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Title

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

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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.

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. 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 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 in fields.

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 according to the PCS1 by the methods described in Iseki et al. (2013a). The evaluation was conducted for 91 breeding populations in order to

estimate genotypic variation in the cross-tolerance. 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-tolerance 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 hours. The light irradiation was conducted for 2 hours under growth lights for the MV treatment and for 3 hours 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 minutes 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 with some modification. The leaf was detached and placed into a 2 mL tube with distilled water at 40ºC 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).

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.

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 and 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 were arranged in a randomized block design with three replications. The plot sizes were 12 m^2 and 9 m^2 for the flooded and aerobic conditions, respectively. For the dry season experiment, seeds were sown on 18 November 2011, and the seedlings were transplanted to the experimental fields where flooded and aerobic conditions were to be established on 19 December 2011. Each plot size was 3 m^2 for both the flooded and the aerobic conditions. The planting density was 25 plants m^{-2} for all the experiments. To avoid transplanting damage under the aerobic conditions, surface water was introduced until two weeks after transplanting and then drained. For the aerobic conditions, any rainfall was immediately drained, and irrigation was only conducted when the soil water potential decreased to -20 kPa.

The meteorological environments and soil water potentials (SWPs) under the aerobic conditions during each of the experimental periods are shown in Table 2. Solar radiation, air temperature and relative humidity

were measured every 5 minutes at a weather station near the experimental fields and stored in a data logger. The SWPs under the aerobic conditions were measured with a pF meter every 3 days. The vapor pressure deficit (VPD) was calculated from the air temperature and relative humidity. In the 2011 wet season experiment, the average VPD and solar radiation were lower than in the 2010 due to high precipitation. The high precipitation also brings the higher SWP under the aerobic condition. The VPD and solar radiation were higher in the dry season. Under the aerobic conditions, the average values of the SWP of 20 cm in depth were from -6 kPa to -13 kPa, indicating that the soil water deficiency was relatively mild.

2.2.2 Measurements

The experimental periods for the measurements of stomatal conductance (g_s) and leaf water potential (LWP) were from 6 to 8 weeks after transplanting which corresponded to the maximum tillering stage for all experiments. We assumed that the rice plants received the most severe damage at midday due to high solar radiation and high evapotranspiration. To obtain the midday values, the measurements were made from 1000 to 1400 on sunny days. At 9 weeks after transplanting, 4 plants with average growth were sampled for each genotype under both water conditions. The shoot materials were dried at 70 °C for 48 hours. Total shoot biomass was calculated by multiplying biomass per plant by planting density and expressed as grams per square meter (g m^{-2}).

2.2.3. Stomatal conductance

The measurements of g_s were obtained with a leaf porometer (SC-1, Decagon Devices, Pullman, WA, USA) 2 or 3 times every week. The leaf face that was not oriented toward the sunlight was used for the measurement. The measurements were conducted once a week during the experimental periods. For a measurement, 3 leaves were randomly selected as replications from each genotype from both water conditions.

2.2.4. Leaf water potential

LWP was measured with the pressure chamber method. Detached leaves were immediately put into a plastic bag and mounted in a cylinder chamber for the measurement. The measurement was performed once every week during the experimental periods. For a measurement, 6 leaves were randomly selected as replications from each genotype from both water conditions.

2.2.5. Data analysis

For analysis of data from the field experiments, the genotypes of DHLs and BCLs were firstly classified into the 2 groups depending on the PCS1. In the wet season experiments, top 5 genotypes which have a positive value in PCS1 were classified into positive PCS1 (+PCS1) group, bottom 5 genotypes which have a negative value in PCS1 were classified into negative PCS1 (–PCS1) group (Table 1). 8 genotypes tested in dry season experiments were also divided into +PCS1 and –PCS1 groups. The genotypes in +PCS1 and –PCS1 were partly different between the wet and dry season experiments. For both of the wet season and

dry season experiments, the mean PCS1 value of each group was almost 4.0 and -1.0 for +PCS1 and –PCS1 groups, respectively. All the measurements with replications during the experimental period were averaged for all the genotypes in each group. For the data analysis of the wet season experiments, a part of the data set already published in Iseki et al. (2013b) were used. The significance of the differences between the water conditions, genotype groups and experimental seasons were evaluated with a 3-way ANOVA using the same statistical software used for the PCA.

