07g0694700), heat shock protein (OsHSP90.1, Os04g0107900) and FK506-binding protein (OsFKBP65, Os04g0352400), which would generally be upregulated to provide protection against chilling stress during the reproductive stage. Our shade or salt stress at vegetative stage might share a similar mechanism for affecting chilling tolerance at the reproductive stage. Further study will be required to confirm whether these changes occurred in our study system.

The present results have strong implications for rice production since they can be used to establish guidelines for growing rice during periods with low light intensity or in areas at risk of high salinity (Shimono et al., 2012). In these areas, farmers must take measures to improve the tolerance of chilling stress, such as using more tolerant cultivars and management practices that will mitigate the salty water. In terms of measured gs in our study (Figure 3(a) and (b)) was less affected by salt treatment on later growth stage, but we observed severe damage of lower leaves at later growth stages (Figure S2). Second, the length of the recovery time after exposure of salt or shade might have different responsibilities. As shown in Figure S3, leaves of salt-treated plants at phase III were still bleached or brown at the start of chilling test. Also, different physiological stages may have different degrees of sensitivity, although underlying physiological and genetic mechanisms are not known.

In the present study, we did not measure physiological and molecular responses in developing pollen and anther. Abiotic stresses regulate plant responses through similar gene networks. Low temperature is commonly reported to induce responses via a network similar to that involved in drought and salt responses (Nakashima et al., 2009). In the case of low temperature at vegetative stage, Suzuki et al. (2015) described the molecular and physiological mechanisms responsible for the negative impacts of pre-conditioning stress (low temperatures during vegetative growth) on chilling tolerance. A low temperature during vegetative growth can downregulate the expression of the stress response genes for ascorbate peroxidase (Os07g0694700), heat shock protein (OsHSP90.1, Os04g0107900) and FK506-binding protein (OsFKBP65, Os04g0352400), which would generally be upregulated to provide protection against chilling stress during the reproductive stage. Our shade or salt stress at vegetative stage might share a similar mechanism for affecting chilling tolerance at the reproductive stage. Further study will be required to confirm whether these changes occurred in our study system.

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Figure 7. Spikelet fertility of rice cultivars exposed to salt treatments at different phases during vegetative growth. Values are means ± standard errors (n = 6–9). Controls with no salt treatment; none, no chilling treatment. For a given cultivar and year, bars labelled with different letters differ significantly (p < 0.05).
effects of these stresses. Our results will also be useful in analyses that identify the cause of yield losses by identifying the effects of pre-conditioning by exposure to salt or low solar radiation on the response to chilling during the reproductive stage.

5. Conclusions

We found evidence that exposure of low light intensity and salt during vegetative growth can increase the susceptibility to low temperatures during the reproductive stage, leading to increased male sterility.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was partly supported by Iwate University and MEXT (Ministry of Education, Culture, Sports, Science and Technology, Japan) project, ‘Research Program on Climate Change Adaptation (RECCA)’.

References

Abe, A., Oikawa, A., & Shimono, H. (2013). Variation of sterility induced by cool-irrigation tolerance test depending on years and period of cool irrigation in rice cultivars. _Japanese Journal of Crop Science_, 82, 176–182 (in Japanese with English abstract).

Andaya, V. C., & Mackill, D. J. (2003). QTLs conferring cold tolerance at the booting stage of rice using recombinant inbred lines from a _japonica × indica_ cross. _Theoretical and Applied Genetics_, 106, 1084–1090.

Dai, L., Lin, X., Ye, C., Ise, K., Saito, K., Kato, A., ... Zhang, D. (2004). Identification of quantitative trait loci controlling cold tolerance at the reproductive stage in yunnan landrace of rice, kunmingxiaobaigu. _Breeding Science_, 54, 253–258.

IPCC. (2007). Summary for Policy makers. In: S. Solomon, D. Qin, M. Manning, Z. J. Chen, M. Marquis, K. B. Avert, M. Tignor, & H. L. Miller (Eds.), _Climate change 2007: the physical science basis_. Contribution of working group i to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, pp. 1–18. Cambridge.

