Impact of Supra-Optimal Temperatures on Physiology and Yield in Rice Field

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Abstract
Heat stress is an increasing constraint for the productivity of rice (Oryza sativa L.) worldwide. In this context, a study was carried out to quantify the supra-optimal temperature effects on rice yield-components and secondly to investigate its effects on plant physiological attributes when different genetic backgrounds are submitted to contrasting environment air-temperatures during the anthesis, the most sensitive growth phase to heat stress. Three Embrapa's cultivars were used, BRS Pampa, BRS Sinuelo CL and IAS 12-9 Formosa which represent indica/japonica, indica and essentially japonica subspecies, respectively. One day before anthesis phase, subplot plants were submitted to heat stress via polythene shelters for 96 h. Photosynthesis and respiration parameters were measured at 24 and 48 h after stress, and at physiological maturity, grain carbon isotope fractionation as well as yield components and grain yield were quantified. There were significant differences among genotypes for some gas exchange parameters at ambient and under increased temperatures at 24 and 48 h after stress, such as carbon assimilation and respiration rate. Heat-stress also affected yield components, especially for BRS Sinuelo CL showing the highest spikelet sterility (54%) while BRS Pampa had the lowest value (20.80%) and the highest 1000-grain weight and grain yield. These results demonstrate that although heat tolerance has been more frequently found in indica spp, this trait can also be present in genotypes combining indica/japonica genetic background, as shown by BRS Pampa cultivar.

Keywords: Oryza Sativa L., carbon isotope discrimination, high-temperature, heat tolerance, rice gas exchange

1. Introduction
Abiotic and biotic stresses represent the major constraints that result in agricultural losses on the global scale and projected climate changes could increase their negative effects in the future (Allen et al., 2010; Brito, Soifiatti, Lima, Carvalho, & Silva-Filho, 2011; De Brito et al., 2010; Diola, Brito, Caixeta, Pereira, & Loureiro, 2013; Diola et al., 2011; Fang, Cammarano, Zhou, Tan, & Ren, 2015; Marengo & Camargo, 2008; Weber et al., 2014). There are differences in the magnitude and uncertainties still persist among the various climate models published and agricultural implications of such extreme climate changes over the twenty-first century.

However, historical climatic data show that global temperatures rose by about 0.5 °C between the preindustrial period and 1980-1999, and current climate projections encompass a range of 1.6-6.9 °C for the end of the twenty-first century, relative to the preindustrial period (Sanderson, Hemming, & Betts, 2010). In Southern Brazil, the Rio Grande do Sul state has a major rice production area, with 1.2 million ha (70% of rice production Brazil) (Conab, 2015) the average minimum temperature has also increased in the last 40 years, largely due to the greater increase in nighttime temperatures rather than daytime temperatures (Marengo & Camargo, 2008). Additionally, the drastic changes in temperature in recent years have resulted in more frequent occurrence of extreme-weather events such as heat waves during the initial reproductive phase of rice, when optimal temperatures at booting and flowering phases should be situated between 25-28 °C and 30-32 °C, respectively (Yoshida, 1981).
In South region of Brazil, heat wave events generally coincide with the critical phases of rice development, decreasing crop yield. Although almost all growth phases are affected by high temperature, flowering (anthesis/fertilization) is the most sensitive phenological phase to heat stress. In this phase, high temperature affects anther dehiscence, pollination and pollen germination, increasing spikelet sterility and consequently leading to yield reduction of rice grains (Jagadish, Crauford, & Wheeler, 2007; Yoshida, 1981). According to these authors, the exposure, even for a short time (less than one hour), can lead to an increase in rice spikelet sterility. Additionally, supra-optimal temperatures can affect photosynthesis, since the fixation of CO₂ via rubisco enzyme and regeneration of the ribulose-1-5-bisphosphate (RuBP) through the Calvin Cycle are highly influenced by temperature variation. Limitations for RuBP regeneration are generally associated with the membrane-bound reactions closely to electron transport activity, ATP and NADPH production and Calvin Cycle enzymes, which are responsible for RuBP regeneration (Scafaro, Haynes, & Atwell, 2010).