3. Results

3.1. Genotypic variation of cross-tolerance to oxidative stress and drought stress in rice breeding lines

Large genotypic variations in F_v/F_m and MSI were observed for both the BCLs and the DHLs in the MV and PEG treatments (Fig. 1). Genotypic variations in morphological traits such as plant height and root length were not found in the BCL and DHL seedlings. In both of the stress treatments, there were strong relationships between F_v/F_m and MSI: the correlation coefficients for the combined populations of BCLs and DHLs were 0.65 in the MV treatment and 0.85 in the PEG treatment. Both correlations were significant at the 0.01 level. Lower F_v/F_m and MSI values were found in Surin1, the recurrent parent of the BCLs, than in DHL143, the donor parent. Weak relationships between in MV treatment and the PEG treatment were observed for both F_v/F_m and MSI. The correlation coefficients were 0.36 for F_v/F_m and 0.47 for MSI, and both were significant at the 0.01 level (Fig. 2).

PC1 explained 64.9 % of the total variance. Due to the high percentage of the variance explained by

PC1, the results and discussion will focus on the value of PCS1, which is assumed to represent an indicator of cross-tolerance. In PC1, the factor loadings did not differ substantially among the factors, ranging from 0.45 to 0.54. The distribution of each line in the BCLs and DHLs is illustrated in Fig. 3. Large genotypic variation was observed in PCS1 for both the BCLs and DHLs. The mean PCS1 was higher in the DHLs (1.57) than in the BCLs (-0.41). In the population of DHLs, the relative position of DHL143 in the PCS1 was higher than those of the other DHLs. The PCS1 of DHL143 (3.31) was also higher than that of Surin1 (-0.19).

3.2. The effects of cross-tolerance on the field performance of rice

For both the +PCS1 and –PCS1 groups, the mean values of g_s, LWP and total shoot biomass were lower under the aerobic conditions than under the flooded conditions (Table 3). Only in the 2011 wet season, LWP showed the same level between under flooded and aerobic conditions. Lower LWP value and total shoot biomass under the aerobic conditions were observed in the dry season experiment than in the wet season experiment. A comparison of the results between the two groups showed that the values of g_s and total shoot biomass tended to be higher for the genotypes of the –PCS1 groups than those in the +PCS1 groups in both water conditions. Differences in total shoot biomass between the two groups were greater under the flooded conditions than under the aerobic conditions. The relative shoot biomass under the aerobic conditions were, on average, 8 % higher in the +PCS1 groups than the –PCS1 groups but the differences were not statistically significant. The values of LWP in the +PCS1 groups were slightly lower than those in the –PCS1 groups.

4. Discussion

The evaluation of cross-tolerance to oxidative stress and drought stress using measurements of F_v/F_m and MSI was successfully applied to the BCLs and DHLs in this study. Antioxidant capacity is one of the physiological mechanisms serving to reduce oxidative damage under MV treatment (Gill and Tuteja, 2010) and is strongly controlled by modifications of a single gene (Mohamed et al., 2003; Sunkar et al., 2003). Tripathy et al. (2000) examined genotypic variation in cell oxidative damage for the screening of drought tolerance in double haploid rice lines and detected a quantitative trait locus (QTL) which accounted for more than 40 % variation of the phenotype. Because the mechanisms of oxidative stress tolerance commonly moderate the oxidative damage in both the PEG and the MV treatments, the oxidative stress tolerance evaluated in the MV treatment confers cross-tolerance. These results suggest that oxidative stress tolerance is a trait whose heredity is relatively less complex and is, therefore, suitable for a breeding objective that involves drought tolerance.

The drought stress tolerance expressed by F_v/F_m and MSI in the PEG treatment largely depends on the characteristics of dehydration avoidance, such as stomatal response and osmotic adjustment (Lilley and Ludlow, 1996; Silva et al., 2010), each of which was affected by the assortments of multiple QTLs demonstrated by Zhang et al. (2001). The weak relationships between the MV and PEG treatments for F_v/F_m and MSI (Fig. 2) indicated that the dehydration avoidance in the PEG treatment functioned independently from the oxidative stress tolerance evaluated in the MV treatment. By using PCS1, we could eliminate the

effects of drought avoidance and evaluate the sole effects of the cross-tolerance on drought stress.

