Genotypic Diversity of Cross-Tolerance to Oxidative and Drought Stresses in Rice Seedlings Evaluated by the Maximum Quantum Yield of Photosystem II and Membrane Stability

Kohtaro Iseki¹, Koki Homma¹, Tsuyoshi Endo² and Tatsuhiko Shiraiwa¹

¹Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan; ²Graduate School of Biostudies, Kyoto University, Kyoto 606-8502, Japan)

Abstract: The genotypic variation of oxidative damage under oxidative and drought stresses was evaluated for a total of 67 rice cultivars consisting of 61 from the rice diversity research set of germplasm and 6 high-yielding varieties. The maximum quantum yield of photosystem II (Fv/Fm) and the membrane stability index (MSI) were measured to assess the oxidative damage induced by methyl viologen (MV) for oxidative stress and polyethylene glycol (PEG) for drought stress. Considerable variations in Fv/Fm and MSI among the cultivars in MV treatment indicated the existence of genotypic diversity in the susceptibility to oxidative damage. The weak relationships of Fv/Fm and MSI between MV and PEG treatment suggested that mechanisms other than oxidative stress tolerance affected the genotypic diversity of oxidative damage in PEG treatment. We used principal component analysis to quantify the cross-tolerance to oxidative damage under MV and PEG treatments: cross-tolerance was higher in cultivars in the japonica group than in the indica groups and higher in the improved cultivars than in the landraces. These results suggest that genotypic diversity of cross-tolerance is related to adaptation to the ecosystem where the genotypes originated and that the characteristics responsible for the tolerance to oxidative damage have been selected during breeding for improved grain yield.

Key words: Cross-tolerance, Drought stress, Genotypic diversity, Oryza sativa L., Oxidative stress, Principal component analysis, Rice diversity research set of germplasm.

Plants showing tolerance to one stress can also have tolerance to other stresses. This phenomenon is called cross-tolerance. Because much of the injury to plants caused by stress exposure is associated with oxidative damage at the cellular level (Allen, 1995), tolerance to oxidative stress is thought to be responsible for cross-tolerance to a wide range of environmental stresses. Oxidative damage due to the generation of reactive oxygen species (ROS) in the photosynthetic cells under environmental stress is caused by a disturbance in the prooxidant-antioxidant balance. A higher level of prooxidants than antioxidants in a plant leads to potential damage, and an excess of ROS in relation to the available antioxidants is considered a state of oxidative stress (Halliwell, 2006). The thylakoid membranes in the chloroplast are the major sites of ROS generation in plant tissues. Under a high reductive condition of the thylakoid membrane, singlet oxygen (¹O₂) is generated in photosystem II (PSII) through the interaction of triplet-state chlorophyll with ground-state oxygen, and superoxide radicals (O₂⁻) are also produced in photosystem I (PSI) via the Mehler reaction (Foyer et al., 1994). The production of ROS is largely affected by physiological and environmental factors. The rate is enhanced under conditions in which the light absorption exceeds dissipation, which includes more than simply carbon fixation (Asada, 2006). The accumulation of ROS affects many cellular functions via degradation of the nucleic acids, protein oxidation, and lipid peroxidation. The oxidative damage to PSII decreases the photosynthetic rate at different light intensities because of the limitation of the electron transport rate (Hikosaka et al., 2004), and the
photosynthetic carbon metabolism is also depressed by severe oxidative damage, especially under high intensity light conditions (Flexas et al., 2004). Such a limitation of photosynthesis derived from oxidative damage restricts the plant primary production under environmental stress. Thus, tolerance to oxidative stress plays an important role in environmental stress tolerance.

Drought stress is a major impediment to crop production in the world. Genetically modified plants with enhanced antioxidant enzymes or excess energy dissipation demonstrate a higher tolerance to drought stress compared to their wild-type counterparts (Mohamed et al., 2003; Sunkar et al., 2003). Genotypic differences in oxidative damage under drought stress have been studied in several plant species, such as rice, wheat and sesame, and the genotypes tolerant to drought stress have higher antioxidant capacities (Sairam et al., 1998; Guo et al., 2006; Fazeli et al., 2007). Pastori and Trippi (1992) also reported a relationship between drought stress tolerance and oxidative stress tolerance: a drought-resistant maize strain showed a high rate of glutathione reductase synthesis under the oxidative stress mediated by methyl viologen (MV). Accumulating information about such genotypic differences is important for understanding the mechanisms of cross-tolerance with drought stress, and the evaluation of genotypic diversity provides fundamental knowledge to develop new cultivars that are tolerant to environmental stresses.

Rice (*Oryza sativa* L.) is an important crop that is cultivated worldwide. Although some studies have revealed that the antioxidant capacity is closely related to the environmental stress tolerance (Dionisio-Sese and Tobita, 1998; Guo et al., 2006; Bonnecarrere et al., 2011), information about the generic diversity in oxidative stress tolerance remains limited. Because rice has a large genotypic variation, depending on the cultivation area, and is divided into several subspecies and ecotypes, conducting a comprehensive survey of the genotypic diversity for specific characteristics is quite difficult. Kojima et al. (2005) developed a rice diversity research set of germplasms (RDRS) that consisted of 69 accessions based on a restriction fragment length polymorphism (RFLP) survey of 332 accessions of cultivated rice. RDRS covered the variation of several agro-morphological traits in the initial accessions, suggesting that this set is a good tool for investigating the potential diversity of physiological traits in rice.

