

The Effects of Night-time Temperatures on Physiological and Biochemical Traits in Rice

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Abstract

High nighttime temperatures impair rice yield. Additionally, heat stress periods have increased during the last years in the rice areas of the tropics. The aim of this study was to physiologically characterize six genotypes of rice (a commercial cultivar ('F60') and five selected lines ('IR 1561', 'FLO 2764', 'LV447-1', 'CT19021', and 'LV1401') subjected to two nighttime temperatures (24 and 30 °C), based on different physiological traits. When the collar formed on leaf 6 of the main stem, one group of six plants in each genotype was subjected to 30 °C from 18:00 to 24:00 hours for eight days, while the other group remained at 24 °C. Differences were found in the interaction between genotype and nighttime temperatures, where a high night temperature reduced leaf photosynthesis by approximately 50% in all genotypes compared to the controls (20 μmol vs. 10 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, respectively). In general, higher plant respiration was also observed in almost all genotypes when the plants were exposed to 30 °C. However, rice plants of the genotype 'F60' showed a constant respiration under two different night temperatures. A high nighttime temperature increased the electrolyte leakage and malondialdehyde content only in the 'LV1401' plants. Plant growth and F_v/F_m ratio were separately conditioned by the night temperature or the genotype factor. A lower total plant dry weight was found at 30 °C (620.36 mg) than in rice plants exposed to 24 °C (254.16 mg). The F_v/F_m ratio was slightly diminished at a high nighttime temperature. These results suggest that physiological variables, such as leaf photosynthesis, plant respiration, malondialdehyde content and leaf photosynthetic pigments, can be considered markers for characterizing tolerant genotypes in earlier growth phases during plant breeding programs.

Keywords: F_v/F_m ratio, leaf photosynthetic pigments, *Oryza sativa* L, oxidative stress, proline

Introduction

Global warming has generated adverse weather conditions, such as heavy and prolonged periods of drought and more frequent periods of high temperatures, in several regions of the world in recent years (FAO, 2002). In Colombia, climate change has led to an approximately 1 °C increase in the mean temperature during the last twenty years (Ballesteros and Enciso, 2012). Additionally, periods of high nighttime temperatures have reduced grain yields in the rice-growing areas of Colombia, thus reducing the crop productivity of one of the most economically important cereals during the last decade (FEDEARROZ, 2013).

High night temperatures (HNT) cause plant responses at the biochemical, physiological and/or genetic level. A high night temperature can reduce the leaf photosynthesis rate and increase plant respiration (Mohammed and Tarpley, 2010).

Earlier studies have shown that rice plants exposed to high night temperatures (35 °C) had low CO_2 assimilation due to lower PSII efficiency and ribulose 1-5 bisphosphate carboxylase/oxidase activity (Yin *et al.*, 2010). In rice, higher plant respiration was observed in the vegetative stage due to HNT (Kanno *et al.*, 2009).

On the other hand, plant acclimatization is also associated with genotype (Wahid *et al.*, 2007; Jha *et al.*, 2014). Several studies have shown that tolerant genotypes have a series of biochemical and physiological changes to cope with heat stress (Bita and Gerats, 2013; Kumar *et al.*, 2016). Kumar *et al.* (2012) stated that susceptible rice genotypes could show greater membrane damage, greater malondialdehyde and proline levels, and a loss of chlorophyll. Therefore, the use of different biochemical and physiological traits has been helpful to screen, and then select, genotypes that are tolerant to high night temperatures (Araus *et al.*, 2008).

The physiological, biochemical and molecular plant responses to heat stress are regulated by a cascade of events (Hasanuzzaman *et al.*, 2013). Knowledge of the physiological mechanisms associated with the interaction between genotype (G) and environment (E) has been one of the bottlenecks in breeding programs (Jha *et al.*, 2014). In this regard, rice seedling studies have become an important tool to understand, in depth, the physiological mechanisms involved in plant tolerance to heat stress conditions during the last years (Xue *et al.*, 2012; Sánchez-Reinoso *et al.*, 2014).

Laza *et al.* (2015) also stated that most studies during the vegetative phase have provided supporting evidence of how HNT might affect rice physiology and an understanding of the physiological mechanisms that are involved. Consequently, it is important to expand our knowledge of the G x E interaction by gaining a better understanding of the physiological and biochemical processes that are involved; this has allowed the use of different techniques to screen genotypes for the development of new, tolerant genotypes in Colombia (FEDÉARROZ, 2013). Therefore, our hypothesis was that high night temperatures would increase plant respiration and decrease photosynthesis rates, which would induce oxidative damage by increasing leaf MDA and proline levels as well as causing changes in leaf photosynthetic pigments, resulting in reduced plant growth in susceptible genotypes. Consequently, the objective of this study was to evaluate the use of a series of physiological and biochemical variables to screen the plant tolerance of six rice genotypes in two nighttime temperatures.