Considering that stability of complex membrane and rubisco enzyme can be affected by temperature variations, photosynthesis reactions are significantly constrained at supra-optimal temperatures (Scafaro et al., 2010). In this context, supra-optimal temperatures have caused a decrease in rice yield in different tropical and subtropical regions, such as in India, Pakistan, Australia, Bangladesh, Thailand, China, Japan and United States (Das, Krishnan, Nayak, & Ramakrishnan, 2014; Guan-fu et al., 2015; Manigbas, Lambio, Madrid, & Cardenas, 2014; Matsushima, Ikwada, Maeda, Honda, & Niki, 1982; Osada, Sasiprap, Rahong, Dhammanuvong, & Chakrabandho, 1973; Tian, Luo, Zhou, & Wu, 2009). Nevertheless, in Brazil, the biggest rice grower outside Asia, rice breeding programs have made no efforts to quantify the yield losses caused by heat stress and/or to establish physiological phenotyping procedures to identify superior genotypes suitable as parents for crossings (personal communications).

The objective of the study was to quantify the effects of high-temperature on different genetic background of rice relative to grain yield and its physiological responses, as a bottom line for orchestration of strategies that could be adopted by Brazilian breeding programs to develop cultivars with increased heat tolerance in a climate change scenarios.

2. Material and Methods

2.1 Genotypes, Edaphoclimatic Conditions and Experimental Design

Three Embrapa’s cultivars were used (BRS Pampa, BRS Sinuelo CL and IAS 12-9 Formosa) representing genetic background of subspecies of indica/japonica, indica and essentially japonica, respectively. The study was carry out under field conditions at Low-Land Embrapa Experimental Station (LLEES), located in Pelotas, RS, Brazil (31°46′19″S, 52°20′33″W), at 17 m altitude, a traditional region for rice production under flooding irrigated system. Before planting, this area was left fallow for three years. Soil chemical parameters and climatic data are shown in Table 1 and Figure 1, respectively.

Table 1. Soil chemical characteristics from 0-20 cm of the soil profile in lowland Experimental Station (LLEES), Embrapa Temperate Climate, Pelotas, RS, Brazil

<table>
<thead>
<tr>
<th>LLEES</th>
<th>M.O.</th>
<th>pH</th>
<th>P*</th>
<th>K+</th>
<th>Ca2+</th>
<th>Mg2+</th>
<th>Al3+</th>
<th>CTC</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample†</td>
<td>%</td>
<td>water</td>
<td>-------mg/dm³-------</td>
<td>---------------cmol/dm³---------</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31141</td>
<td>1.1</td>
<td>5.7</td>
<td>24.7</td>
<td>91</td>
<td>2.1</td>
<td>1.4</td>
<td>0.0</td>
<td>5.5</td>
<td>67</td>
</tr>
<tr>
<td>31142</td>
<td>0.8</td>
<td>5.6</td>
<td>25.7</td>
<td>83</td>
<td>1.9</td>
<td>1.3</td>
<td>0.1</td>
<td>5.8</td>
<td>59</td>
</tr>
<tr>
<td>31143</td>
<td>0.9</td>
<td>5.8</td>
<td>21.7</td>
<td>68</td>
<td>1.8</td>
<td>1.2</td>
<td>0.0</td>
<td>5.2</td>
<td>62</td>
</tr>
</tbody>
</table>

Note. *P was extracted using Mellish solution and pH was measured in water. †Three samples replicates were analyzed.
To investigate the effects of heat stress on yield-components and physiological attributes on different genetic backgrounds, the study was conducted by sowing these genotypes on October 17th, 2014, (best sowing time for rice sowing in Brazilian south region) in a split plot on a randomized complete block design with four replicates, being sub plot constituted by six rows spaced at 0.175 m from each other and measuring 2.5 m long content 40 plants m density. The management practices and fertilization were conducted according to technical recommendations for the crop (Sosbai, 2014). Starter fertilizer was 15 kg of N ha\(^{-1}\), 60 kg of P\(_2\)O\(_5\) ha\(^{-1}\), 60 kg of K\(_2\)O ha\(^{-1}\) bank-applied adjacent to each row at planting. The additional nitrogen was 130 kg of N ha\(^{-1}\) applied at V4 growth stage (tillering initiation) and 70 kg of N ha\(^{-1}\) at R1 growth stage (panicle differentiation) (Counce, Keisling, & Mitchel, 2000).

Three cultivars were tested in the main plots and two temperature levels were tested under sub plots, which were imposed via use of polythene field-shelters constructed specifically for this purpose. These structures are made of 150 micron clear transparent plastic film for greenhouses sustained by PVC tubes; each shelter had 2.5 m length, 1.1 m width and 1.4 m height, with an opening on the upper surface as shown in the Figure 1D.