The objective of this study was to evaluate the genotypic variation of oxidative damage under oxidative and drought stresses using RDRS to examine the cross-tolerance to oxidative stress and drought stress in a wide range of diverse genotypes. Since RDRS includes few high-yielding varieties (HYVs), we also examined some HYVs to evaluate recent improvements in cultivar breeding.

### Materials and Methods

#### 1. Genotypes and plant preparation

The genotypic variation in oxidative damage was evaluated using RDRS developed at the National Institute of Agrobiological Sciences (NIAS), Japan. A total of 67 accessions in RDRS are divided into 3 genotype groups: *japonica*, *aus* type *indica* (*indica* 1) and non-*aus*-type *indica* (*indica* 2) (Kojima et al., 2005). Seven accessions in RDRS are improved cultivars, and the others are landraces. We divided the *japonica* group into *temperate japonica* and *tropical japonica* cultivars for data analysis according to the NIAS accession information (http://www.gene.affrc.go.jp). In this study, 8 accessions of RDRS (accession numbers 52, 55, 56, 57, 58, 59, 62, and 66) were not used because of insufficient seed. We also examined 6 HYVs, shown in Table 1. Under irrigated conditions, Takanari and IR72 are commonly known as *indica* HYVs. IR72158-16-3-3-1 is a second-generation new plant type line developed by crossing elite *indica* with improved *tropical japonica*. Liangyoupeijiu is an *indica*-*japonica* hybrid rice with a higher yield than traditional hybrid rice (Wang et al., 2005). B6144-MR-6-0-0 and IR55423-01 are also *indica* HYVs that are well adapted to upland and aerobic cultivation (Atlin et al., 2006). The germinated rice seeds of a total of 67 cultivars (shown in Table 5) were sown on 96-well PCR plate, one seed per well. The bottom of each well was cut, and the plate put on the small plastic case (similar to the plate in size) filled by the 1/2000 diluted Hyponex solution (N : P : K = 6% : 10% : 5%, Hyponex Japan, Japan). The solution pH was adjusted to 6.5 with 1 N HCl. Seedlings were hydroponically grown at 25ºC and

| Cultivar       | Origin     | Variety type | Germplasm group |
|---------------|------------|--------------|-----------------|
| Takanari      | Japan      | Improved     | *Indica*        |
| B6144-MR-640  | Indonesia  | Improved     | *Indica*        |
| IR55423-01    | Philippines| Improved     | *Indica*        |
| IR72          | Philippines| Improved     | *Indica*        |
| IR72158-16-3-3-1| Philippines| Improved     | *Indica*/*japonica* |
| Liangyoupeijiu | China      | Improved     | *Indica*/*japonica* |

| Table 1. Description of the cultivars included in the HYV group. |
297

Iseki et al. — Genotypic Diversity of Cross-Tolerance to Oxidative Stress and Drought Stress in Rice

100 μmol m⁻² s⁻¹ of photosynthetic photon flux density (PPFD) at the leaf surface under a 12 hr photoperiod. Seedlings grown for 10 d, approximately 2 or 3 leaf age, were used for the oxidative stress, drought stress, and control (no stress) treatments. Then, the quantum yield of PSII and membrane stability were measured as the indicator of the leaf oxidative damage. The measurements were also done for seedlings with no stress treatment to evaluate the control value in each cultivar.

2. Oxidative stress treatment
In the presence of MV, the Mehler reaction is promoted due to the auto-oxidation of the photoreduced MV radicals to form O₂⁻ in PSI. The seedling roots were soaked in 1 mM MV solution and kept in the dark for 7 hr; the seedlings were then transferred to the light condition at 100 μmol m⁻² s⁻¹ of PPFD. After exposure to light for 2 hr, the leaf oxidative damage was assessed.

3. Drought stress treatment
Drought stress was imposed by water deficit and high intensity light treatment. The water deficit was induced by the addition of polyethylene glycol 6000 (PEG), which inhibits root water uptake. The PEG concentration was decided according to Zhou et al. (2007) who examined the stomatal conductance and leaf water content under various PEG concentration using rice seedlings. The seedling roots were placed directly into 25% PEG solution, corresponding to osmotic potential of 0.1 MPa at 25°C, and kept in the dark for 7 hr; the seedlings were then transferred to a high intensity light condition of 600 μmol m⁻² s⁻¹ of PPFD at the leaf surface. After exposure to light for 2 hr, the leaf oxidative damage was assessed.

4. Quantum yield of PSII
The chlorophyll fluorescence was measured using a chlorophyll fluorometer (OS-30p, OPTI-SCIENCES, Hudson, NH, USA). The topmost fully expanded leaf was used for the measurement of the maximum quantum yield of PSII (Fv/Fm). The seedlings were dark adapted for more than 20 min prior to the Fv/Fm measurement. After dark adaptation, minimum fluorescence (Fo) was measured under a weak modulating light of red LED at approximately 0.5 μmol m⁻² s⁻¹ of PPFD, thereafter maximum fluorescence (Fm) was determined by applying a saturating pulse at 3000 μmol m⁻² s⁻¹ of PPFD, from the same light source. Fv/Fm was calculated as Fv/Fm = (Fm – Fo) / Fm.