Materials and Methods

General growing conditions

An experiment was conducted under greenhouse and laboratory conditions located at the Faculty of Agricultural Sciences of the Universidad Nacional de Colombia, Bogotá. The growing conditions in the greenhouse were as follows: day/night temperature (30/24 °C), relative humidity (70%) and a natural photoperiod of 12 h. Rice seeds were sown in the superior portion of 450-mL plastic pots containing a loamy soil. After seed germination, seedlings were fertirrigated daily with 80 mL of a nutrient solution containing a complete fertilizer (Wuxal, Bayer CropScience, Bogotá D.C., Colombia) at a rate of 2 mL L⁻¹ H₂O. The fertilizer concentration was as follows: total nitrogen 160 g L⁻¹, (ammonia N, 38 g L⁻¹; nitric N, 12 g L⁻¹; urea N, 110 g L⁻¹), assimilable P (P₂O₅) 160 g L⁻¹; soluble K (K₂O) 120 g L⁻¹; 10 g B L⁻¹; 0.21 g Cu L⁻¹; 0.43 g Fe L⁻¹; 0.36 g Mn L⁻¹; 0.07 g Mo L⁻¹; 10 g Zn L⁻¹ (* Chelated with EDTA - pH of 10% 6.5; density at 25°C 1.40 g cm⁻³).

Treatments

A susceptible genotype ('F60') to high daytime temperatures (Sánchez-Reinoso *et al.*, 2014) and five genotypes used by the National Rice Growers Association in their breeding programs ('IR 1561', 'FLO 2764', 'LV447-1', 'CT19021', and 'LV1401') were evaluated. At 32 days after seed germination (DAG), when the rice plants showed collar formation on leaf 6 on the main stem (V6 stage), rice seedlings were split into two groups of six plants per genotype. The first group was always placed under greenhouse conditions (control plants). In the second group of plants, rice plants were moved into a growth chamber (MLR-351H, Sanyo, Bensenville, Illinois, USA) at

30 °C between 1800 and 2400 h (HNT plants). Then, plants were returned to the greenhouse conditions between 0000 and 1800 h for 8 days. In general, rice plants were exposed to nighttime temperature treatments for eight days because of the physiological and biochemical alterations that were observed in a previous experiment (Alvarado-Sanabria *et al.*, 2017). Finally, we used a night temperature of 24 °C as the control temperature because it is the average night temperature in many rice areas, while 30 °C is the most common night temperature registered during heat stress periods in Colombia.

Photosynthesis

A mature leaf from the main stem of each plant was used to determine leaf photosynthesis using a portable photosynthesis meter (LI-COR 6200, Lincoln, Nebraska, USA) between 37 and 40 DAG. During the photosynthesis measurements, the conditions in the instrument chamber were: Photosynthetic Active Radiation (PAR) greater than 800 μmol m⁻² s⁻¹, leaf temperature 27 ± 5 °C, vapor pressure deficit 1.8 ± 0.5 kPa. The readings were carried out between 0900 and 1200 h. Finally, six plants per treatment (temperature x genotype) were taken collected to determine the CO₂ assimilation rate by putting a rice leaf into the chamber of the instrument.

Plant respiration

The methodology described by Sánchez-Reinoso *et al.* (2014) was followed to estimate the total plant respiration in total darkness, either in greenhouse or chamber conditions. This method consisted of introducing plants in 2-L airtight plastic chambers. Subsequently, CO₂ infrared sensors (CO₂-BTA, Vernier, Beaverton, OR, USA) were inserted into the chambers and connected to a portable interface (LabQuest, Vernier, Beaverton, OR, USA). Before placing the plants in the sealed chambers, all pots were covered with a plastic wrap to prevent soil respiration, which allowed us to quantify the plant tissue respiration only. Then, the sensor estimated a CO₂ (mg L⁻¹) concentration in the chamber every 4 seconds (s) for 300 s, resulting in a linear trend line. Then, the linear regression slope (ppm s⁻¹) was determined to obtain the total plant respiration rate using the total plant fresh weight (mmol CO₂ kg FW⁻¹ h⁻¹). The plant respiration readings were performed between 2100 and 2400 h on the same dates and in the same plants that were used in the photosynthesis measurements.

