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CHILLING/FREEZING STRESS

Physiological Response of Multiple Contrasting Rice (*Oryza sativa* L.) Cultivars to Suboptimal Temperatures

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Abstract

The physiological response of multiple rice cultivars, eighteen initially and eight cultivars later on, to suboptimal temperatures (ST) conditions was investigated in laboratory and outdoor experimental conditions. Treatment with ST decreased growth in different extents according to the cultivar and affected the PSII performance, determined by chlorophyll fluorescence fast-transient test, and stomatal conductance, regardless the experimental condition. Two groups of cultivars could be distinguished on the base of their growth and physiological parameters. The group of cultivars presenting higher growths displayed optimal JIP values, and higher instantaneous water use efficiency (WUE_i), due to a lower Gs under ST, unlike cultivars showing lower growth values, which presented worse JIP values and could not adjust their Gs and hence their WUE_i. In this work, we detected at least two cultivars with superior tolerance to ST than the cold tolerant referent Koshihikari. These cultivars could be used as parents or tolerance donors in breeding for new crop varieties. On other hand, positive and significant correlations between data obtained from laboratory and outdoor experiments suggest that laboratory measurements of most of the above mentioned parameters would be useful to predict the response of rice cultivars to ST outdoor.

Introduction

Rice (*Oryza sativa* L.) is regarded as the most important crop in terms of human nutrition and caloric consumption worldwide, providing more than one-fifth of the calories consumed by humans (Smith 1998, FAO 2013). This species has evolved in tropical and subtropical areas, and hence, it is vulnerable to cold weather (Imin et al. 2006). Several million ha of rice cultivation are affected globally by low temperature every year, resulting in annual yield losses of 1–3.9 t ha^{-1} (Jena and Hardy 2012). Plant physiologists use the term freezing to mean temperatures below 0 °C, chilling for temperatures between 0 °C and the minimum temperature for growth, and suboptimal temperatures for growth (ST) for those between the former and the optimum temperature (Menéndez et al. 2013). Under field conditions, rice seedlings are exceptionally subjected to freezing periods, more regularly they endure chilling, whereas the most common situation is ST (Greaves 1996, Morsy et al. 2005, Zhang et al. 2012). Although some early studies demonstrated that rice growth rate and metabolism are noticeably inhibited in the range of

15–20 °C (Kabaki et al. 1982, Takanashi et al. 1987), the physiological bases for rice growth delay by ST remain fairly unexplored. In contrast, numerous authors have focused on morphological, biochemical and molecular responses to chilling and freezing in this crop (Peng and Ismail 2004, Bertin et al. 1996, Bonnecarrère et al. 2011, Wang et al. 2013).

Photosystem II (PSII) has been demonstrated to be one of the sensitive targets for low temperature stress (Strauss et al. 2006, 2007, Pagter et al. 2008). Among major effects of chilling on rice plants during early vegetative growth is the inhibition of electron transport through PSII and consequent photoinhibition (Gesch and Heilman 1999, Jeong et al. 2002). In rice, chilling tolerance was consistent with a reduced level of photoinhibition and oxidative damage (Bonnecarrère et al. 2011). Low temperature may also affect chlorophyll (Chl) fluorescence of PSII in several species (Long et al. 1994, Strasser et al. 1995). The ratio of variable to maximal Chl_a fluorescence or maximum quantum efficiency of PSII (F_V/F_M) decreased in plants subjected to low temperatures (Renaut et al. 2005, Pagter et al. 2008). Moreover, this ratio has been widely used as parameter to screen for chilling tolerance (Neuner and Larcher 1990, Sthapit et al. 1995, Agati et al. 1996, Fracheboud et al. 1999).

Breeding and genetic manipulation to increase crop yields require of rapid and reliable procedures to select the best adapted, among numerous cultivars. It has been suggested that specific selection techniques under controlled conditions would be useful to speed up the development of cultivars with higher chilling tolerance (Wery et al. 1994, Bertin et al. 1996, Strauss et al. 2006). In addition, the obtaining of quantitative data for statistical testing should be privileged over visual or subjective measurements for cultivar selection. Thus, the finding of rice cultivars with objective contrasting responses to ST could contribute to develop cultivars with improved ST tolerance and also to increase the current knowledge of this growth constraint.

The aim of the present work was to gain insight into the physiological response of rice to ST and to detect rice cultivars with contrasting performance under a ST range. For this purpose, we characterized plant growth, PSII functioning, net photosynthesis and gas exchange of several rice cultivars when confronting ST conditions. In the Argentinian rice planting region, typical daily temperatures during the early seedling stage of the rice cycle oscillate between 21 and 13 °C (Quintero 2009). To mimic the real field ST conditions for rice cropping in Argentina, we used this temperature range in laboratory experiments. This temperature range is considered as suboptimal as the minimum temperature for rice growth has been established at 10 °C (Yoshida 1981).