5. Membrane stability
The cell membrane stability was assessed by measuring the membrane stability index (MSI) according to Sairam and Saxena (2000), with some modification. After the measurement of Fv/Fm, 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 MSI = 1 – (C1 / C2).

6. Data analysis
All of the experiment was conducted 6 times. The data for the Fv/Fm and MSI in each MV and PEG treatment are the averages of 6 replications (6 seedlings). The effects of the variety type and germplasm group on the data were tested for significance using a single-factor analysis of variance (ANOVA). To obtain an overview of the genotypic variation of the cross-tolerance to the oxidative damage induced by the MV and PEG treatments, 4 data sets of Fv/Fm-MV, MSI-MV, Fv/Fm-PEG and MSI-PEG, each including 67 cultivars, were used for the principal component analysis (PCA). Information on using PCA for the analysis of the genotypic variation in crop plants has been presented by Mohammadi and Prasanna (2003). We applied this procedure to standardized variables. The ANOVA and PCA were performed using the statistical software Ekuseru-Toukei 2006 (Social Survey Research
Results

1. Genotypic variation of the quantum yield and membrane stability

Large genotypic variations in $F_v/F_m$ and MSI under both MV and PEG treatments were detected for the 67 cultivars used in this study (Fig. 1), although $F_v/F_m$ and MSI were at almost the same level under the non-stress condition. Genotypic variations were also seen in the plant height and root length, but these morphological characteristics did not affect the difference of $F_v/F_m$ and MSI under MV and PEG treatments (data not shown). Under MV treatment, $F_v/F_m$ varied from 0.357 to 0.712, and MSI varied from 0.26 to 0.78. Compared to MV treatment, a larger variation was observed under PEG treatment, in which $F_v/F_m$ ranged from 0.189 to 0.697 and MSI from 0.15 to 0.85. The genotypic variation of $F_v/F_m$ was highly correlated to MSI, regardless of the type of stress treatment. The correlation coefficients were 0.79 and 0.90 in MV and PEG treatments, respectively. The regression lines of MSI against $F_v/F_m$ differed slightly but not significantly with the germplasm groups (Fig. 1). The genotypic variation of $F_v/F_m$ and MSI in PEG treatment could be partially explained by those variations in MV treatment; the correlation coefficients between MV and PEG treatment were 0.43 for the $F_v/F_m$ and 0.50 for MSI at a significance level of 0.01 (Fig. 2).

2. Oxidative damage in the different variety types and germplasm groups

Significant differences in $F_v/F_m$ and MSI were observed among the different germplasm groups under both MV and PEG treatments (Table 2). The average values of $F_v/F_m$ and MSI were higher in the japonica groups than in the indica groups, including the HYVs, under MV treatment. Under PEG treatment, the $F_v/F_m$ and MSI in the temperate japonica and HYVs were higher than in the other groups, and those of the indica 1 group were the lowest. Lower $F_v/F_m$ and MSI values were observed in tropical japonica compared to temperate japonica under MV and PEG treatments.

Table 2. Mean values of $F_v/F_m$ and MSI in the different germplasm groups under MV and PEG treatments.

| Germplasm group | n  | Control | MV | PEG |
|-----------------|----|---------|----|-----|
|                 |    | $F_v/F_m$ | MSI | $F_v/F_m$ | MSI | $F_v/F_m$ | MSI |
| Temperate japonica | 4  | 0.806 ± 0.007 | 0.94 ± 0.02 | 0.636 ± 0.073 | 0.64 ± 0.18 | 0.561 ± 0.096 | 0.55 ± 0.15 |
| Tropical japonica | 10 | 0.807 ± 0.009 | 0.94 ± 0.02 | 0.603 ± 0.052 | 0.60 ± 0.07 | 0.420 ± 0.095 | 0.47 ± 0.12 |
| Indica 1         | 23 | 0.803 ± 0.005 | 0.94 ± 0.02 | 0.544 ± 0.098 | 0.50 ± 0.13 | 0.390 ± 0.109 | 0.42 ± 0.15 |
| Indica 2         | 24 | 0.806 ± 0.007 | 0.94 ± 0.02 | 0.519 ± 0.064 | 0.55 ± 0.08 | 0.462 ± 0.126 | 0.53 ± 0.16 |
| HYV             | 6  | 0.800 ± 0.007 | 0.93 ± 0.05 | 0.573 ± 0.044 | 0.53 ± 0.11 | 0.593 ± 0.051 | 0.66 ± 0.11 |
| Total           | 67 | 0.804 ± 0.007 | 0.94 ± 0.02 | 0.552 ± 0.082 | 0.54 ± 0.11 | 0.449 ± 0.125 | 0.49 ± 0.15 |
| ANOVA           |    | NS       | NS  | ** | *   | ** | ** |

The numbers of cultivars are shown in n. The data are expressed as the mean ± SD of the genotypes in each group. ** and * indicate significant difference at $P<0.01$ and $P<0.05$, respectively, and NS indicates not significant.
treatments. Under PEG treatment, indica 1 showed the lower Fv/Fm and MSI than indica 2, while the relation was not consistent under MV treatment. The degree of oxidative damage in tropical japonica in relation to that in other germplasm groups was not consistent between PEG and MV treatments. The Fv/Fm and MSI under PEG treatment were lower in tropical japonica than temperate japonica, indica 2 and HYVs, although the values under MV treatment were relatively high compared to the other groups. The Fv/Fm and MSI values of the improved cultivars were significantly larger than those in the landraces under both MV and PEG treatments (Table 3).