The higher cross-tolerance (PCS1) of DHL143 among the DHLs (Fig. 3) was consistent with the results of our previous study, in which DHL143 was confirmed as a drought-tolerant line (Jongdee et al., 2006). In the MV and PEG treatments, both the oxidative stress tolerance and the drought stress tolerance were higher in the DHLs than in Surin1 (Fig. 1). This result suggested that the higher drought tolerance in DHL143 was closely related to the higher cross-tolerance. The lower cross-tolerance of the BCLs than the DHLs might be a result of the lower tolerance of Surin1 than DHL143. The values of PCS1 in the BCLs were segregated in the BCLs, indicating that cross-tolerance is a heritable trait that can be improved by crossbreeding.

The values of the total shoot biomass in the +PCS1 groups were smaller than those in the –PCS1 groups, especially under flooded conditions (Table 3). The lower values of g_s in the +PCS1 groups were one of the causes of low shoot biomass because a lower g_s is the principal cause of photosynthesis reduction under a mild soil water deficiency (Medrano et al., 2002). In contrast, a lower g_s is a favorable characteristic for maintaining the water condition of the leaf under severe drought stress (Lizana et al., 2006; Sinclair et al., 2008). Another cause of the smaller shoot biomass in the +PCS1 groups is expected to be the high energy cost of cross-tolerance mechanisms such as high antioxidant enzyme activity and excess energy dissipation, which compete with energy use for photosynthetic carbon fixation and potentially decrease plant growth (Raven, 2011).

The effect of the cross-tolerance on mitigation of biomass reduction was restricted (8 % on average)

and not statistically significant in this study. However the tendency of smaller decrease in biomass in the +PCS1 groups under aerobic conditions (Table 3) suggests that the lower g_s levels contributed to the maintenance of plant growth even when the LWP was relatively higher under the mild soil water deficiency (Table 2). The higher cross-tolerance capacity in the genotypes of the +PCS1 groups was expected to moderate the oxidative damage resulting from the low g_s. In our previous study, we compared the values of F_v/F_m and MSI between the flooded and aerobic conditions of the same field experiments, and a significant difference was only observed in F_v/F_m, not in MSI (Iseki et al. 2013b). The reason for this result is that the drought intensity was moderate, approximately -10 kPa, under the aerobic conditions. In contrast, the drought intensity in the screening was 25 % PEG, corresponding to -100 kPa of osmotic pressure. The advantage of the traits related to high PCS1 in terms of plant water status and plant growth might be distinguished under severe drought stress. Chenu et al. (2011) reported that certain characteristics associated with drought tolerance are advantageous for yield in drought-prone environments and also tend to produce decreases in yield performance in the more favorable environments. The genotypes in the +PCS1 groups showed smaller biomass decreases under the aerobic conditions but less shoot biomass under the flooded conditions. An optimal balance between tolerance and stress intensity may serve to allow high biomass production under a given drought stress environment.

5. Conclusions

The DHLs, which show extensive genotypic variation in osmotic adjustment and root morphology

(Zhang et al., 2001; Kamoshita et al., 2002), also showed genotypic variation in the cross-tolerance measure represented by PCS1 in this study. The higher PCS1 of DHL143 than that of Surin1 improved the cross-tolerance of several BCLs. The biomass reduction in the genotypes of +PCS1 group tended to be mitigated under mild drought conditions. In view of the small shoot biomass in the genotypes of the +PCS1 groups, it is important to develop rice varieties that have an optimal cross-tolerance to a target environment because the degree and pattern of drought stress occurring under rainfed conditions is highly environmentally dependent (Jongdee et al., 2006). The characteristics associated with high PCS1 values are expected to be more effective for plant growth under severe drought stress.