Materials and Methods

Plant material

Rice cultivars Koshihikari, IR24, IR28, IR50, Japonesito three meses, Japonesito eight meses, Japonesito Prolifico, General Rossi, H313-1-2, H313-26-2, Bombilla, Cini 754, Cnia 948, CT-6742-10-10-1, Oro, Cala PA, Ayasmi, Honezhaosen were kindly provided by the Rice Breeding Program of the Universidad Nacional de La Plata, Argentina. Seeds of these cultivars were placed within petri plates on two layers of Whatman N° 5 filter paper rinsed with 7 ml carbendazim 0.025 %p/v (Yoshida 1981) and incubated in growth chamber at 30 °C in darkness until germination. The resultant seedlings were transplanted to different substrates for laboratory and outdoor assays.

Laboratory assays

The following A and B assays were performed in growth chamber with 12-h photoperiod, 80 % humidity and 200 μmol photons m^{-2} s^{-1} of photosynthetically active radiation (PAR). The day/night temperatures for ST and optimal temperatures of growth (OT) were 21 of 13 and 28 of 24 °C, respectively.

(i) Short-term assay. Seedlings were hydroponically cultured at OT by transplanting them on plastic net frames placed over a 4-l black tray containing 3 l distilled water. After three days of culture, the water was replaced by 3 l Yoshida solution (Glenn et al. 1997). Therein, the Yoshida solution was renewed every three days until the end of the experiment. When the third leaf (Yoshida 1981) emerged (T_0 stage), half of the seedlings were transferred to the ST condition. Seedlings were further cultivated for another 3 d under the OT or ST conditions (Figure S1A).

(ii) Long-term assays. Seedlings were transplanted to 0.5-l plastic pots, containing sterile organic soil extract as substrate. Pots were introduced within 4-l trays with 3 l distilled water and kept at either ST or OT during 24 d. Water was periodically added to the trays in order to maintain the water level invariable during the time-lapse experiment. In the case of plants maintained at OT, the following procedure was undertaken: at the 25th experimental day, when the light within the growth chamber turned on, temperature was kept at 24 °C during 2 h only, and then it was gradually diminished, until reaching 11.5 °C after 3 h (Figure S1B).

Outdoor assay

Seedlings were transplanted to 4-l pots with holes in the bottom and filled with sterile organic soil extract. Pots were introduced within 10-l trays with 5 l distilled water (Figure

S1C). Trays were kept in a greenhouse, with 21/13 ± 2 °C day/night, 550 PAR, for 12 d. Afterwards, trays were transferred outside in the open, where plants were further grown for another 12 d, under the temperature and PAR conditions depicted in Figure S2.

Plant growth determination for the short-term laboratory assay

Seedlings from the hydroponic culture were scanned at 600 ppi resolution (HP PSC 1510 Hewlett Packard Development Company, LP, USA) at the T_0 stage after three days of culture under OT or ST. The digitalized images were analysed with the software package OPTIMAS (Optimas 6.1, Corporation. Bothell, WA, USA) to determine the third leaf length. Net leaf elongation was calculated from the equation:

$$\textit{Net leaf elongation} = \textit{length at day 3} - \textit{length at } T_0 \textit{ stage}$$

Chlorophyll fluorescence fast-transient test

Non-invasive chlorophyll fluorescence fast-transient test (JIP test) was performed on blades of expanded leaves with a portable chlorophyll fluorometer (Pocket PEA, Hansatech Instrument, UK) in laboratory and outdoor experiments. The JIP analysed parameters were density of active reaction centre (RC) per excited cross section at time zero (RC/CS_0), number of quinone *a* reducing RC per PSII antenna Chl (RC/ABS), maximum quantum yield of primary PSII photochemistry (F_V/F_M), specific energy flux of absorbed photon per PSII RC (ABS/RC), specific energy flux of dissipated excitation energy at time zero per PSII RC (DI_0/RC) and performance index for energy conservation from photons absorbed by PSII antenna, to the reduction of quinone *b* (PI_{ABS}). For this purpose, leaves were covered with leaf clips to adapt them to darkness for 20 min. Then, leaf clips were opened, and samples were exposed during 3 s to 3500 μmol photons m^{-2} s^{-1} (637 nm peak wavelength). The pocket PEA software (PEA plus v1.1, Hansatech Instrument Ltd., UK) was used to analyse PSII properties according to Strasser et al. (2000).

Determination of chlorophyll content

Chlorophyll content was measured on blades of expanded leaves of plants grown outdoor, with a non-invasive portable chlorophyll spectrophotometer (Clorofilio, Cavadevices, BA, Arg.). Data were expressed in SPAD units.