### 3. Principal component analysis

The first and second principal components (PC1 and PC2) accounted for 67.6% and 24.9% of the total variance, respectively. Since the high percentage of the total variance was explained by PC1 and PC2, they will be focused in the results and discussion. Table 4 shows the PC1 and PC2 scores (PCS1 and PCS2) for each cultivar by the values of Fv/Fm and MSI in MV and PEG treatments. In PC1, the factor loadings did not differ greatly with the factors, ranging from 0.75 to 0.85. In PC2, positive loadings were observed for Fv/Fm-PEG (0.44) and MSI-PEG (0.51), and negative loadings were observed for Fv/Fm-MV (-0.60) and MSI-MV (-0.43). Accordingly, PC1 indicates the cross-tolerance to oxidative and drought stresses, and PC2 indicates the difference between the tolerance to oxidative and drought stresses. A positive value of PC2 indicates that the relative values of Fv/Fm and MSI in the 67 cultivars in PEG treatment were larger than in MV treatment. The distribution of the cultivars in the different germplasm group was illustrated by a scatter plot of PCS1 and PCS2 (Fig. 5). A higher PCS1 was observed for the japonica groups and HYVs, almost all of which were positive, whereas indica 1 and indica 2 were distributed throughout a wide range of PCS1, ranging from negative to positive. Compared to the indica groups, the japonica groups were distributed in a lower range of PCS2. In the same scatter plot, the cultivars were divided into two variety types: improved and landrace (Fig. 4). On average, the improved cultivars have a higher PCS1 than the landraces; in contrast, the distribution of the improved cultivars in PC2 was not distinct from that of the landraces.

### Discussion

In this study, oxidative damage was assessed by measuring Fv/Fm and MSI, which exhibited considerable variations in RDRS and HYVs. Fv/Fm is used as a sensitive indicator of photosynthetic performance, which decreases due to the slowly relaxing quenching process and PSII photoinhibition (Baker and Rosenqvist, 2004). The molecular mechanisms of photoinhibition have been well studied: Krieger-Liszky (2005) and Keren and Kreiger-Liszky (2011) showed that increased generation of the 1O2 derived from the excess energy in the reaction center of PSII perturbs D1 protein turnover, which reduces the quantum yield of photosynthesis. Photoinhibition is enhanced under environmental stresses through the excessive reduction of plastoquinone (PQ) due to the suppression of energy use for carbon fixation (Tambussi et al., 2000; Murata et al., 2007). Faraloni et al. (2011) used chlorophyll fluorescence as a rapid tool to screen olive cultivars tolerant to drought stress, selecting high-Fv/Fm cultivars as drought tolerant, and indicated that the tolerant cultivars maintain a high leaf water content under in vivo drought stress. The high correlation between Fv/Fm and MSI observed in this study (Fig. 1) indicates that the ROS damage to PSII and the cell membrane occurred simultaneously due to the excess of light energy in the electron transport chain. Membrane stability is also easily estimated through the measurement of electrolyte leakage from the cells. Techniques to assess membrane stability have been applied to quantify the oxidative damage to cell membranes under several stress conditions, such as salt and drought (Bajji et al., 2002; Farooq and Azam, 2006). Tripathy et al. (2000) used MSI as a stress indicator for QTL analysis in rice under drought stress. The wide use of Fv/Fm and MSI indicates that these techniques are valid tools to evaluate oxidative damage, and, due to the simple procedures involved, these indicators are useful in large-scale screening. In this study, Fv/Fm alone could be used to
rapidly evaluate the cellular oxidative damage. However, because the $F_v/F_m$ is hardly affected by mild drought stress or low irradiance (Baker and Rosenqvist, 2004; Razavi et al., 2008), the careful selection of an appropriate fluorescence parameter is important to evaluate the plant stress under the given environments. Furthermore, the value of $F_v/F_m$ is dependent on the measurement of chlorophyll fluorescence emanated from only the top few layers of leaf chlorenchyma, whereas the oxidative damage occurs across the thickness of leaf (Maxwell and Johnson, 2000). Accordingly, using both $F_v/F_m$ and MSI may favor more precise evaluation of the oxidative damage even under severe drought stress.

