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Heat Shocks Increase the Chilling Tolerance of Rice (*Oryza sativa*) Seedling Radicles

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The growth of rice (*Oryza sativa* L., cv. M202) seedling radicles, initially 10 ± 1 mm long, was linear for the 96 h it took them to grow to 150 mm at 25 °C. Exposure to 5 °C for 24 h reduced the rate of growth by about 50%, and longer exposures caused a progressive reduction in growth. Initial radicle length significantly affected chilling sensitivity: with 2 days at 5 °C inhibiting growth at 25 °C by 23% for 1-mm radicles, 63% for 10-mm radicles, and 87% for 40-mm radicles. Heat shocks of 35 °C for 4 min, 40 °C for 3 min, 45 °C for 2 min, or 50 °C for 1 min, prior to chilling, reduced the 75% inhibition of radicle growth caused by 2 days at 5 °C to 34%, 25%, 14%, and 13%, respectively. The length of exposure that conferred chilling tolerance increased from less than 2 min for 50 °C to over 8 min for 35 °C. This increase in effective treatment duration was accompanied by a reduction in the maximum induced chilling tolerance. Practical application of heat-shock treatments to increase the chilling tolerance of rice seedlings may sacrifice a small reduction in maximum chilling tolerance at the lower inductive temperatures for a larger margin of safety in their application.

KEYWORDS: Abiotic stress; radicle growth; cross-protection; rice

INTRODUCTION

Most plants indigenous to the tropics and semitropics, and some temperate plants, are sensitive to a physiological disorder called chilling injury (*1*−*3*). Exposing sensitive plants, such as maize, rice, sorghum, and sugarcane, to nonfreezing temperatures below ∼12 °C induces a variety of symptoms which include reduced vigor, abnormal ripening, stimulated respiration and ethylene production, increased membrane permeability, and increased disease susceptibility. The severity of injury is dependent on species, growing conditions, duration of exposure, and temperature. Symptom development can be reduced by a number of pre- and postchilling treatments (*2*, *3*).

A number of abiotic stresses (e.g., cold-shock, ethanol, heat-shock, osmotic-shock, and salinity) applied before chilling can increase chilling tolerance (*4*−*6*). A significant increase in chilling tolerance is conferred in tomato tissue (*6*, *7*), excised cucumber cotyledon disks (*8*), and seedling radicles of cucumber, maize, okra, and tomato by a heat shock applied before chilling (*5*, *9*, *10*). Heat-shock proteins are produced in response to stress and are thought to protect plants against the deleterious effects of subsequent stresses (*11*). Correlations have been reported between the appearance of heat-shock proteins and the persistence of induced chilling tolerance (*12*, *13*).

Rice is a major agronomic crop that produces food for a large proportion of humanity. Production can be reduced by exposure to chilling temperatures at most stages of growth (*14*). Understanding the basis of chilling injury in rice and developing procedures to efficiently select chilling-tolerant rice lines could help reduce the effect of chilling on yield. Seedling radicle growth is an effective and rapid method to quantitatively screen individual plants for chilling tolerance. Computer analysis of video images is an efficient approach to measuring radicle length, but we found that results were often erratic when radicles curved or when lateral roots were present. Manual measurements are better at estimating the actual length, but measuring radicle lengths is exacting and time-consuming, especially when they are thin and hard to see. The time needed to measure the lengths of hundreds of radicles within a short period of time during an experiment limits the number of treatments and replicates that can be used. Previously, scientists have overcome the need for immediate and continuous measurements of experimental parameters by using photographic methods to capture images that could be archived and analyzed later.

Research reported in this paper was done to study the effect of chilling and heat shock on the growth kinetics of rice seedling radicles. The use of an office copier to make enlarged images of rice seedling radicles was verified as effective and innocuous.

MATERIALS AND METHODS

Plant Material. Rice (*Oryza sativa* L., cv. M-202) seeds were obtained from a local vendor. A total of 5 g of seeds was imbibed in 1 L of aerated water overnight at 20 °C. Imbibed seeds were transferred to moist paper toweling overlying capillary cloth that was sandwiched between two 15 × 30 cm Plexiglas plates (6 mm thick) that were held together with rubber bands. The plates were held in a vertical position

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at 25 °C in a humid, ethylene-free atmosphere for about 36 h, or until the radicles were about 10 mm long.