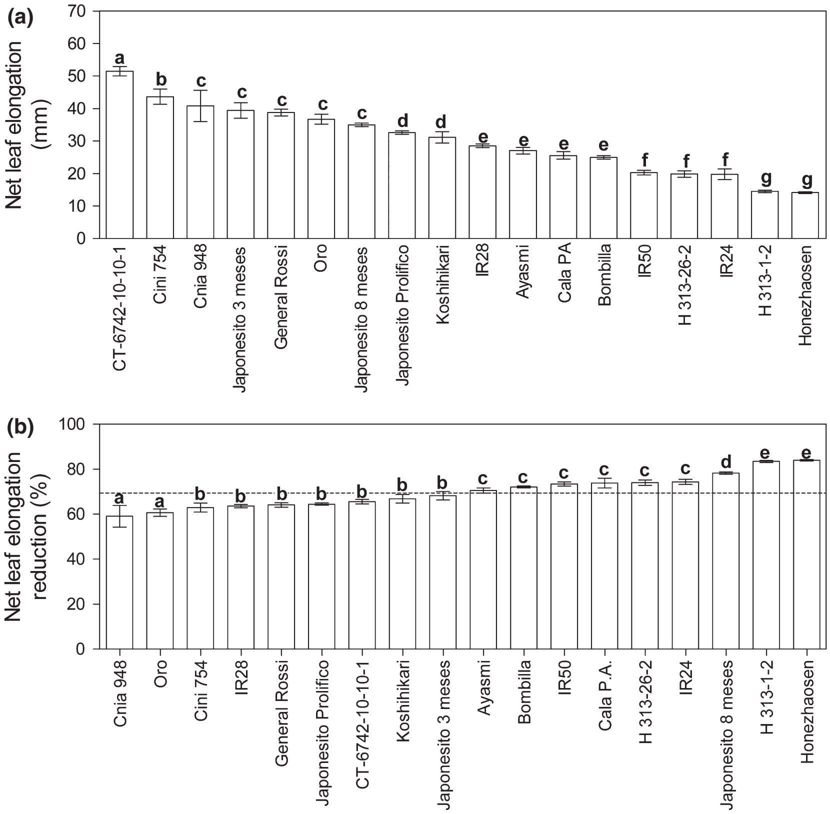

Fig. 1 Net leaf elongation in rice cultivars. Seedlings were grown hydroponically under suboptimal temperatures (ST) and optimal temperatures in short-term laboratory assays. Net leaf elongation of the third leaf was determined by the analysis of digitalized images of shoots. (a) Net leaf elongation under ST. (b) Percentage of net leaf elongation reduction of plant grown under ST with respect to that elongation in plant grown under OT. Dashed lines represent the mean of percentage of net leaf elongation reduction of all cultivars. Bars with the same letter are not statistically different (DGC test; P < 0.01; data represents mean ± S.E.; n = 15).

Determination of gas exchange parameters

The gas exchange parameters, net photosynthesis rate (Pn), stomatal conductance (Gs) and internal CO_2 concentration (Ci) were measured in blades of expanding leaves at light saturation (1200 μmol photons m^{-2} s^{-1} illumination, LED light) at the same air temperature of each particular experimental conditions, using a portable photosynthesis system (TPS-2 Portable Photosynthesis System, MA, USA).

Determination of relative water content

The relative water content (RWC) was estimated on the leaf blade. For this purpose, fresh tissue was weighed and floated for 24 h to achieve turgidity and then oven-dried at 70 °C for 48 h. RWC was calculated from the equation:

$$RWC = [(\text{fresh weight} - \text{dry weight}) / \text{turgid weight - dry weight}] \times 100$$

Statistical analyses

Data were subjected to ANOVA and post hoc analyses, DGC test (Di Rienzo et al. 2002) and T test using the INFOSTAT statistical software package (InfoStat version 2010. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. URL http://www.infostat.com.ar).

Results

Screening of rice growth performance under ST and OT conditions

We carried out a screening aimed at detecting rice cultivars with contrasting growth performance under ST. For this purpose, a short-term, hydroponic culture was established at the laboratory, subjecting seedlings with their third leaf in expansion to ST or OT conditions. Results revealed variations among the 18 different cultivars regarding the net leaf elongation of seedling grown under ST during three days (Fig. 1a). The percentage of net leaf elongation reduction, related to that registered at OT, averaged 70 % for all cultivars (Fig. 1b). Cultivars Cnia 948, Oro, Cini 745, IR28, General Rossi, Japonesito Prolífico, CT-6742-10-10-1 and Koshihikari presented the lowest percentage of net leaf elongation, in that order, whereas the sequence with cultivars presenting the highest values of this parameter was Honezhaosen, H313-1-2, Japonesito 8 meses, IR24, H 313-26-2, Cala P.A. IR50 and Bombilla.

Afterwards, plants of eight cultivars representative of the two groups delimited by this average (above and below) were subjected to ST conditions in two long-term (24 d) experiments, performed either at the laboratory or outdoor. Results from the last experiments showed that rice cultivars varied their shoot height response to ST (Fig. 2a, b), being the pattern of this variation similar to that found for net leaf elongation values in the former experiment (Fig. 1): cultivars IR24, Honezhaosen and IR50 (hereafter, the L group) presented lower shoot heights and net leaf elongation than CT-6742-10-10-1, General Rossi, Bombilla, Koshihikari and Cnia 948 (hereafter, the H group).

Effect of ST treatment on PSII performance (JIP test)

We performed a laboratory experiment where F_V/F_M, RC/ABS, PI_{ABS}, RC/CS_0, ABS/RC and DI_0/RC parameters were evaluated at 10 and 24 days after temperature treatments were initiated. Results showed that cultivars of the L group presented F_V/F_M values equal or lower than 0.75 (Fig. 3a), with the exception of cultivar IR24 (which showed an optimum at day 10). In contrast, cultivars of the H group displayed F_V/F_M values mostly falling within the optimum range (0.75–0.85, according to Bolhàr-nordenkampf et al.