The lower susceptibility to oxidative stress in japonica rice cultivars seems to correlate with their adaptability to the higher latitude ecosystems. The $O. sativa$ cultivars around the world are roughly grouped into two subspecies, indica and japonica, according to geographical distribution and a series of morphological characteristics (Kato, 1930). Indica rice is mainly distributed at lower latitudes in tropical and...
subtropical areas, ranging from Southern China to Bengal, whereas *japonica* rice is found at high latitudes, such as Japan, Korea and Northern China. The differences in the tolerance to environmental stress between *indica* and *japonica* rice have been studied with regard to salinity, low temperature and drought (Glaszmann et al., 1990; Lilley and Ludlow, 1996; Lee et al., 2003). For oxidative stress, Jiao and Ji (2001) reported that the *japonica* subspecies was more tolerant than *indica* to photoinhibition and that the higher maintenance of the D1 protein in *japonica* was induced by a higher activity of superoxide dismutase. This finding is consistent with our findings that the cultivars of *temperate japonica* have higher \( \frac{F_v}{F_m} \) and MSI than the *indica* 1 and *indica* 2 cultivars, regardless of the stress treatment (Table 2), suggesting that oxidative stress tolerance is important for adaptability to the environment where the *japonica* cultivars survive.

**Table 4. Continued.**

| Name         | Origin | V(1) | G(2) | PCS1 | PCS2 | Control | MV | PEG |
|--------------|--------|------|------|------|------|---------|-----|-----|
|              |        |      |      |      |      | MSI     | F_v/F_m | MSI | F_v/F_m | MSI | F_v/F_m |
| Tupa 729     | Bangladesh | L   | TJ   | 0.18 | -0.20 | 0.96 | 0.813 | 0.579 | 0.559 | 0.519 | 0.427 |
| Khao Mac Kho | Viet Nam | L   | TJ   | 0.17 | -1.11 | 0.91 | 0.820 | 0.389 | 0.625 | 0.453 | 0.377 |
| IR72         | Philippines | I   | HYV  | 0.15 | 1.57  | 0.89 | 0.796 | 0.411 | 0.521 | 0.618 | 0.573 |
| Jhona 2      | India   | U   | I1   | 0.08 | -0.29 | 0.96 | 0.808 | 0.580 | 0.559 | 0.515 | 0.404 |
| Lebed        | Philippines | L   | I1   | 0.05 | -0.58 | 0.95 | 0.807 | 0.533 | 0.604 | 0.368 | 0.502 |
| Qingyu       | Taiwan  | L   | I2   | 0.02 | 0.92  | 0.95 | 0.803 | 0.574 | 0.463 | 0.624 | 0.440 |
| Basalianon   | Philippines | L   | I1   | -0.16 | -0.64 | 0.96 | 0.802 | 0.577 | 0.571 | 0.468 | 0.372 |
| Urasan 1     | Japan   | L   | TJ   | -0.23 | 0.15  | 0.96 | 0.810 | 0.498 | 0.549 | 0.439 | 0.490 |
| Ma Sho       | Myanmar | L   | TJ   | -0.26 | -1.66 | 0.93 | 0.797 | 0.564 | 0.649 | 0.291 | 0.395 |
| Pulii Arang  | Indonesia | L   | I2   | -0.34 | -0.28 | 0.94 | 0.796 | 0.558 | 0.540 | 0.450 | 0.396 |
| Hong Cheuh Zai | China | L   | I2   | -0.38 | -0.52 | 0.94 | 0.812 | 0.571 | 0.550 | 0.460 | 0.354 |
| ARC 11094    | India   | L   | I1   | -0.48 | -1.17 | 0.89 | 0.796 | 0.503 | 0.632 | 0.296 | 0.427 |
| Tupa 121-3   | Bangladesh | L   | I1   | -0.53 | -1.23 | 0.91 | 0.803 | 0.506 | 0.635 | 0.358 | 0.362 |
| Khao Nam Jen | Laos    | L   | J    | -0.80 | 0.20  | 0.93 | 0.796 | 0.389 | 0.572 | 0.386 | 0.483 |
| VANDARAN     | Sri Lanka | L   | I2   | -0.86 | 1.89  | 0.95 | 0.810 | 0.408 | 0.421 | 0.522 | 0.544 |
| Khao Tan Chiem | Viet Nam | L   | TJ   | -1.17 | -1.84 | 0.95 | 0.805 | 0.592 | 0.583 | 0.302 | 0.229 |
| Shwe Hang Gyi | Myanmar | L   | I2   | -1.31 | -0.68 | 0.90 | 0.812 | 0.533 | 0.516 | 0.332 | 0.324 |
| Ryon Suisan Koumai | China | L   | I2   | -1.43 | -0.09 | 0.94 | 0.816 | 0.550 | 0.448 | 0.353 | 0.352 |
| Jena 035     | Nepal   | L   | I1   | -1.43 | 1.43  | 0.92 | 0.812 | 0.360 | 0.448 | 0.495 | 0.443 |
| Padi Kuning  | Indonesia | L   | I2   | -1.44 | -0.41 | 0.89 | 0.784 | 0.486 | 0.516 | 0.355 | 0.328 |
| ARC 7291     | India   | L   | I1   | -1.45 | -0.55 | 0.96 | 0.793 | 0.453 | 0.544 | 0.301 | 0.363 |
| Bei Khe      | Cambodia | L   | I2   | -1.46 | 0.12  | 0.95 | 0.800 | 0.509 | 0.457 | 0.393 | 0.346 |
| Deejaoahualuo | China   | L   | I2   | -1.57 | 0.91  | 0.93 | 0.803 | 0.441 | 0.428 | 0.413 | 0.416 |
| Asu          | Bhutan  | L   | I2   | -1.61 | 0.16  | 0.96 | 0.809 | 0.478 | 0.464 | 0.440 | 0.296 |
| Jarjan       | Bhutan  | L   | I1   | -1.88 | -2.36 | 0.93 | 0.804 | 0.541 | 0.602 | 0.154 | 0.294 |
| Kaluheenati  | Sri Lanka | L   | I1   | -2.13 | 0.57  | 0.89 | 0.806 | 0.402 | 0.440 | 0.389 | 0.328 |
| Bingala      | Myanmar | L   | I2   | -2.13 | 0.50  | 0.88 | 0.798 | 0.399 | 0.445 | 0.341 | 0.359 |
| ARC 5955     | India   | L   | I1   | -2.17 | 0.97  | 0.96 | 0.806 | 0.377 | 0.421 | 0.418 | 0.346 |
| Nepal 8      | Nepal   | L   | I1   | -2.17 | -0.73 | 0.94 | 0.802 | 0.440 | 0.517 | 0.286 | 0.255 |
| Tadukan      | Philippines | L   | I2   | -2.90 | -0.93 | 0.95 | 0.815 | 0.456 | 0.469 | 0.208 | 0.189 |
| Ratul        | India   | L   | I1   | -2.99 | -0.38 | 0.96 | 0.809 | 0.384 | 0.466 | 0.266 | 0.205 |
| Kasalath     | India   | L   | I1   | -3.08 | 1.89  | 0.94 | 0.803 | 0.259 | 0.357 | 0.427 | 0.337 |
| Nepal 555    | Nepal   | U   | I1   | -3.43 | 0.43  | 0.92 | 0.805 | 0.323 | 0.402 | 0.205 | 0.296 |
| Badari Dhan  | Nepal   | L   | I1   | -4.03 | 0.23  | 0.96 | 0.808 | 0.306 | 0.388 | 0.206 | 0.190 |