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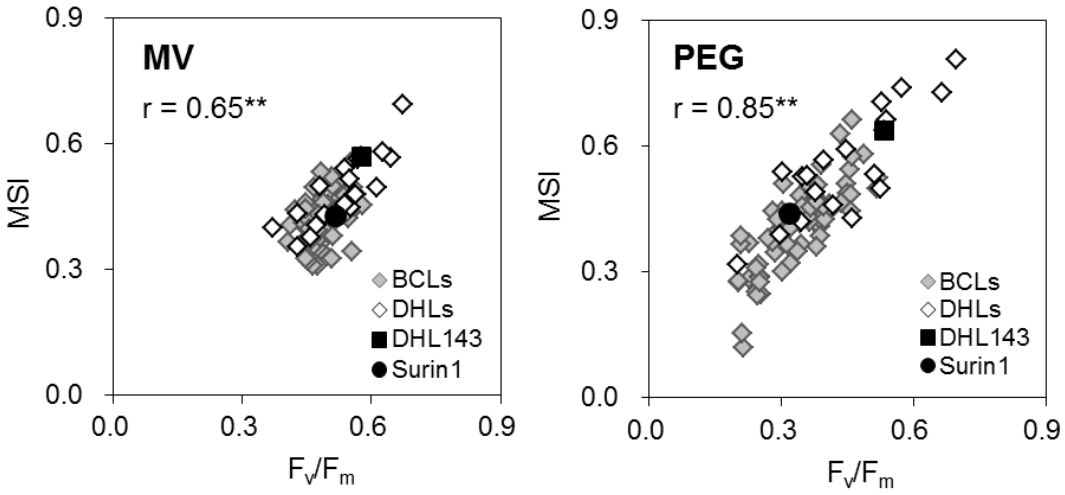
Table and figure captions

Fig. 1. Relationships between F_v/F_m and MSI in BCLs and DHLs in the MV and PEG treatments. DHL143 and Surin1 are the donor parent and recurrent parent of the BCLs, respectively. ** Significant at $p < 0.01$.

Fig. 2. Relationships between the MV and PEG treatments for F_v/F_m and for MSI. ** Significant at $p < 0.01$.

Fig. 3. Distribution of the first principal component scores (PCS1) in the BCLs and DHLs. The positions of Surin1 and DHL143 in PCS1 were indicated by black triangles.



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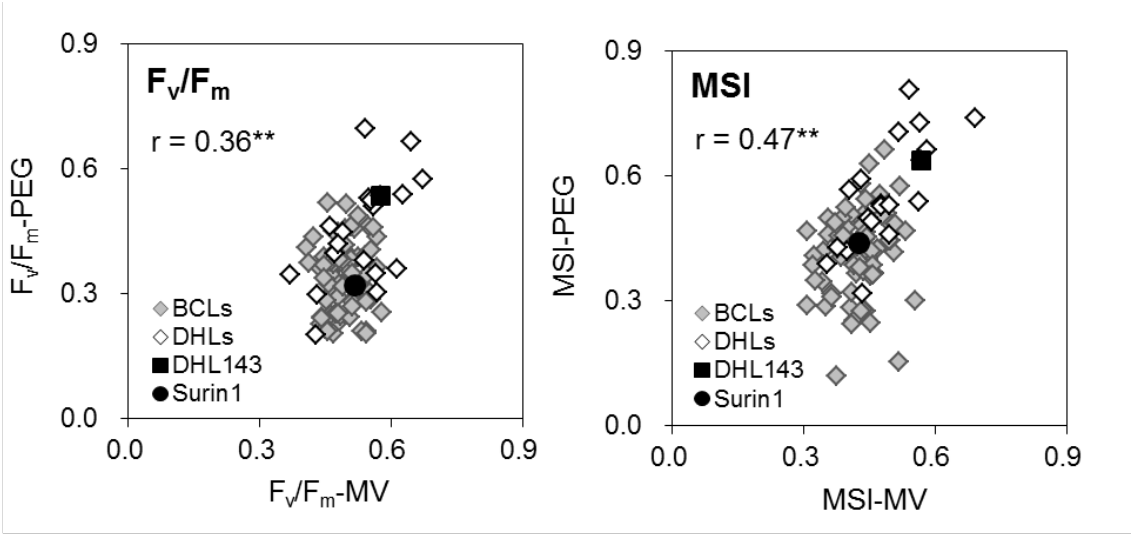

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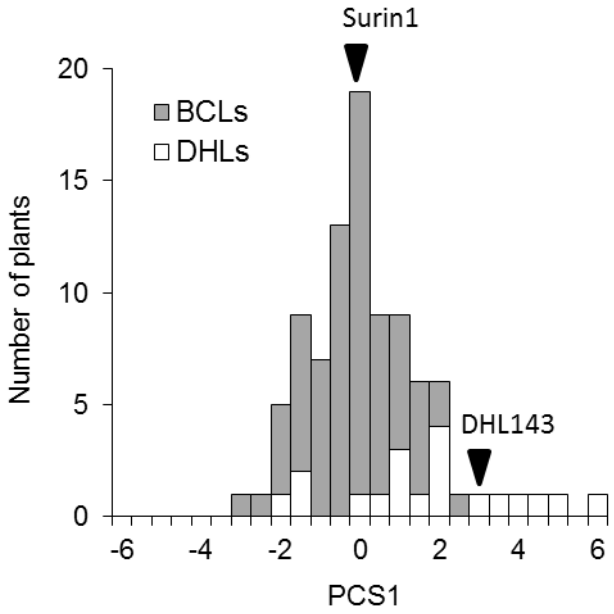