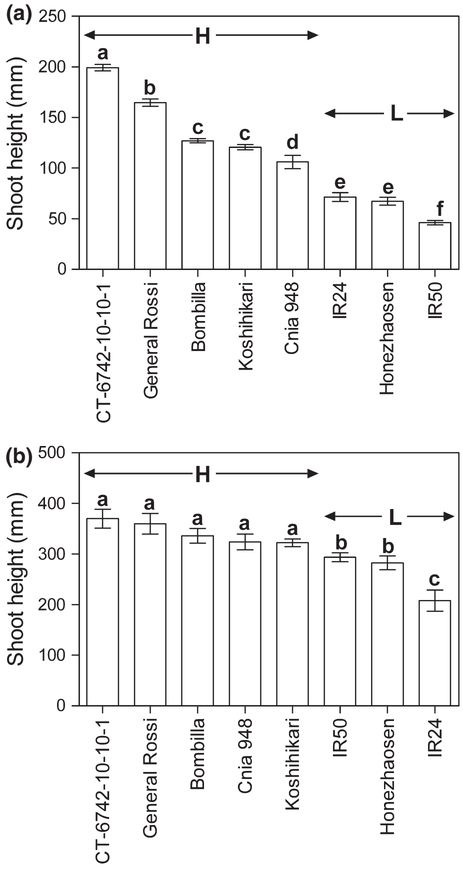

Fig. 2 Shoot height of selected rice cultivars. Seedlings were grown under (a) laboratory and (b) outdoor suboptimal temperatures conditions in long-term experiments. H: H group of cultivars; L: L group of cultivars. Bars with the same letter are not statistically different (DGC test; $P < 0.01$; data represents mean ± S.E.; n = 15).

