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The effects of cross-tolerance to oxidative stress and drought stress on rice dry matter production under aerobic conditions

Kohtaro Iseki[a], Koki Homma[a,*], Tatsuhiko Shiraiwa[a], Boonrat Jongdee[b], Poonsak Mekwatanakarn[b]

[a] Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan
[b] Ubon Rice Research Center, Ubon Ratchathani 34000, Thailand

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ABSTRACT

Oxidative damage occurring in plant cells under drought stress is a known cause of reduced plant primary production. Decreasing oxidative damage through oxidative stress tolerance is expected to confer drought stress tolerance. In this study, we estimated cross-tolerance to oxidative stress and drought stress for breeding populations and analyzed the effects of the cross-tolerance on dry matter production in field experiments. For a total of 91 rice genotypes, including 72 backcross lines (BCLs), cross-tolerance was estimated from the first principal component score (PCS1) derived from a principal component analysis using a data set with a parameter of chlorophyll fluorescence and cell membrane stability index in both the oxidative and the drought stress treatments as the factors. The higher cross-tolerance was represented by the higher PCS1, and generally the values of PCS1 were segregated in the BCLs, suggesting that cross-tolerance is a heritable trait that can be improved by crossbreeding. The effects of positive and negative PCS1 on dry matter production under flooded and aerobic conditions were tested in field experiments. The decrease in dry matter production under aerobic conditions was smaller for the positive PCS1 genotypes. However, these genotypes also showed a lower stomatal conductance and smaller shoot biomass, especially under flooded conditions. We concluded that cross-tolerance is a useful trait for improving dry matter production, especially under severe drought stress. In view of the trade-offs between cross-tolerance and dry matter production, it is important to develop rice varieties with an optimal level of cross-tolerance for a target environment characterized by drought stress.

1. Introduction

Oxidative damage is one of the major causes of plant injury under drought stress. As drought stress proceeds, stomatal closure induces the limitation of photosynthesis by carbon, and the use of energy for photosynthesis then becomes lower than the absorbed light energy (Zhou et al., 2007). The over-reductive state in the electron transport chain derived from the excess light energy increases the reduction of molecular oxygen and produces reactive oxygen species (ROS). ROS oxidize DNA, RNA, proteins and lipids and disturb plant cellular functions (Gill and Tuteja, 2010). To date, in certain crop species, genotypes with high antioxidant capacity are known to show higher tolerance to drought stress compared with genotypes of low antioxidant capacity (Pastori and Trippi, 1992; Sairam and Saxena, 2000; Guo et al., 2006; Fazeli et al., 2007). Because plants make use of common pathways and components in exhibiting tolerance to drought stress and oxidative stress, tolerance to oxidative stress also confers tolerance to drought stress. This phenomenon is termed cross-tolerance.

In our previous study, we developed a method to evaluate cross-tolerance in seedlings belonging to a rice diversity germplasm research set (RDRS) (Iseki et al., 2013a). The tolerance of the seedlings to oxidative stress and to drought stress was evaluated with measurements of the maximum quantum yield of photosystem II (F_v/F_m, a parameter of chlorophyll fluorescence) and of the membrane stability index (MSI) under both oxidative stress and

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Abbreviations: BCLs, Back-cross lines; DHLs, Double haploid lines; F_v/F_m, Maximum quantum yield of photosystem II; g_s, Stomatal conductance; LWP, Leaf water potential; MSI, Membrane stability index; MV, Methyl viologen; PCA, Principal component analysis; PC1, First principal component; PCS, Principal component score; PEG, Polyethylene glycol; PPFD, Photosynthetic photon flux density; ROS, Reactive oxygen species; SWP, Soil water potential; VPD, Vapor pressure deficit.

[*] Corresponding author at: Kyoto University, Graduate School of Agriculture, Kitashirakwa-Oiwake, Sakyo, Kyoto 606-8502, Japan. Tel.: +81 75 753 6042; fax: +81 75 753 6065.

E-mail address: homma@kais.kyoto-u.ac.jp (K. Homma).

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drought stress. Based on a principal component analysis (PCA) using the F_v/F_m and MSI measurements in both stress treatments as the factors, the cross-tolerance to oxidative stress and drought stress was evaluated from the first principal component score (PCS1). A high intraspecific diversity of cross-tolerance was observed in the RDRS. We found that cross-tolerance was higher in japonica varieties than in indica varieties and higher in improved varieties than in landraces. A higher oxidative stress tolerance in japonica varieties was also reported by Jiao and Ji (2001). The results of their study indicated that genotypic diversity in oxidative stress tolerance in rice resulted from differences in antioxidant enzyme activity. The PCS1 derived from a PCA in our evaluation method is an appropriate indicator of cross-tolerance and may be suitable for a selection criterion for genotypes showing better plant growth under stress conditions.

To date, the relationship between oxidative stress and photosynthetic rate or biomass production has been studied by using simulation models (Werner et al., 2001; Hikosaka et al., 2004). The results of these studies indicated that oxidative stress is to be mitigated to achieve sufficient biomass production under drought prone environment such as aerobic rice cultivation. Recently, the importance of aerobic rice cultivation is increasing with increasing agricultural water use in drought prone area. Our previous study (Iseki et al., 2013b) examined the occurrences of oxidative damage in a field experiment and reported that F_v/F_m of rice leaves under aerobic conditions was lower than that under flooded conditions. However, the relation between F_v/F_m and biomass production was unclear.