1) Variety type; I: Improved, L: Landrace, and U: Unknown.
2) Germplasm group; J: *Temperate Japonica*, TJ: *Tropical Japonica*, I1: Indica 1, I2: Indica 2 and HYV: High yielding variety.
which adapted to different growing seasons. The differences in the physiological characteristics among these ecotypes are not well known. Aus is generally characterized as an early-maturity drought tolerant ecotype grown in the late dry season (Khush, 1997). However, even though the indica 1 germplasm group in RDRS consists mostly of aus cultivars (Kojima et al., 2005), the differences in the oxidative damage between indica 1 and indica 2 were not obvious under MV and PEG treatments (Table 2).

To examine the relationship between oxidative stress tolerance and cultivar improvement, we divided the cultivars into improved genotypes and landraces and compared their oxidative damage under MV and PEG treatments. The Fv/Fm and MSI values in the improved cultivars were significantly higher than those in the landraces (Table 3). It is worth noting that the oxidative damage of HYVs was milder compared to indica 1 and indica 2, although HYVs belong to the indica group. A more severe oxidative damage in the landraces as compared with the improved cultivars was also reported by Kumagai et al. (2009), who concluded that the higher photoinhibition of PSII in the traditional variety affected the low sustainability of photosynthesis under nitrogen-deficient conditions. Wang et al. (2005) compared higher-yield super-rice hybrids with traditional hybrid rice cultivars and reported that the super-rice hybrids were well protected against midday photoinhibition. These results suggest that physiological traits related to oxidative stress tolerance have been unintentionally selected during rice breeding aimed at improving the grain yield.

Water deficit and MV are known to enhance O2•− production in chloroplasts. In the light, bipyridinium herbicides, such as MV, generate oxygen radicals directly. The PSI-mediated reduction of MV compounds results in the formation of radical cations, which then react with molecular oxygen to produce O2•−, with the subsequent production of H2O2 (Babbs et al., 1989). On the other hand, under PEG treatment, which induces CO2-limiting conditions with excess light energy, the major site of O2•− production is the thylakoid membrane-bound primary electron acceptor of PSI (Foyer et al., 1994). O2•− and H2O2 easily oxidize the lipid membrane (Blokhina et al., 2003). Both MV and PEG treatments restrict the linear electron flow and induce the over-reduction of the PQ pool, which increases the energy flux for O2•− generation in PSII, resulting in an increase of photoinhibition with the start of lipid peroxidation. The common cause of oxidative damage by either stress was the production of ROS in PSI, which occurred concomitantly with the photoinhibition of PSII, and this may have resulted in the observed cross-tolerance.