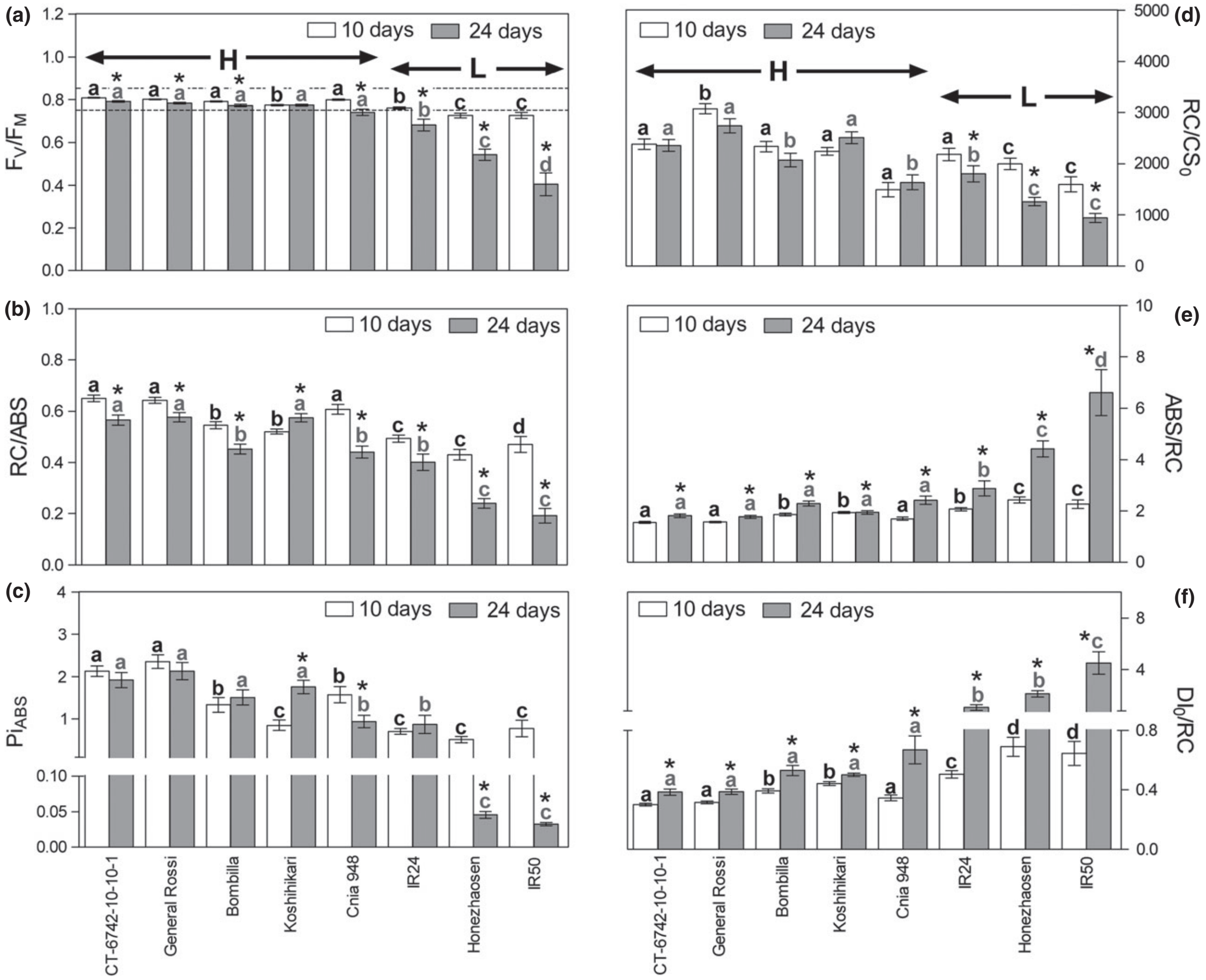

Fig. 3 JIP test in selected rice cultivars grown under suboptimal temperatures condition, in laboratory experiment. Seedlings were grown during 24 d. JIP test was performed with a portable chlorophyll fluorometer at the 10th and 24th days. The JIP analysed parameters were (a) F_V/F_M, (b) RC/ABS, (c) PI_{ABS}, (d) RC/CS_0, (e) ABS/RC and (f) DI_0/RC. Dashed lines in A represent the maximum (0.85) and minimum (0.75) F_V/F_M optimum values. H: H group of cultivars; L: L group of cultivars. Bars (mean ± S.E.; n = 15) with the same letter are not statistically different (DGC test; $P < 0.01$). White and grey letters correspond to 10th and 24th days, respectively. Asterisks represent significant differences between the sampling days (T test; $P < 0.01$).

1989) until the end of the experiment (excepting the slight decrease observed in cultivar Cnia 948 at day 24). Our results revealed that the RC/ABS decreased overtime for all cultivars excepting Koshihikari, for which an increase of this ratio was found (Fig. 3b). The performance index PI_{ABS} combines structural and functional criteria of the PSII, so it is a good vital index (Moreno et al. 2008). The PI_{ABS} decreased over time for cultivars Cnia 948, Honezhaosen and IR50, whereas it increased in Koshihikari and did not change in the remaining cultivars (Fig. 3c). On other hand, all cultivars from the L group diminished their RC/CS_0 over time (Fig. 3d), whereas cultivars from the H group did not change this ratio, indicating that RCs inactivation may have occurred in the first, but not in the second cultivar group. Under ST stress, all cultivars increased their ABS/RC ratio over time (Fig. 3e). However, these increments tended to be lower in the H group, compared with those of the L group. The ABS/RC is dependent on the ratio of active to non-active reaction centres and representative of the average antenna size (Strasser et al. 2000). Such results, along with those of the RC/CS_0 ratio (Fig. 3d) would indicate an inactivation of some RCs in the L group. Likewise, the DI_0/RC ratio, which is indicative of the amount of energy absorbed that is not trapped by the RC, but dissipated, tended to increase in both cultivar groups, although the magnitude of the increment was higher in the L than in the H group (Fig. 3f). Next, the F_V/F_M, Chl content, RC/ABS, PI_{ABS}, RC/CS_0, ABS/RC and DI_0/RC were measured on these same cultivars subjected to ST, in an outdoor, long-term experiment, at final time (day 24, Fig. 4). Again, F_V/F_M values were optimal for group H and suboptimal for group L (Fig. 4a). Besides, group H showed higher RC/ABS and PI_{ABS} values than group L (Fig. 4b,c). Inversely, the ABS/RC and DI_0/RC values in the H group of cultivars were lower than those of the L group (Fig. 4e,f), whereas both cultivars groups could not be separated by their RC/CS_0 (Fig. 4d). Interestingly, high and significant correlation coefficients were found between values obtained at the laboratory and outdoor experiments for growth data and for most of the above measured JIP parameters (Table 1).

In parallel, Chl content did not showed differences among cultivars in this outdoor assay (Figure S3).

Influence of ST treatment on gas exchange parameters

Gas exchange analyses were performed in an outdoor experiment, on seedlings of the same cultivars as above. Our results showed that Gs and Pn differently varied among cultivars: cultivars from to the L group presented significantly higher Gs values than those of the H group (Fig. 5a), whereas both cultivars groups could not be separated by their Pn (parameters in a Fig. 5b), Ci and RWC (Figure S4). An additional laboratory, long-term assay was