For ambient and within polythene shelter structure, the day-night temperature intensity inside and outside polythene sheets were recorded at each five minutes during this period using a Hobbo station with Pendant temp/light (Onset Computer Corporation, Bourne, MA, USA). Hobbo solar radiation shield were used inside and outside of polythene sheets to protect the data loggers and sensor of the direct or reflected solar radiation and allowing maximum air flow around the sensor avoiding an overestimate of registered air temperature during the crop season duration.
2.2 Phenological Phases Monitoring and Physiological Measurements

After emergence, ten plants of each cultivar per plot were targeted using a wood cutting to monitor the phenological phases according to Counce’s scale (Counce et al., 2000); this procedure was crucial to determine the exact phase to induce heat stress. At anthesis stage of the plants (R4 growth stage), plants were covered using a shelter per subplot.

At 24 and 48 h after stress, the net assimilation rate \((P_n)\), stomatal conductance \((g_s)\), transpiration rate \((E)\) and \(C_i/C_a\) were measured between 12 and 14 h under artificial photosynthetic photon flux (PPF) \((1,100 \, \mu\text{mol m}^{-2} \, \text{s}^{-1})\) using a portable photosynthesis system infrared gas analyzer (LI-COR 6400XT, LI-COR Biosciences, Lincoln, NE, USA). For measurements, a CO₂ cartridge was used and a 6400-01 CO₂ Injector System controlled the CO₂ partial pressure entering the cuvette \((C_a, \mu\text{bar})\).

For all subplots treatments, measurements were started on flag expanded leaf on three different plants \((n = 3)\). The leaves used for gas exchange measurements were tagged to use the same leaves in all analyses. The mid to distal portion of each leaf blade was inserted into the leaf chamber for gas-exchange measurements. For respiration measurements, a leaf was acclimated in dark for 40 min after gas exchange analyses avoiding any light-enhanced dark respiration. Then, all respiration procedures were done using an LI-6400 gas exchange system (LI-COR 6400XT, Biosciences, Lincoln, NE, USA); the same leaf used for gas exchange measurements was also inserted in the closed respiration chamber for respiration analysis.

2.3 Carbon Isotope Fractionation and Yield-Components Determinations

At physiological maturity, to determine whether carbon isotope discrimination (CID) could be affected by high-temperature as result of different genetic backgrounds used in this study, ten panicles per subplot were randomly harvested. Subsequently, the grains were oven-dried and ground to a fine powder for analysis of carbon isotope composition \((\delta^{13}C)\) and CID. For this purpose, the analysis of \(\delta^{13}C\) was carry out using a mass spectrometer (Delta-S Finnigan Mat, Bremen, Germany) at Biosciences Institute in the Universidade Estadual de São Paulo (Unesp), Botucatu, São Paulo, Brazil. The atmospheric isotopic composition was considered to be -8‰ in relation to the international standard, Pee Dee Belemnite (PDB). A second standard calibrated against a fossil belemnite from the Pee Dee formation was used for comparison.

The CID values were calculated according to Farquhar and co-workers (Farquhar, Ehleringer, & Hubick, 1989); where \(\delta a\) refers to the atmospheric isotopic composition and \(\delta p\) refers to the plant isotopic composition:

\[
\Delta^{13}C = \frac{\delta a - \delta b}{1 + \frac{\delta p}{1000}}
\]

2.4 Statistical Procedures

The homogeneity of variances was tested by the Bartlett test and Normality of data via Shapiro-Wilk test; subsequently data were subjected to analysis of variance (ANOVA) (SigmaPlot 13.0). The Least Significant Difference (LSD) among the means was statistically analyzed using Student-Newman-Keuls method \((p \leq 0.05)\). Additionally, when interaction effects were significant, unfold statistical procedures were done aiming to quantify the effects of treatments within each ambient temperature and comparisons for all genetic background within each temperature regime (ambient and under shelter).

3. Results

According to the historic weather record, from years 1971-2000 (http://agromet.cpact.embrapa.br/estacao/mensal.html, Figure 1A), the hottest period at Low-Land Embrapa Experimental Station (LLEES), at 17 m altitude, is from December to February. Historic weather data show an increase in the frequency of absolute maximum temperature higher than 32 °C, during the hottest months (December up to February), which are above the optimal threshold for rice flowering (Yoshida, 1981). Thus, sowing period was adjusted to assure that the rice varieties would flower during the hottest months (mid-December to February) (Figures 1B and 1C). At ambient temperature, the maximum absolute temperature during stress was 37.5 °C whereas under shelters the maximum values reached 42.8 °C. During stress, the mean maximum and minimum air temperatures reached 26.6 °C and 20.4 °C, respectively, for those plants maintained under ambient conditions, while for those submitted to heat-stress these means reached 36.8 °C and 21.8 °C, respectively.