Germinating seeds with 10 ± 1-mm radicles were removed from the large Plexiglas sandwich and gently transferred to moist paper toweling overlying capillary cloth and sandwiched between 7 × 13 cm Plexiglas plates (3 mm thick) as before. Each smaller plate held 7 to 10 seedlings and was treated as a unit of replication. The plates were positioned vertically in a 20 × 26 × 14 cm plastic tub, which was then lightly covered with aluminum foil. The trays were either held at 25 °C for the initial measurements of radicle growth, or chilled at 5 °C before being moved to 25 °C for the growth measurements. In a separate series of preliminary experiments, 24-h chilling treatments were administered to radicles 1−40 mm long. All growth and chilling treatments were done in the dark.

Application of Heat Shocks. Each small plate of rice seedlings with 10 ± 1 mm long radicles was placed in a plastic bag and immersed in water at 25, 35, 40, 45, or 50 °C for up to 8 min. The bags were left open at the top to allow sufficient gas diffusion to prevent anaerobic conditions from developing. The 25 °C treatment was considered the nonheat-shock control. All heat treatments were applied before chilling treatments. The bagged plates were then held for 15 min in room-temperature water (ca. 20 °C) before being removed from the bags and placed in plastic tubs lined with wet capillary cloth.

Measurement of Chilling Injury. Growth of the radicle after chilling was measured by a method modified from that previously described (*5*, *9*). Images of the small plates were periodically made on a Kodak Ektaprint 30 copier set at 200% enlargement and the lightest image darkness (Eastman Kodak Co., Rochester, NY). Radicle length was measured to the nearest mm, with a clear ruler, from images taken before and after treatment, after chilling, and periodically during growth at 25 °C. In some experiments, the small plates were disassembled and the radicles were gently straightened before measurement. Measurements for each seedling were regressed over time and the slope and correlation coefficient were calculated.

Statistical Analysis. All experiments were repeated at least twice and gave similar results. Each treatment had at least three replicates that were individual plates with from 7 to 10 seedlings per plate. Means and standard errors were calculated. Linear regressions were calculated for the change in radicle length over time to determine the rate of radicle growth.

RESULTS AND DISCUSSION

Measurement of Radicle Length. Plates containing rice seedlings with radicles from 10 to 110 mm long were first photocopied at 200%, and then the plates were disassembled and the actual length of each radicle was measured with a clear plastic ruler. The length of the radicles was 52.2% of the length measured off the enlarged image (data not shown). The correlation coefficient was 0.996. The slight difference in radicle length between the measurements of the actual seedling and those taken off the coped images probably represent the difficulty of accurately measuring curved radicles. Radicles could be gently straightened to get an accurate measure of their length, whereas the ruler had to be repeatedly rotated and repositioned to follow all the curves of the radicles on the image. However, making the enlarged copies was faster than doing the radicle length measurements on the plants themselves, so the periodic measurements needed for the kinetic analysis could be quickly accomplished with the actual measurements done later.

Repeated Copying has No Effect on Radicle Growth. Plates containing rice seedlings with radicles 10 ± 1 mm long were held at 25 °C and periodically copied at 200% over a period of 96 h. Over the 4 days of these experiments, plates were either copied once a day (i.e., few images) or 4 times per day (i.e., many images). The rate of radicle elongation was linear during the time it took the radicles to grow from 10 ± 1 mm to 145 ± 11 mm in length. The rate of radicle growth was unaffected by the number of copies made: 1.41 mm h^{-1} with an R^2 of 0.999 when 4 images were made, and 1.42 mm h^{-1} with an R^2 of 0.997 when 18 images were taken over the 4-day experiment. Making many copies in a short time (i.e., a copy every 1.5 h for 9 h; 7 copies) had no significant effect on either the short-term or long-term growth of the radicles (data not presented). It appears that the very bright and hot light used in copy machines did not adversely affect the subsequent growth of the copied growing plant tissue and that periodic copying of the seedlings can be used in studies of rice seedling radicle growth kinetics. The ability to make enlarged copies of seedling radicles without causing significant growth changes was helpful in studying the kinetics of chilling injury.

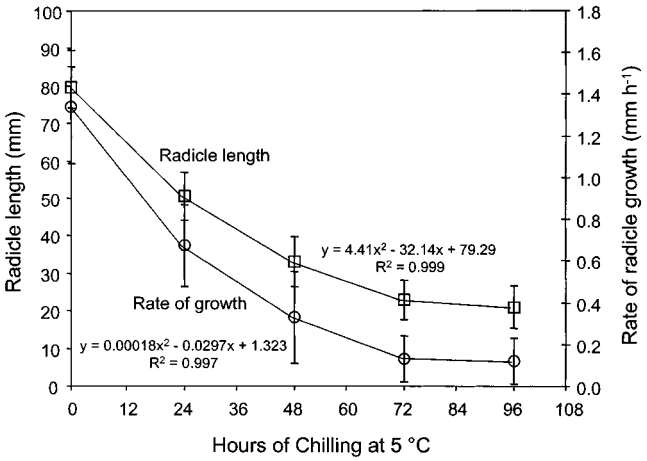

Figure 1. Radicle length and rate of rice seedling radicle growth for 2 days at 25 °C after being chilled at 5 °C for 0–4 days. The seedlings had radicles initially 10 ± 1 mm long. Quadratic equations were fit to the data and are shown with their R^2 values. Vertical bars represent the SE about that mean.