PCA showed cross-tolerance to oxidative damage under both oxidative and drought stresses (PC1) and the difference in the tolerance to the two stresses (PC2). The high factor loading (more than 0.75) of PC1 for Fv/Fm and MSI in PEG treatment suggested that the cross-tolerance is partially effective in lowering damage under drought stress and is thought to be a useful trait to improve drought stress tolerance. However, the fact that the relationships of Fv/Fm and MSI between MV and PEG treatments were not strong (Fig. 2) suggests that mechanisms other than the cross-tolerance, which specifically functioned in PEG treatment and reduced the induction of oxidative damage, existed. The bias in PC2 was positive for the indica cultivars and negative for the japonica cultivars (Fig. 3). Compared to indica 2 and the HYVs, the tropical japonica cultivars sustained severe oxidative damage under PEG treatment, although the oxidative damage was relatively mild under MV treatment (Table 2). The reason for this inconsistence was expected to be the difference in the mechanism other than the oxidative stress tolerance, such as osmotic adjustment, which reduces the induction of oxidative damage and seems to be represented by the PCS2. Lilley and Ludlow (1996) reported that a japonica background conferred a low osmotic adjustment, whereas indica lines displayed a greater osmotic adjustment. Thus, the lower
levels of dehydration in the cultivars of indica 2 and HVs (belonging to indica) are thought to maintain the photosynthetic rate and linear electron flow in PEG treatment, and consequently mitigate ROS generation in PSI and PSII. Another reason for the genotypic variation in PCS2 might be the differences in photosopiration rate, which increases under drought stress and contributes to dissipate excess energy, resulting in the decrease of ROS generation (Wingler et al., 1999). Although the information about genotypic differences in photosopiration rate is restricted, Yeo et al. (1994) reported a large variation in photosopiration rate in the genotype Oryza.

Nearly all of the top 10 cultivars with a high PCS1 are improved type and consist of the temperate japonica, tropical japonica and the indica 2 groups, whereas most of the cultivars with a low PCS1 belong to the landrace type of the indica 1 group (Table 4). Although the mechanisms other than the oxidative stress tolerance, such as osmotic adjustment, might mask the effect of cross-tolerance, the oxidative stress tolerance was partially effective in reducing the oxidative damage sustained under the drought stress induced by PEG treatment. Oxidative damage occurred under many types of environmental stresses, and the cross-tolerance evaluated in this study is also thought to be effective for the tolerance to other stresses. Here, the question arises as to why the cross-tolerance was higher in the japonica cultivars than in the indica cultivars, although the environment of the lower latitude where most of the indica cultivars originated is more stressful than in the areas where the japonica cultivars originated. From the viewpoint of a stress acclimation, the subspecies-dependent genotypic variation of oxidative damage in rice could be considered as the result of an adaptive strategy. To understand the development of the genotypic diversity in oxidative stress tolerance in rice and to optimize the stress tolerance under a given environmental condition, further analyses of the relation between the cross-tolerance and the traits of biomass production such as photosynthetic performance under various types of environmental stress are needed.

References
Allen, R.D. 1995. Dissection of oxidative stress tolerance using transgenic plants. Plant Physiol. 107: 1049-1054.
Asada, K. 2006. Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol. 141: 391-396.
Atlin, G.N., Lafitte, H.R., Tao, D., Laza, M., Amante, M. and Courtois, B. 2006. Developing rice cultivars for high-fertility upland systems in the Asian tropics. Field Crop Res. 97: 43-52.
Babbs, C.F., Pham, J.A. and Goodbaugh, R.C. 1989. Lethal hydroxyl radical production in paraquat-treated plants. Plant Physiol. 90: 1267-1270.
Bajji, M., Kinet, J.M. and Lutts, S. 2002. The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. Plant Growth Regul. 36: 61-70.
Baker, N.R. and Rosenqvist, E. 2004. Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. J. Exp. Bot. 55: 1607-1621.
Blokhina, O., Virolainen, E. and Fagerstedt, K.V. 2003. Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann. Bot. 91: 179-194.
Bonnecarrere, V., Borsani, O., Diaz, P., Capdevielle, F., Blanco, P. and Monza, J. 2011. Response to phototoxic stress induced by cold in japonica rice is genotype dependent. Plant Sci. 180: 726-732.
Dionisio-Sese, M.L. and Tobita, S. 1998. Antioxidant responses of rice seedlings to salinity stress. Plant Sci. 135: 1-9.
Faraloni, C., Cutino, L., Petruccelli, R., Leva, A.R., Lazzeri, S. and Torzillo, G. 2011. Chlorophyll fluorescence technique as a rapid tool for in vitro screening of olive cultivars (Olea europaea L.) tolerant to drought stress. Environ. Exp. Bot. 73: 49-56.
Farooq, S. and Azam, F. 2006. The use of cell membrane stability (CMS) technique to screen for salt tolerant wheat varieties. J. Plant Physiol. 163: 629-637.
Fazeli F., Ghorbani, M. and Niknam, V. 2007. Effect of drought on biomass, protein content, lipid peroxidation and antioxidant enzymes in two sesame cultivars. Biol. Plant. 51: 98-103.
Flexas, J., Bota, J., Loreto, F., Cornic, G. and Sharkey, T.D. 2004. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C₃ plants. Plant Biol. 6: 269-279.
Foyer, C.H., Lelandais, M. and Kunert, K.J. 1994. Photooxidative stress in plants. Physiol. Plant. 92: 696-717.
Glazmann, J.C., Kaw, R.N. and Khush, G.S. 1990. Genetic divergence among cold tolerant rice (Oryza sativa L.). Euphytica 45: 95-104.
Guo, Z., Ou, W., Lu, S. and Zhong, Q. 2006. Differential responses of antioxidant system to chilling and drought in four rice cultivars differing in sensitivity. Plant Physiol. Biochem. 44: 828-836.
Halliwell, B. 2006. Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. Plant Physiol. 141: 312-322.
Hikosaka, K., Kato, M.C. and Hirose, T. 2004. Photosynthetic rates and partitioning of absorbed light energy in photoinhibited leaves. Plant Physiol. 121: 699-708.
Jiao, D. and Ji, B. 2001. Photoinhibition in indica and japonica subspecies of rice (Oryza sativa) and their reciprocal F₁ hybrids. Aust. J. Plant Physiol. 28: 299-306.
Kato, S. 1930. On the affinity of cultivated varieties of rice plants, Oryza sativa L. J. Dept. Agric. Kyushu Univ. 2: 241-276.
Keren, N. and Krieger-Liszay, A. 2011. Photoinhibition: molecular mechanisms and physiological significance. Physiol. Plant. 142: 1-5.
Khush, G.S. 1997. Origin, dispersal, cultivation and variation of rice. Plant Mol. Biol. 35: 25-34.
Kojima, Y., Ehana, K., Fukuoaka, S., Nagamine, T. and Kawase, M. 2005. Development of an RFLP-based rice diversity research set of germplasm. Breed. Sci. 55: 431-440.
Krieger-Liszay, A. 2005. Singlet oxygen production in photosynthesis. J. Exp. Bot. 56: 337-346.
Kumagai, E., Araki, T. and Kubota, F. 2009. Characteristics of gas exchange and chlorophyll fluorescence during senescence of flag leaf in different rice (Oryza sativa L.) cultivars grown under nitrogen-deficient condition. Plant Prod. Sci. 12: 285-299.
Lee, K.S., Choi, W.Y., Ko, J.C., Kim, T.S. and Gregorio, G.B. 2003. Salinity tolerance of japonica and indica rice (Oryza sativa L.) at
the seedling stage. *Planta* 216: 1043-1046.