In general, when compared to plants under ambient temperatures, gas exchange performance of treatments submitted to polythene shelters (Figure 1D) showed a photosynthesis decrease of 14.7% at 24 h, whereas non-significant differences were shown at 48h after (Figure 2A).
Figure 2. CO₂ assimilation rate – $P_n$ (A); stomatal conductance – $g_s$ (B), transpiration rate – $E$ (C); the ratio of internal to atmospheric CO₂ concentration – $C_i/C_a$ (D) and respiration rate – CO₂ (E) in rice genotypes submitted to supra-optimal temperatures for twenty-four hours.

Note. For black columns (ambient conditions) and for white crosshatched columns (submitted to supra-optimal temperatures) means followed by the same capital letter and by same lower case letters, respectively, are not significantly different at the $p < 0.05$ level by Newman-keuls test. *, ** and n.s. symbols mean significant differences at $p < 0.05$, $p < 0.01$ and non-significant, respectively, for a specific genotype growing at ambient conditions comparing its performance when submitted to supra-optimal temperatures. Values are means ± s.e. ($n = 4$).

The other gas exchange parameters $g_s$, $E$ and $C_i/C_a$ increased, at 24 h under heat-stress under shelters, by 36.0, 12.0 and 8.0%, respectively, for all cultivars except BRS Pampa, which maintained unchanged performance under the two temperature regimes (Figures 2B, 2C and 2D). Analysis of respiration rate indicated an increase of 16.0% at 24h after heat-stress (Figure 2E). At 48 h after stress, there were no significant differences for gas exchange parameters, except for BRS Pampa, that showed an increased transpiration ratio when submitted to heat-stress (Figure 3).
Figure 3. CO₂ assimilation rate – $P_n$ (A); stomatal conducance – $g_s$ (B); transpiration rate – $E$ (C); the ratio of internal to atmospheric CO₂ concentration – $C_i/C_a$ (D) and respiration rate – CO₂ (E) in rice genotypes submitted to supra-optimal temperatures for forty-eight hours.

Note. For black columns (ambient conditions) and for white crosshatched columns (submitted to supra-optimal temperatures) means followed by the same capital letter and by same lower case letters, respectively, are not significantly different at the $p < 0.05$ level by Newman-keuls test. n.s., * and ** symbols means non-significant, significant differences at $p < 0.05$ and $p < 0.01$, respectively, for a specific genotype growing at ambient conditions comparing its performance when submitted to supra-optimal temperatures. Values are means ± s.e. ($n = 4$).

For Carbon isotope discrimination, BRS Pampa and BRS Sinuelo CL showed higher values when compared to IAS 12-9 Formosa, under ambient temperature, whereas under heat-stress BRS Pampa showed the highest CID values and lower variation between ambient and heat temperature (Figure 4A). Relative to yield components, BRS Sinuelo CL showed the highest values for spikelet sterility regardless of temperature regime (Figure 4B). At heat-stress condition, this cultivar showed about 54.0% of spikelet sterility, whereas under ambient temperature this percentage was around 26.0%. For 1000-grains weight, BRS Pampa showed the highest values, independent of the treatment, and the lowest reduction at heat-stress when compared to other genotypes (Figure 4C).
Figure 4. Grain rice carbon isotope discrimination – CID (A); spikelet sterility – % (B); 1000-grain weight – g(C) and grain yield – g m⁻² (D)

Note. For black columns (ambient conditions) and for white crosshatched columns (submitted to supra-optimal temperatures) means followed by the same capital letter and by same lower case letters, respectively, are not significantly different at the p < 0.05 level by Newman-keuls test. n.s., * and ** symbols means non-significant, significant differences at p < 0.05 and p < 0.01, respectively, for a specific genotype growing at ambient conditions comparing its performance when submitted to supra-optimal temperatures. Values are means ± s.e. (n = 4).

In general, plants submitted to heat-stress presented a reduction in yield grain of 19.9%. In this way, compared to other cultivars, BRS Pampa showed the highest grain yield, independent of temperature regime; under ambient temperature grain yield increased about 52.0% when compared to other cultivars. Comparing each cultivar under different treatments, it was verified that grain yield of BRS Pampa, BRS Sinuelo CL and IAS 12-09 Formosa were reduced at 6.0%, 20.0% and 19.5%, respectively.