Kanda, E., Kimura, T., Oikawa, A., Ookawa, S., Sasaki, J., Asano, M., ... Shimono, H. (2012). Factors responsible for regional variation of cold tolerance in rice in northern Japan. _Japanese Journal of Crop Science_, 81, 190–193. (in Japanese with English abstract).

Kanda, E., & Shimono, H. (2009). Cold tolerance for sterility induced by low temperature in rice can be affected by water temperature at two weeks before the panicle initiation stage. _Jpn. J. Crop Sci._, 78, (extra1), 227th annual meeting of Jpn. Soc. Crop Sci., 280–281 (Japanese).

Kanno, H. (1997). Estimation of daily-mean air temperatures on a 1km2 mesh during the occurrence of a Yamase wind. _Journal of Agricultural Meteorology_, 53, 11–19. (in Japanese with English abstract).

Kuroki, M., Saito, K., Matsuba, S., Yokogami, N., Ando, T., Sato, Y., ... Shimizu, H. (2011). Detection of quantitative trait loci for cold tolerance at the booting stage in a rice breeding line, Hokkai-PL9. _Breeding Research_, 13, 11–18. (in Japanese with English abstract).

Li, H. B., Wang, J., Liu, A. M., Liu, K. D., Zhang, Q., & Zou, J. S. (1997). Genetic basis of low-temperature-sensitive sterility in _indica-japonica_ hybrids of rice as determined by RFLP analysis. _TAG Theoretical and Applied Genetics_, 95, 1092–1097.

Matsumura, H., Suzuki, K., & Shimono, H. (2012). Water temperatures during vegetative growth affect cold tolerance at the booting stage of rice under controlled environmental conditions. _Journal of Agricultural Meteorology_, 68, 159–164.

Matsunaga, K. (2005). Establishment of an evaluation method for cold tolerance at the booting stage of rice using deep water irrigation system and development of highly cold tolerant rice varieties by combining cold tolerance genes. _Bull. Miyagi Furukawa Agric. Exp. Sta._, 4, 1–78. (in Japanese with English abstract).

Matsushima, S., Tanaka, T., & Hoshino, T. (1964). Analysis of yield-determining process and its application to yield-prediction and culture improvement of lowland rice: LXX. Combined effects of air-temperatures and water-temperatures at different stages of growth on the grain yield and its components of lowland rice. _Japanese journal of crop science_. 33, 53–58. (in Japanese with English abstract).
Mori, M., Onishi, K., Tokizono, Y., Shinada, H., Yoshimura, T., Numao, Y., ... Sato, T. (2011). Detection of a novel quantitative trait locus for cold tolerance at the booting stage derived from a tropical japonica rice variety Silewah. *Breeding Science, 61*, 61–68.

Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology, 59*, 651–681.

Nakashima, K., Ito, Y., & Yamaguchi-Shinozaki, K. (2009). Transcriptional regulatory networks in response to abiotic stresses in arabidopsis and grasses. *Plant Physiology, 149*, 88–95.

Oh, C. S., Choi, Y. H., Lee, S. J., Yoon, D. B., Moon, H. P., & Ahn, S. N. (2004). Mapping of quantitative trait loci for cold tolerance in weedy rice. *Breeding Science, 54*, 373–380.

Saito, K., Miura, K., Nagano, K., Hayano-Saito, Y., Araki, H., & Kato, A. (2001). Identification of two closely linked quantitative trait loci for cold tolerance on chromosome 4 of rice and their association with anther length. *TAG Theoretical and Applied Genetics, 103*, 862–868.

Sakata, T., Oda, S., Tsunaga, Y., Shomura, H., Kawagishi-Kobayashi, M., Aya, K., ... Higashitani, A. (2014). Reduction of gibberellin by low temperature disrupts pollen development in rice. *Plant Physiology, 164*, 2011–2019.

Satake, T. (1976). Determination of the most sensitive stage to sterile-type cool injury in rice plants. *Res. Bull. Hokkaido Nat. Agric. Exp. Stn.*, 113, 1–44.