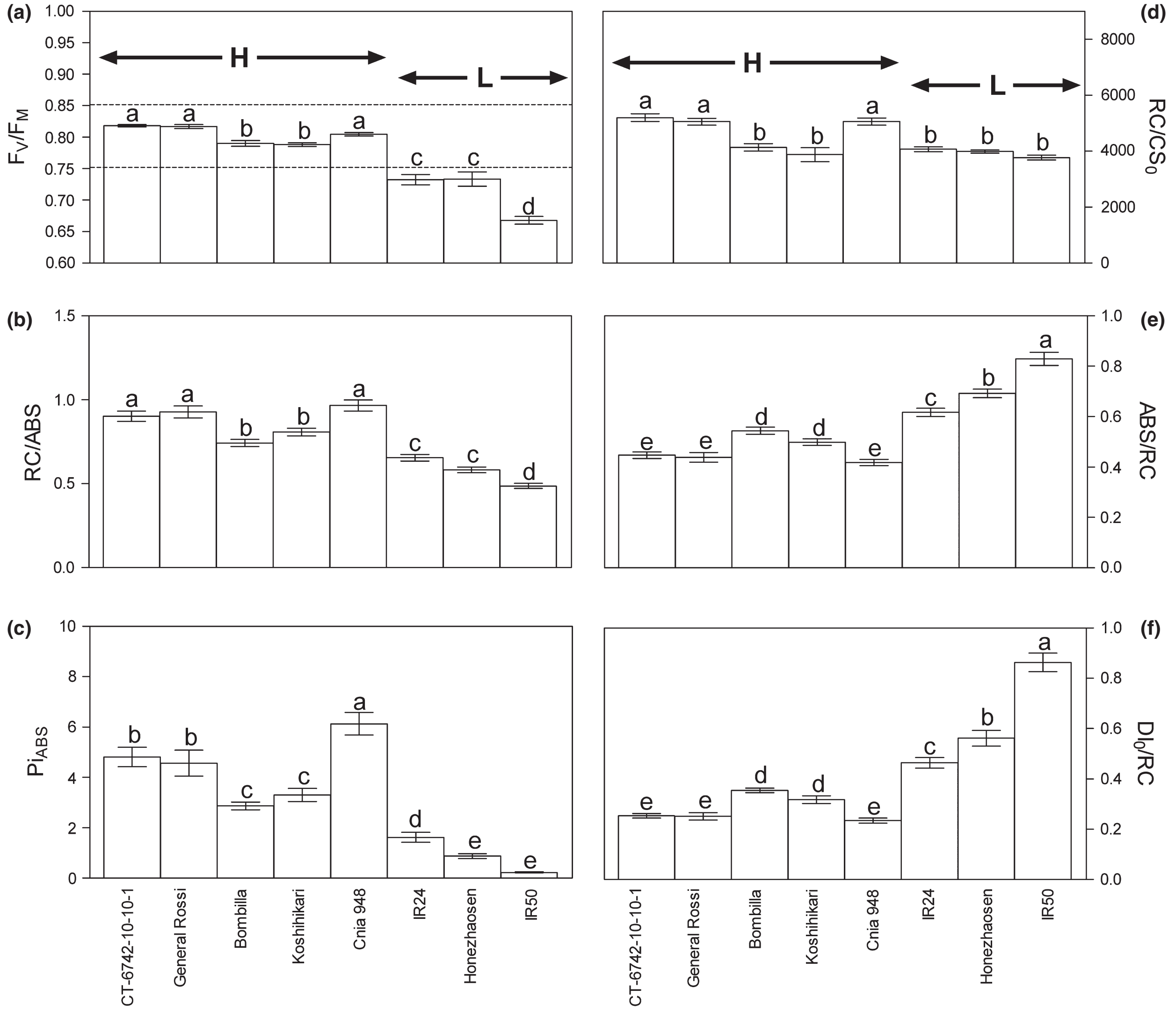

Fig. 4 JIP test in selected cultivars under suboptimal temperatures condition, in outdoor experiment. JIP test was performed with portable chlorophyll fluorometer at the end of the assay (24 d). The JIP analysed parameters were (a) F_V/F_M, (b) RC/ABS, (c) PI_{ABS}, (d) RC/CS_0, (e) ABS/RC and (f) DI_0/RC. Dashed lines in A represent the maximum (0.85) and minimum (0.75) F_V/F_M optimum values. H: H group of cultivars; L: L group of cultivars. Bars with the same letter are not statistically different (DGC test; $P < 0.01$; data represents mean ± S.E.; n = 15).

Table 1 Correlation between values obtained at laboratory and outdoor experiments for shoot height and JIP parameters

Parameter	Pearson R	P value
Shoot height	0.78	0.0160
F_V/F_M	0.94	0.0005
RC/ABS	0.86	0.0160
PI_{ABS}	0.70	0.0350
RC/CS_0	0.49	0.2210
ABS/RC	0.94	0.0006
DI_0/RC	0.97	<0.0001

performed to describe gas exchange ST range. Seedlings of groups H and L were grown under OT during 24 days. At the 25th day, when the light turned on, the temperature was set at 24 °C during 2 h and then diminished gradually during 3 h to 11.5 °C. Our results showed that Gs tended to diminish when the temperature was lowered, and again, cultivars of the H group had lower Gs values than those of the L group, in the whole temperature range (Fig. 6a). On other hand, Pn values also tended to decrease in all cultivars with temperature diminution and, as in the outdoor assay, groups H and L could not be clearly distinguished (Fig. 6b).

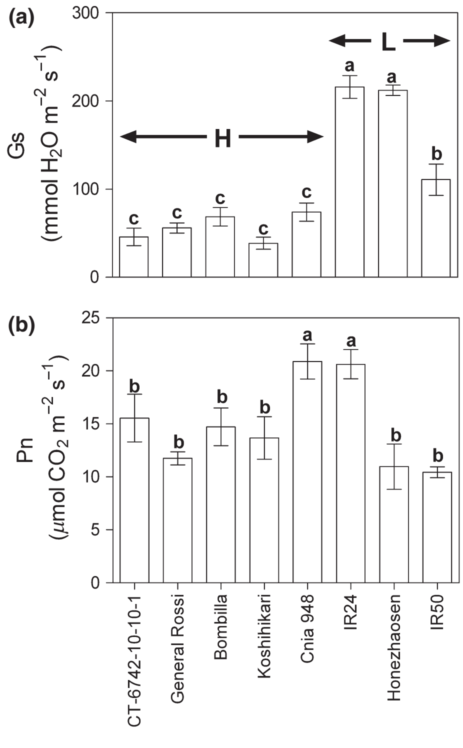

Fig. 5 Gas exchange parameters in selected rice cultivars grown under suboptimal temperatures condition, in outdoor experiment. Gs (a) and Pn (b) were determined with a portable photosynthesis system at the end of assay (24 d). H: H group of cultivars; L: L group of cultivars. Bars with the same letter are not statistically different (DGC test; P < 0.01; data represents mean ± S.E.; n = 15).