4. Discussion

Quantification of heat-stress effects on rice physiological and yield components and identification of genetic variability are important steps for breeding new varieties in those areas affected by high temperatures during critical development phases, especially at initial reproductive phase, when supra-optimal temperatures occurrence (≥ 32 °C) can affect rice plant performance (Yoshida, 1981). Concerning the physiological heat-stress effects, different authors found that photosynthesis and/or yield components decline with increase of supra-optimal temperatures level and time duration of stress imposing (Bahuguna, Jagadish, Coast, & Wassmann, 2014; Ishimaru et al., 2015; Jagadish et al., 2007; Šebela, Quiñones, Olejníčková, & Jagadish, 2015). In the present study, photosynthesis rates declined drastically, especially in the IAS 12-9 Formosa, an essentially japonica cultivar submitted to heat-stress, whereas in those genotypes having indica genetic background, rate decrease was less evident. Photosynthetic process has been reported as highly sensitive to heat, showing inhibition by stomatal and non-stomatal limitations (Higuchia, Sakuratania, & Utsunomiya, 1999). Although, the
negative effects of the supra-optimal temperatures on gas exchange variables were evident, decreasing the photosynthesis values; on the other hand, there was an increase of gs, E and ci:ca, indicating that photosynthesis reduction cannot be attributed to progressive decrease in the fluxes of CO2 concentration reaching the chloroplasts. In this sense, there are studies showing that photosynthesis declines at moderate heat-stress can result from a decrease in the activation state of rubisco, via a loss of carbamylation capacity due to changes in stromal pH and Mg2+ concentration (Fukayama et al., 2011; Hammond, Andrews, & Woodrow, 1998; Salvucci & Crafts-Brandner, 2004; Scafaro et al., 2010).

Therefore, temperature effects are generally attributed to variation of ion concentration in the stroma because of unspecified changes in the properties of the thylakoid membranes (Vani, Saradhi, & Mohanty, 2001; Weis, 1981). In recent years, advances have been done for better understanding of the biochemical cross-talk on Rubisco activation, including the involvement of Rubisco activase as an important integrant in this process. These advances have uncovered the impairment of Rubisco’s chaperone activase enzymes leading to the deactivation of Rubisco (DeRidder, Shybut, Dyle, Kremling, & Shapiro, 2012) and whether consider Rubisco chaperone activase as highly sensitive to thermal denaturation, even to short heat-wave periods. The occurrence of supra-optimal temperature events, as such imposed to rice genotypes used in this study, can contribute to justify the decreases on photosynthesis rate found in our report.

Enzymatic reactions generally increase with temperature until a physiological threshold, what can lead to an increase in the flux through metabolic pathways (Salvucci & Crafts-Brandner, 2004). The results indicate that IAS 12-9 Formosa showed the highest values for respiration rate, independently of the temperatures regime. However, in agreement with those results found for cotton (Salvucci & Crafts-Brandner, 2004), there was a decrease of photosynthesis relative to those rice plants submitted to heat-stress; while it was again more evident in the IAS 12-9 Formosa, a japonica cultivar.

In the last decade, there was an increase of carbon isotope discrimination (CID) use as a promising indirect selection or proxy for higher yield and for abiotic stress tolerance performance in plants (Brito et al., 2011, 2014a, 2014b; Farquhar et al., 1989). In this report, BRS Pampa showed highest CID values, independent of heat temperature conditions. This cultivar also showed the highest grain yield levels at ambient and under heat stress conditions. In other reports, a positive correlation between CID and productivity has been found (Araus et al., 1997; Brito et al., 2014a; Wu, Chang, & Jing, 2011). The relationship between higher CID and yield has been established by variation in assimilation-weighted gs (Farquhar et al., 1989). As proposed by other authors, our data show that the relationship in plants between CID, yield and heat tolerance is mediated by increases in gs and can be associated with decrease of leaf temperatures, as result of heat avoidance strategies (Percy, Lu, Radin, Turcotte, & Zeiger, 1996; Radin, Lu, Percy, & Zeiger, 1994). In this sense, BRS Pampa showed the highest values for CID, 1000-grain weight, grain yield and lower spikelet sterility values, independent of temperature regime imposed during initial reproductive phase.

In conclusion, this study indicates that the supra-optimal temperatures (under shelter – mean maximum air temperatures reaching 36.8 °C) during the initial flower phase, even during a short period (48 h) can lead to physiological changes, decreasing the photosynthetic performance and reducing yield components and grain productivity. Additionally, these results suggest that although heat tolerance has been more frequently found in indica spp, this trait can also be present in genotypes combining indica/japonica genetic background, as shown by BRS Pampa cultivar.

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