Fig. 3. Distribution of the first principal component scores (PCS1) in the BCLs and DHLs. The positions of Surin1 and DHL143 in PCS1 were indicated by black triangles.

Table 1

Principal component scores (PCS) of the genotypes used for the field experiments. The genotypes were divided into groups with positive and negative values based on the first PCS (PCS1).

Year	Season	Group	Pedigree	PCS1	Group	Pedigree	PCS1
2010, 2011	Wet season	+PCS1	IR68586-FA-CA-82	5.68	–PCS1	IRUBN030070-9-32	-0.32
			IR69644-AC-65	4.95		IRUBN030062-1-9	-0.37
			IR69644-AC-56	4.40		IRUBN030056-10-42	-0.43
			IR68586-FA-CA-143	3.31		IR68586-CA-14	-2.03
			IR68586-FA-CA-115	1.72		IRUBN030063-9-4	-2.26
			Mean PCS1	4.01		Mean PCS1	-1.08
2011-2012	Dry season	+PCS1	IR68586-FA-CA-82	5.68	–PCS1	IRUBN030055-5-190	-0.28
			IR69644-AC-65	4.95		IRUBN030070-9-32	-0.32
			IR68586-FA-CA-143	3.31		IRUBN030062-1-33	-0.67
			IR68586-CA-24	1.93		IRUBN030054-8-73	-3.09
			Mean PCS1	3.97		Mean PCS1	-1.09

Table 2

Means of day temperature, day vapor pressure deficit (VPD), solar radiation and soil water potential under the aerobic conditions during the experimental periods.

Year, season	Day temperature [a)] (°C)	Day VPD (hPa)	Solar radiation (MJ m^{-2} day^{-1})	Soil water potential (kPa)	
				-20cm	-40cm
2010, wet season	27.9	4.8	14.3	-11.4	-10.8
2011, wet season	27.4	3.9	12.8	-6.3	-4.4
2011-2012, dry season	27.5	19.0	17.9	-12.7	-11.6

a) Day temperature and day VPD were averaged for the mean values of the time from 600 to 1800 every day during the experimental periods.

Table 3

Average values of stomatal conductance, leaf water potential, total shoot biomass and relative shoot biomass in the groups with positive and negative values of the first principal component score (PCS1) under flooded and aerobic conditions.

Year, Season	Water condition	Stomatal conductance (mmol H_2O m^{-2} s^{-1})		Leaf water potential (MPa)		Total shoot biomass (g m^{-2})		Relative shoot biomass (aerobic/flooded)	
		+PCS1[a]	–PCS1[b]	+PCS1	–PCS1	+PCS1	–PCS1	+PCS1	–PCS1
2010, wet season	Flooded	223	257	-0.78	-0.80	401.5	463.1	0.89	0.81
	Aerobic	207	212	-0.85	-0.85	355.0	371.5		
2011, wet season	Flooded	298	294	-0.79	-0.78	308.9	369.4	0.66	0.58
	Aerobic	227	234	-0.78	-0.77	204.5	212.8		
2011-2012, dry season	Flooded	372	396	-0.85	-0.78	249.5	288.3	0.54	0.48
	Aerobic	216	265	-1.25	-1.15	133.7	140.3		
Means	Flooded	298	315	-0.81	-0.79	320	374	0.70	0.62
	Aerobic	216	237	-0.96	-0.92	231	242		
ANOVA	Group (G)	**		**		*		ns	
	Water condition (W)	**		**		**			
	Season (S)	**		**		**		**	
	G×W	ns		ns		ns			
	G×S	ns		**		ns		ns	
	W×S	**		**		ns			
	G×W×S	ns		ns		ns			

[a] +PCS1: groups of the genotypes with positive PCS1

[b] –PCS1: groups of the genotypes with negative PCS1

ns: not significant

* Significant at $p < 0.05$

** Significant at $p < 0.01$