Finally, we calculated the water use efficiency (WUE_i = Pn/Gs, Ripullone et al. 2004) in our rice plants from values obtained in former outdoor and laboratory assays. Results from the outdoor assay indicated that cultivars from the H group presented higher WUE_i values than those of the L group, which is related to the fact that they had a reduced Gs, compared with the L cultivar group (Fig. 7a). Accordingly, the laboratory assay showed that the WUE_i was raised by temperatures below 20 °C, but only in cultivars of the H group (Fig. 7b).

Discussion

One of the aims of the present work was to detect rice cultivars with contrasting performance under a ST range. Our results showing that the extent of the ST-induced growth

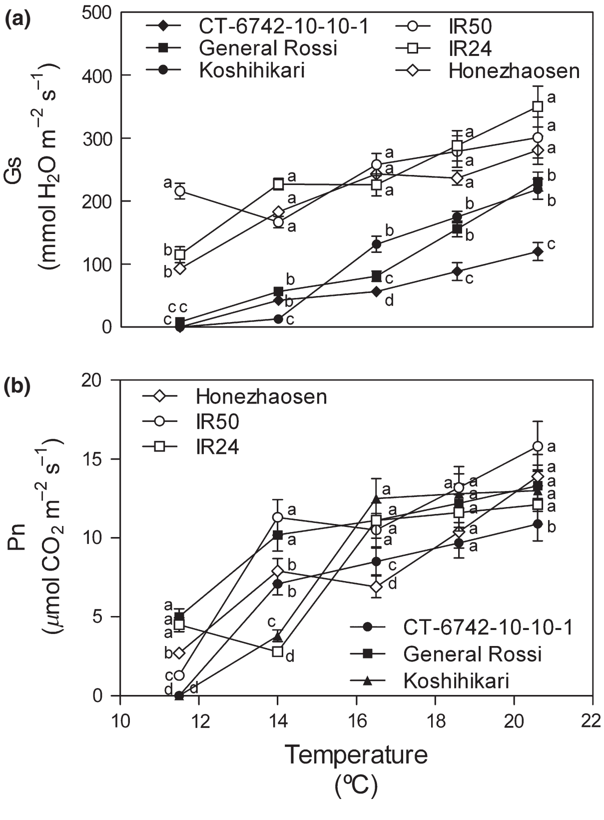

Fig. 6 Gas exchange parameters in selected rice cultivars grown under a suboptimal temperatures range, in a laboratory experiment. Seedlings were grown during 24 d under optimal temperatures condition. At day 25, the temperature of the growth chamber was diminished gradually during 3 h from 24 °C to 11.5 °C. Gs (a) and Pn (b) were determined in the 21–11.5 °C range. Different letters represent significant differences among cultivars at each temperature (DGC test; P < 0.01; data represents mean ± S.E.; n = 6).

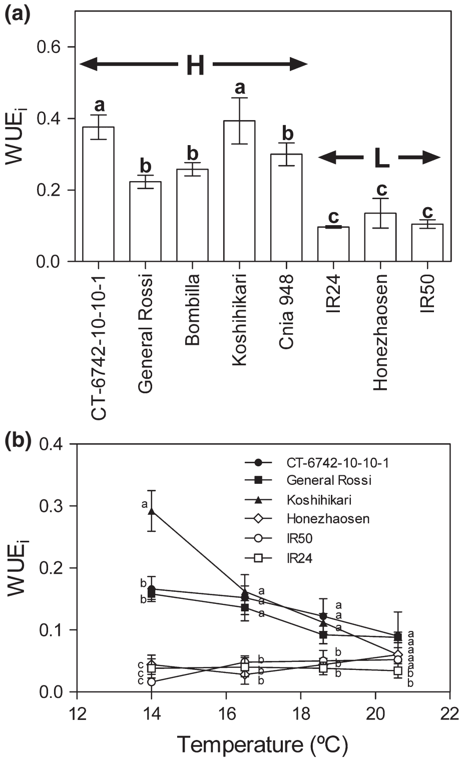

Fig. 7 Instantaneous water use efficiency in selected rice cultivars grown under laboratory and outdoor suboptimal temperatures at long-term experiments. WUE$_i$ was calculated from Gs and Pn data (Figs 5 and 6). H: H group of cultivars; L: L group of cultivars. Bars with the same letter are not statistically different in (a). Different letters represent significant differences among cultivars at each temperature in (b) (DGC test; $P < 0.01$; data represents mean ± S.E.; n = 15 and 6 for (a) and (b), respectively).

reduction was lower in the H than in the L group of cultivars led us to consider these groups as tolerant and sensitive, respectively. This view finds support in most of the JIP parameters measured in both laboratory and outdoor experiments, which suggested a higher ST-derived detrimental effect on the PSII functionality in the L, compared with the H group of cultivars. On one hand, the outcome of measurements from the laboratory experiment indicated that the PSII performance of the L group of cultivars, in contrast with those of the H group, tended to diminish from days 10 to 24 when treated with ST. On the other hand, results on F_V/F_M, Chl content, RC/ABS, PI_{ABS}, RC/CS_0, ABS/RC and DI_0/RC, obtained in the outdoor experiment, indicated that the amount of active chlorophyll diminished with temperature decrease. Besides the fact that such diminution was more obvious in the L group contributes at explaining its lower tolerance to ST.