Shimono, H., Abe, A., Aoki, N., Koumoto, T., Sato, M., Yokoi, S., ... Nagano, K. (2016). Combining mapping of physiological quantitative trait loci and transcriptome for cold tolerance for counteracting male sterility induced by low temperatures during reproductive stage in rice. *Physiologia Plantarum, 157*, 175–192.

Shimono, H., Hasegawa, T., & Iwama, K. (2002). Response of growth and grain yield in paddy rice to cool water at different growth stages. *Field Crops Research, 73*, 67–79.

Shimono, H., Ishii, A., Kanda, E., Suto, M., & Nagano, K. (2011). Genotypic variation in rice cold tolerance responses during reproductive growth as a function of water temperature during vegetative growth. *Crop Science, 51*, 290–297.

Shimono, H., & Kanda, E. (2008). Does regional temperature difference before the panicle initiation affect the tolerance for low temperature-induced sterility in rice? *Plant Production Science, 11*, 430–433.

Shimono, H., Okada, M., Kanda, E., & Arakawa, I. (2007). Low temperature-induced sterility in rice: Evidence for the effects of temperature before panicle initiation. *Field Crops Research, 101*, 221–231.

Shimono, H., Suto, M., & Nagano, K. (2012). Cold tolerance for sterility induced by low temperature at booting stage can be improved by warmer water temperature during vegetative growth. *Climate in Biosphere, 12*, 1–5. (in Japanese with English abstract).

Shirasawa, S., Endo, T., Nakagomi, K., Yamaguchi, M., & Nishio, T. (2012). Delimitation of a QTL region controlling cold tolerance at booting stage of a cultivar, ‘Lijiangxintuanheigu’, in rice, *Oryza sativa L. Theoretical and Applied Genetics, 124*, 937–946.

Suh, J. P., Jeung, J. U., Lee, J. I., Choi, Y. H., Yea, J. D., Virk, P. S., ... Jena, K. K. (2010). Identification and analysis of QTLs controlling cold tolerance at the reproductive stage and validation of effective QTLs in cold-tolerant genotypes of rice (*Oryza sativa L.)*. *Theoretical and Applied Genetics, 120*, 985–995.

Suzuki, K., Aoki, N., Matsumura, H., Okamura, M., Ohsugi, R., & Shimono, H. (2015). Cooling water before panicle initiation increases chilling-induced male sterility and disables chilling-induced expression of genes encoding OsFKBP65 and heat shock proteins in rice spikelets. *Plant, Cell & Environment, 38*, 1255–1274.

Takeuchi, Y., Hayasaka, H., Chiba, B., Tanaka, I., Shimano, T., Yamagishi, M., ... Yano, M. (2001). Mapping quantitative trait loci controlling cool-temperature tolerance at booting stage in temperate japonica rice. *Breeding Science, 51*, 191–197.

Tsuda, M. (2007). Genotypic differences in water stress susceptibility and panicle water potential at meiosis stage in rice. *Bulletin of the Faculty of Bioresources, Mie University, 18*, 1–6. (in Japanese with English abstract).

Wada, S. (1992). Cool weather damage in rice plants. Tokyo: Youkenndo.

Xu, L. M., Zhou, L., Zeng, Y. W., Wang, F. M., Zhang, H. L., Shen, S. Q., & Li, Z. C. (2008). Identification and mapping of quantitative trait loci for cold tolerance at the booting stage in a japonica rice near-isogenic line. *Plant Science, 174*, 340–347.

Ye, C., Fukai, S., Godwin, I., Koh, H., Reinke, R., Zhou, Y., ... Redoña, E. (2010). A QTL controlling low temperature induced spikelet sterility at booting stage in rice. *Euphytica, 176*, 291–301.

Yoshida, S. (1981). *Fundamentals of rice crop science*. Los Banos: IRRI.

Zhou, L., Zeng, Y., Zheng, W., Tang, B., Yang, S., Zhang, H., ... Li, Z. (2010). Fine mapping a QTL qCTB7 for cold tolerance at the booting stage on rice chromosome 7 using a near-isogenic line. *Theoretical and Applied Genetics, 121*, 895–905.