Chilling Reduces Subsequent Radicle Growth. Exposure to 5 °C for 0−4 days resulted in a progressive reduction in the subsequent growth of rice seedling radicles (**Figure 1**). Radicles did not grow in length while at 5 °C. Radicle growth at 25 °C, after 24 h of chilling at 5 °C, was only 58% of that of the nonchilled control. Extending the period of chilling to 2, 3, and 4 days reduced subsequent growth to 33%, 19%, and 16% of that of the nonchilled control, respectively. The chilling-induced decline in radicle growth is described by the following quadratic equation: radicle length (mm) = 4.41 (hours of chilling)^2 − 32.14 (hours of chilling) + 79.29; with an R^2 of 0.999.

Chilling also reduced the rate of radicle growth at 25 °C by about 50%, from 1.34 ± 0.27 mm h^{-1} for the nonchilled controls to 0.67 ± 0.19 mm h^{-1} for seedlings chilled at 5 °C for 24 h (**Figure 1**). Increasing the duration of chilling further reduced the rate of radicle growth: e.g., 75%, 90%, and 91% by 2, 3, and 4 days of chilling at 5 °C, respectively. The chilling-induced decline in the rate of radicle growth is described by the following quadratic equation: rate of radicle length (mm h^{-1}) = 0.00018 (hours of chilling)^2 − 0.0297 (hours of chilling) + 1.323; with an R^2 of 0.997.

Initial radicle length significantly affected chilling sensitivity (**Figure 2**). Rice seedlings with radicles initially 1−40 mm were chilled at 5 °C for 24 h and then grown at 25 °C for 48 h. Radicle growth declined rapidly as the initial length of the radicle increased from 1 to 15 mm, and then more slowly until the initial length reached 40 mm. For example, chilling reduced the growth of 1-mm radicles from 86 ± 6.4 mm to 67 ± 9.2 mm, the growth of 10-mm radicles from 81 ± 5.5 mm to 32 ± 7.1 mm, and the growth of 30-mm radicles from 83 ± 7.3 mm

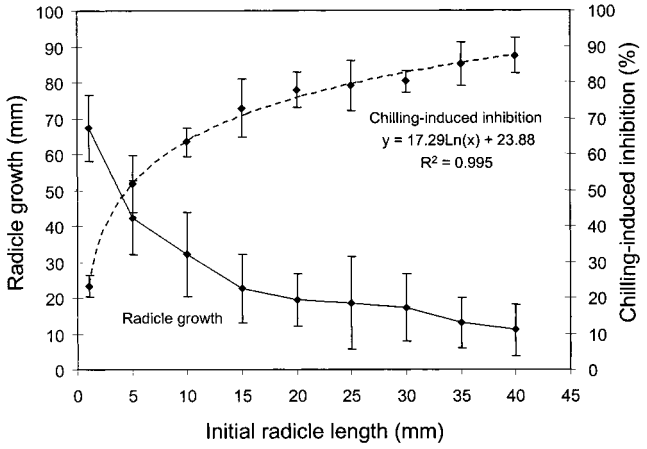

Figure 2. Growth of rice seedling radicle for 2 days at 25 °C after being chilled at 5 °C for 24 h. The seedlings had radicles initially 1–40 ± 1 mm long. The dashed line represents the percent inhibition caused by chilling for each specific initial radicle length. Vertical bars represent the SE about that mean.

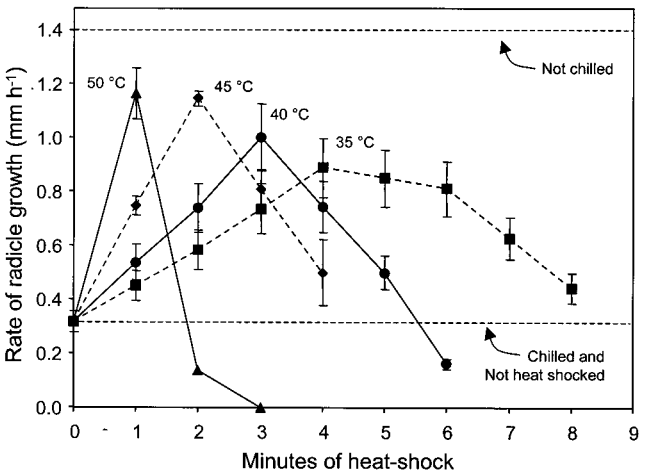

Figure 3. Rate of rice seedling radicle growth for 2 days at 25 °C after being heat-shocked for various combinations of duration and temperature, and then chilled at 5 °C for 2 days. Radicles were initially 10 ± 1 mm long. Vertical bars represent the SE about that mean.