Our F_V/F_M results from laboratory and outdoor experiments are in line with previous works in chilled rice reporting higher F_V/F_M values in tolerant than in sensitive cultivars (Guo-li and Zhen-fei 2005, Li et al. 2010, Bonnecarrère et al. 2011), although in contrast with other reports indicating that F_V/F_M is frequently insensitive to chilling (Van Heerden et al. 2003). Also at difference with our results, diminutions in the Chl content in sensitive, but not in tolerant rice cultivar grown under chilling stress, were previously reported (Li et al. 2010, Kim et al. 2012). Such apparent contradictions between own and others results could be a reflection of the different physiological nature of chilling and ST constrains.

Results from the outdoor and laboratory experiments here obtained showed that the two groups of cultivars (L and H) could be discerned on the base of their F_V/F_M, RC/ABS, PI_{ABS}, ABS/RC and DI_0/RC. Interestingly, cultivars ranked in a very similar way for each measured parameter (Figs 3 and 4), what stimulated us to propose that laboratory measurements of these parameters would be a valid method to predict the response of rice cultivars to ST in the field. In this regard, the positive and significant coefficients found in a correlation analyses between laboratory and outdoor results suggested that this method might be valid. Likewise, both groups of cultivars could be clearly discriminated by their Gs but not Pn, Ci and RWC profiles. The fact that in the outdoor assay, cultivars from the H group showed higher WUE$_i$ values than those of the L group suggests that WUE$_i$ adjustment through Gs reduction could be part of the mechanisms contributing in these cultivars to growth sustainment during ST constrain.

In parallel, the Gs results here obtained are in line with a report showing that stomata of chilling-sensitive maize cultivars remain open, while those of chilling-tolerant cultivars close under chilling stress (Aroca et al. 2003). A similar phenomenon was observed in chilling sensitive and tolerant tomato cultivars subjected to chilling stress (Bloom et al. 2004). Last reports are congruent with the notion that low temperature provokes leaf dehydration in sensitive plants (Pardossi et al.1992, Janowiak and Markowski 1994, Aroca et al. 2011). Therefore, it is possible that in cultivars of the H group, a higher ability to reduce Gs may have led to improved water balance and lower sensitivity under the ST conditions. Unfortunately, there are relatively few reports on the effect of low temperature on Gs and Pn in rice, and these are specific of chilling stress (e.g.: Li et al. 2010, Aghaee et al. 2011, Hassibi et al. 2011), preventing us from further discussion of our results.

Screening methods frequently identified japonica and indica cultivars, respectively, as tolerant and susceptible to cold (Mackill and Lei 1997). Our results suggest that japonica cultivars also have a better performance under the ST constraint than indica ones. Thus, Koshihikari and Bombilla, described as japonica cultivars (Takeuchi et al.2001, Giarrocco et al. 2007), were included within the group of cultivars most tolerant to ST, whereas IR50 and IR24, typical indica cultivars (Andaya and Mackill 2003), which were regarded as sensitive to cold stress (Ghosh and Singh 1983, Andaya and Mackill 2003, Guo-li and Zhen-fei 2005, Kim and Tai 2011, Kim et al. 2012) were among the most sensitive to ST.

Koshihikari is the leading variety in Japan (40 %; Uehara 2003) and it is used in breeding as donor of cold tolerance trait (Ashikari et al. 2007). Interestingly, we detected at least two cultivars, CT-6742-10-10-1 and General Rossi, with superior tolerance than Koshihikari to the ST condition, which could be used as parents or tolerance donors in breeding for new crop varieties. We also propose that these cultivars, along with the more sensitive cultivars here studied (e.g.: Honezhaosen or IR50) could also be useful for further studies orientated to elucidate the physiological mechanisms leading to improved growth under ST conditions.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Sketch of the laboratory and outdoor experimental setups.

Figure S2. Environmental PAR and air temperature of the outdoor experiment. Graphic represents the PAR and the air temperature registered at the 12th to the 24th day of the experiment. Dotted, solid and dashed lines represent the average of PAR, maximum and minimum temperatures respectively.

Figure S3. Chl content in selected rice cultivars under ST in outdoor experiment. Chl content was determined with a portable chlorophyll spectrophotometer at the end of the assay (24 days). H: H group of cultivars; L: L group of cultivars. Bars with the same letter are not statistically different (DGC test; $P < 0.01$; data represent mean ± SE; $n = 15$).

Figure S4. Intercellular CO_2 concentration and relative water content in selected rice cultivars grown under ST condition, in outdoor experiment. Ci (A) and RWC (B) were determined at the end of assay (24 days). H: H group of cultivars; L: L group of cultivars. Bars with the same letter are not statistically different (DGC test; $P < 0.01$; data represents mean ± SE; $n = 15$).