Lilley, J.M. and Ludlow, M.M. 1996. Expression of osmotic adjustment and dehydration tolerance in diverse rice lines. *Field Crops Res.* 48: 185-197.

Maxwell, K. and Johnson, G.N. 2000. Chlorophyll fluorescence – a practical guide. *J. Exp. Bot.* 51: 659-668.

Mohamed, E.A., Iwaki, T., Munir, I., Tamoï, M., Shigeoka, S. and Wardano, A. 2003. Overexpression of bacterial catalase in tomato leaf chloroplasts enhances photo-oxidative stress tolerance. *Plant Cell Environ.* 26: 2037-2046.

Mohammadi, S.A. and Prasanna, B.M. 2003. Analysis of genetic diversity in crop plants – Salient statistical tools and considerations. *Crop Sci.* 43: 1235-1248.

Murata, N., Takahashi, S., Nishiyama, Y. and Allakhverdiev, S.I. 2007. Photoinhibition of photosystem II under environmental stress. *Biochem. Biophys. Acta* 1767: 414-421.

Pastori, G.M. and Trippi, V.S. 1992. Oxidative stress induces high rate of glutathione reductase synthesis in a drought-resistant maize strain. *Plant Cell Physiol.* 33: 957-961.

Razavi, F., Pollet, B., Steppe, K. and Van Labeke, M.C. 2008. Chlorophyll fluorescence as a tool for evaluation of drought stress in strawberry. *Photosynthetica* 46: 631-633.

Sairam, R.K., Deshmukh, P.S. and Saxena, D.C. 1998. Role of antioxidant systems in wheat genotypes tolerance to water stress. *Biol. Plant.* 41: 387-394.

Sairam, R.K. and Saxena, D.C. 2000. Oxidative stress and antioxidants in wheat genotypes: possible mechanism of water stress tolerance. *J. Agron. Crop Sci.* 184: 55-61.

Sunkar, R., Bartels, D. and Kirch, H.H. 2003. Overexpression of a stress-inducible aldehyde dehydrogenase gene from *Arabidopsis thaliana* in transgenic plants improves stress tolerance. *Plant J.* 35: 452-464.

Tambussi, E.A., Bartoli, C.G., Beltrano, J., Guianet, J.J. and Araus, J.L. 2000. Oxidative damage to thylakoid proteins in water-stressed leaves of wheat (*Triticum aestivum*). *Physiol. Plant.* 108: 398-404.

Tripathy, J.N., Zhang, J., Robin, S., Nguyen, T.T. and Nguyen, H.T. 2000. QTLs for cell-membrane stability mapped in rice (*Oryza sativa* L.) under drought stress. *Theor. Appl. Genet.* 100: 1197-1202.

Wang, Q.A., Lu, C.M. and Zhang, Q.D. 2005. Midday photoinhibition of two newly developed super-rice hybrids. *Photosynthetica* 43: 277-281.

Wingler, A., Quick, W.P., Bungard, R.A., Bailey, K.J., Lea, P.J. and Leegood, R.C. 1999. The role of photorespiration during drought stress: an analysis utilizing barley mutants with reduced activities of photorespiratory enzymes. *Plant Cell Environ.* 22: 361-373.

Yeo, M.E., Yeo, A.R. and Flowers, T.J. 1994. Photosynthesis and photorespiration in the genus *Oryza*. *J. Exp. Bot.* 45: 553-560.

Zhou, Y., Lam, H.M. and Zhang, J. 2007. Inhibition of photosynthesis and energy dissipation induced by water and high light stresses in rice. *J. Exp. Bot.* 58: 1207-1217.

* In Japanese.