The objective of this study is to clarify the effects of cross-tolerance on rice dry matter production under aerobic conditions, and to validate the cross-tolerance as a selection criterion. For the purpose, the cross-tolerance to oxidative stress and drought stress was evaluated for 91 rice breeding lines according to the PCS1 by the methods described in Iseki et al. (2013a). For a part of the 91 lines, the effects of cross-tolerance on dry matter production under field experiments were analyzed by using a part of the data set obtained in our previous study (Iseki et al., 2013b) and by adding a newly conducted dry season trial. Based on all these results, the possibility of improvement in drought tolerance in a drought-sensitive local variety by increasing the cross of the variety is discussed.

2. Materials and methods

2.1. Evaluation of cross-tolerance to oxidative stress and drought stress

2.1.1. Plant materials and preparation

Double haploid lines (DHLs) derived from anther culture were developed from a cross between CT9993-5-10-1-M (upland japonica) and IR62266-42-6-2 (lowland indica). These lines offer a high level of genotypic variation with respect to drought tolerance (Zhang et al., 2001). Based on genotype screening of the DHLs under drought stress, IR68586-FA-CA-143 (DHL143) was identified as a drought-tolerant line (Jongdee et al., 2006). DHL143 backcross introgression lines (BCLs) into Surin1, a rainfed lowland rice variety in Thailand, were developed to improve the drought stress tolerance of the original variety. In this study, a total of 91 rice genotypes consisting of 18 DHLs, 72 BCLs and Surin1 were used for oxidative and drought stress treatments. 15 in 20 genotypes tested in our previous study (Iseki et al., 2013b) were from the DHLs and BCLs. We included these materials into the 91 genotypes.

The germinated rice seed of each rice line was sown on a 96 well PCR plate, one seed for one well. The bottom of each well was cut, and the plate was then put in a plastic case filled with 1/2000 diluted Hyponex nutrient solution (Hyponex 8-12-6, Hyponex Japan, Osaka, Japan). Seedlings were hydroponically grown for 10 days at 25 °C and 100 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) under a 12 h photoperiod. The oxidative stress and drought stress treatments were then imposed.

2.1.2. Stress treatments

Oxidative stress and drought stress were imposed by the addition of methyl viologen (MV) and polyethylene glycol 6000 (PEG), respectively. MV promotes the production of superoxide radical from photosystem I and increase oxidative damage under light irradiation. PEG inhibits root water uptake and promote drought stress especially under high light irradiation. The seedling roots were soaked in 1 mM MV solution to induce oxidative stress and in 25% PEG solution to induce drought stress. Prior to light irradiation, the seedlings were kept in the dark for 7 h. The light irradiation was conducted for 2 h under growth lights for the MV treatment and for 3 h at a high light intensity (greater than 600 μmol m^{-2} s^{-1} of PPFD) for the PEG treatment.

2.1.3. Evaluation of stress tolerance

Plant damage was evaluated from measurements of F_v/F_m and MSI. F_v/F_m and MSI were measured for the topmost fully expanded leaf of each seedling after the stress treatments. After 20 min of dark adaptation, F_v/F_m was measured with a chlorophyll fluorometer (OS-30p, OPTI-SCIENCES, Hudson, NH, USA) and leaf clip under dim light which do not affect the F_v/F_m measurement. On the same leaf used for the measurement of F_v/F_m, MSI was then measured. The leaf was detached and placed into a 2 mL tube with distilled water at 40 °C for 60 min, and then its electrical conductivity was recorded (C1). The same samples were then placed in boiling water for 10 min, and the electrical conductivity was then recorded (C2). The MSI was calculated as $\mathrm{MSI} = 1 - (C1/C2)$.

2.1.4. Data analysis and the interpretation of the principal component scores

All of the experiments were conducted 6 times. The F_v/F_m and MSI values in each MV and PEG treatment were averages of 6 replications (6 seedlings). To obtain an overview of the variation of cross-tolerance in the populations of BCLs and DHLs, four data sets with F_v/F_m–MV, MSI–MV, F_v/F_m–PEG and MSI–PEG, each including 91 genotypes, were used for the PCA. We applied this procedure to standardized variables. Significant differences in F_v/F_m and in MSI between BCLs and DHLs were tested using a single-factor analysis of variance (ANOVA). The ANOVA and PCA were performed with Ekuseru-Toukei 2006 statistical software (Social Survey Research Information, Japan). According to a previous study (Iseki et al., 2013a), we interpreted the first principal component (PC1) as the cross-tolerance to oxidative and drought stresses the higher PCS1 represents the higher cross-tolerance.

2.2. Field experiments

2.2.1. Growth conditions

Field experiments were conducted in experimental fields of the Ubon Rice Research Center, Thailand, in the wet seasons of 2010–2011 and in the dry season of 2011–2012. The wet season experiments were already described in Iseki et al. (2013b) but the experiment in dry season was newly conducted for this study. The soil is light in texture, permitting high percolation and loss of water, and it is classified as loamy sand. 20 and 8 genotypes, including DHLs and BCLs, for the experiments in wet season and dry season respectively, were grown under flooded and aerobic conditions. For the wet season experiments, seeds were sown in a seedling nursery on 23 June 2010 and 17 June 2011. The seedlings were transplanted to two experimental fields, one with flooded conditions and one with aerobic conditions, on 12 August 2010 and 30 July 2011. In each of the water conditions, the rice genotypes