to 17 ± 9.3 mm. The chilling-induced inhibition of growth, calculated using total radicle length was lowest (23%) for radicles initially 1 mm long. As the initial length increased, the percent inhibition increased, reaching 87.5% for 40-mm radicles. The percent chilling-induced inhibition of growth with increasing initial radicle length is given by the following equation: 17.29 Ln (radicle length) + 23.88, with an R^2 of 0.995 (**Figure 2**). This is similar to cucumber seedlings in which the chilling sensitivity of the radicle increased as the initial radicle length increased from 1 to 20 mm (*9*, *15*).

Heat Shocks Increase Chilling Tolerance. Certain combinations of 35−50 °C heat shocks applied for 0−7 min resulted in increased chilling tolerance, whereas other combinations were injurious (e.g., 50 °C for 2 min or 40 °C for 6 min) (**Figure 3**). Nonheat-shocked rice seedling radicles, initially 10 ± 1 mm long, grew at a rate of 0.33 mm h^{-1} at 25 °C after being chilled for 2 days at 5 °C. Radicles that were not chilled grew at 1.40 mm h^{-1}. Treating the seedling radicles before chilling with heat shocks of 35 °C for 4 min, 40 °C for 3 min, 45 °C for 2 min, or 50 °C for 1 min, resulted in rates of growth of 0.92 ± 0.12, 1.03 ± 0.13, 1.18 ± 0.08, and 1.20 ± 0.10 mm h^{-1}, respectively. All of these treatments reduced chilling-induced inhibition of growth from 75% for nonheat-shocked radicles to 34%, 25%, 14%, and 13% for the 35, 40, 45, and 50 °C treatments, respectively. As the heat-shock temperature was reduced from 50 to 35 °C, the length of exposure that conferred chilling tolerance above the control level expanded from less than 2 min for 50 °C to over 8 min for 35 °C. However, this increase in effective treatment duration was accompanied by a slight reduction in the maximum induced chilling tolerance. Practical application of heat-shock treatments to increase the chilling tolerance of rice seedlings may sacrifice a small reduction in maximum chilling tolerance at the lower inductive temperatures for a larger margin of safety in their application.

Rice Seedling Radicles as a Model System to Study Chilling. Chilling-induced reductions of radicle growth is a more sensitive and easily measured indicator of chilling injury than other commonly used measures such as ion leakage or the increased production of ethylene or carbon dioxide (*9*). Methods used in this paper demonstrate how the kinetic study of chilling effects on radicle growth can be facilitated by the use of commonly available photocopiers to make enlarged copies of seedling radicles for later analysis. Data from photocopier images have been used to calculate growth kinetics in studies of enzymatic antioxidant defense systems in chilled and heat-shocked cucumber seedlings radicles (*16*), and in antioxidant enzymes and DPPH-radical scavenging activity in chilled and heat-shocked rice seedlings radicles (*17*).

Rice seedling radicles may be a better model system for the study of chilling injury than other tissues, because the chilling sensitivity of rice seedling radicles is greater than that of cucumber radicles. Chilling at 5 °C for 24 h caused about a 50% reduction in growth of rice radicles, whereas 3 days of chilling at 2.5 °C produced only a 30% reduction in the growth of cucumber radicles (*10*, *16*, *17*). The pattern of chilling-induced inhibition of subsequent radicle elongation is slightly different between cucumber and rice. In cucumber, there was a linear reduction of about 10% in radicle growth at 25 °C with each additional day of chilling at 2.5 °C up to 3 days. Beyond 3 days, chilling caused a rapid decline in radicle growth (*18*). The linear and nonlinear components of chilling-induced inhibition of subsequent radicle growth could be used to investigate the different effects of chilling on sensitive plant tissue. This linear component of chilling was not observed in rice seedlings. However, the chilling sensitivities of both rice and cucumber seedlings increase with increasing radicle length, and both are responsive to protective heat-shock treatments. The physiology and genetics of rice have been more extensively studied than those of cucumber, and this information could be helpful in dissecting the physiological basis for chilling injury and the protective effect of heat-shock treatments.

Practical Applications of this Work. This paper shows that a heat-shock treatment confers chilling tolerance to rice seedling radicles. A prior heat-shock treatment could possibly improve the stand establishment of seedlings grown under low-temperature stress. However, the persistence of heat-shock-induced chilling tolerance in plants growing in the field has not been extensively studied. This paper also details a simple method for studying the growth kinetics of rice seedling radicles and then shows how radicle growth is quantatively affected by chilling. This method can therefore be used to screen large numbers of seedlings for chilling tolerance without permanently damaging them or requiring long periods of growth in expensive greenhouse or growth chamber facilities.

LITERATURE CITED

(1) Lyons, J. M. Chilling injury in plants. *Annu. Rev. Plant Physiol.* **1973**, *24*, 445−466.

(2) Saltveit, M. E.; Morris, L. L. Overview of chilling injury of horticultural crops. In *Chilling Injury of Horticultural Crops*; Wang, C. Y., Ed.; CRC Press: Boca Raton, FL, 1990; pp 3−15.

(3) Saltveit, M. E. Discovery of Chilling Injury. In *Discoveries in Plant Biology***;** Kung, S. D., Yang, S. F., Eds.; World Scientific Publishing Co.: Singapore, 2000; vol. 3, pp 423−448.

(4) Graham, D.; Patterson, B. D. Responses of plants to low, nonfreezing temperatures: proteins, metabolism and acclimation. *Annu. Rev. Plant Physiol.* **1982**, *33*, 347−372.

(5) Jennings, P.; Saltveit, M. E. Temperature and chemical shocks induce chilling tolerance of germinating *Cucumis sativus* (cv. Poinsett 76) seeds. *Physiol. Plant.* **1994**, *91*, 703−707.

(6) Saltveit, M. E. Prior temperature exposure affects subsequent chilling sensitivity. *Physiol. Plant.* **1991**, *82*, 529−536.

(7) Lurie, S.; Klein, J. D. Acquisition of low-temperature tolerance in tomatoes by exposure to high-temperature stress. *J. Am. Soc. Hortic. Sci.* **1991**, *116*, 1007−1012.

(8) Lafuente, M. T.; Belver, A.; Guye, M. G.; Saltveit, M. E. Effect of temperature conditioning on chilling injury of cucumber cotyledons. *Plant Physiol.* **1991**, *95*, 443−449.

(9) Rab, A.; Saltveit, M. E. Differential chilling sensitivity in cucumber (*Cucumis sativus*) seedlings. *Physiol. Plant.* **1996**, *96*, 375−382.

(10) Rab, A.; Saltveit, M. E. Sensitivity of seedling radicles to chilling and heat-shock induced chilling tolerance. *J. Am. Soc. Hortic. Sci.* **1996**, *121*, 711−715.

(11) Vierling, E. The role of heat shock proteins in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1991**, *42*, 579−620.

(12) Collins, G. G.; Nie, X. L.; Saltveit, M. E. Heat shock increases chilling tolerance of mung bean hypocotyl tissue. *Physiol. Plant.* **1993**, *89*, 117−124.

(13) Sabehat, A.; Weiss, D.; Lurie, S. The correlation between heat-shock protein accumulation and persistence and chilling tolerance in tomato fruit. *Plant Physiol.* **1996**, *110*, 536−541.

(14) Lyons, J. M.; Breidenbach, R. W. Strategies for altering chilling sensitivity as a limiting factor in crop production. In *Stress Physiology in Crop Plants*; Mussell, H., Staples, R. C., Eds.; John Wiley & Sons: New York, 1979; pp 179−197.

(15) Saltveit, M. E.; Martinez-Font, R. T.; Mangrich, M. E. Effect of radicle length and osmotic stress on the chilling sensitivity of cucumber (*Cucumis sativus*, L., cv. Poinsett 76) radicles. *Physiol. Plant.* **2002**, in press.

(16) Kang, H.-M.; Saltveit, M. E. Activity of enzymatic antioxidant defense systems in chilled and heat-shocked cucumber seedling radicles. *Physiol. Plant.* **2001**, *113*, 548−556.

(17) Kang, H.-M.; Saltveit, M. E. Antioxidant enzymes and DPPH-radical scavenging activity in chilled and heat-shocked rice (*Oryza sativa* L.) seedlings radicles. *J. Agric. Food Chem.* **2002**, *50*, 513−518.

(18) Mangrich, M. E.; Saltveit, M. E. Effect of chilling, heat shock, and vigor on the growth of cucumber (*Cucumis sativus*) radicles. *Physiol. Plant.* **2000**, *109*, 137−142.

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