Chlorophyll fluorescence parameters

F_v/F_m ratio measurements were estimated using an MINI-PAM modulated fluorometer (Heinz Walz, Effeltrich, Germany). F_v/F_m readings were recorded by adapting leaves to the dark using lightweight leaf clips for at least 20 min before obtaining the measurements. Chlorophyll fluorescence readings were determined with a maximum light intensity of up to 3000 μmol m⁻² s⁻¹ at the same date and time and in the same leaves that were used to estimate photosynthesis.

Growth parameters

Leaf area was determined using a leaf area meter LI-3000C (LI-COR Lincoln, Nebraska, USA) at 40 DAG. Then, plants were harvested and dried at 70°C for 48 h to determine the leaf and stem dry weights. Specific leaf mass (leaf dry weight/leaf area) and leaf/stem ratio (leaf mass/stem mass + leaf sheath per plant) were determined.

Electrolyte leakage (%)

At 40 DAG, 0.3 g of fresh leaves was cut into 1-cm long pieces and placed in test tubes with 30 mL of deionized water. Subsequently, the tubes were incubated in a water bath at 30°C for two h, and the first electrical conductivity (EC_1) was estimated. The samples were incubated again at 95°C in a water bath for 15 min to release all the electrolytes. The sample was allowed to cool, and the final electrical conductivity (EC_2) was recorded. The electrolyte percentage was calculated using the formula described by Sanchez-Reinoso *et al.* (2014):

$$\% \text{ Electrolytes} = \left(\frac{EC_1}{EC_2} \right) \times 100$$

Chlorophyll and carotenoid content

At 40 DAG, three 1-cm long pieces of veinless leaf blades (50 mg fresh weight) from a fully developed leaf were ground using liquid nitrogen and stored at -80°C until the respective analyses were made. Fifty mg of fresh leaves was macerated in a mortar with 5 mL of 80% acetone, and then the sample was centrifuged at 8000 rpm for 5 min. Then, the absorbance readings at wavelengths of 663 nm, 647 nm and 470 nm were recorded, and the chlorophyll a, b and carotenoids content (mg g⁻¹ FW) was determined based on Lichtenthaler (1987).

Proline and Malondialdehyde (MDA)

At 40 DAG, 300 mg of fully developed leaves from the main stem was ground with liquid nitrogen and stored at -80°C until analysis. Proline content was determined following the Bates *et al.* (1973) method, where 100 mg of plant tissue was taken and homogenized in 5 mL of 3% sulfosalicylic acid. Subsequently, the sample was centrifuged at 6000 rpm for 30 min. Then, 1 mL of the supernatant was mixed in a falcon tube with 1 mL of ninhydrin acid and 1 mL of glacial acetic acid. The above mixture was stirred for 1 min, and then it was subjected to a water bath at 98°C for one h. Then, the reaction was stopped with ice. Finally, the reaction mixture was extracted with 3 mL of toluene, and the tubes were vigorously agitated with a vortex mixer. The absorbance was measured at 520 nm using a spectrophotometer (Spectronic BioMate 3 UV - Vis, Thermo, Madison, WI, USA). The proline content was determined using a calibration curve and the following formula:

$$\frac{\mu\text{mol Proline}}{\text{fresh plant material}} = \frac{\left[\frac{(\mu\text{g Proline} \times \text{mL Toluene})}{\text{mL}} \right]}{\left[\frac{115.5 \mu\text{g}}{\mu\text{mol}} \right]} \times \left[\frac{\text{g sample}}{5} \right]$$

MDA content was determined following the Hodges *et al.* (1999) modified protocol, which was as follows: i) 100 mg of plant material was homogenized in 2 mL of 0.1% trichloroacetic acid, ii) the sample was centrifuged at 8000 rpm for 15 min, iii) 1 mL of supernatant was extracted and mixed with 2 mL of 20% trichloroacetic acid and 2 mL of thiobarbituric acid (+TBA) and, in another test tube, 1 mL of supernatant was mixed with 2 mL of 20% trichloroacetic acid (-TBA), iv) both tubes were stirred for one min and subjected to a water bath at 95°C for 30 min, v) the reaction was stopped on an ice bed, and the absorbance values were read at 440, 532 and 600 nm. The MDA content was determined as follows:

$$A = [Abs 532_{+TBA} - Abs 600_{+TBA} - (Abs 532_{-TBA} - Abs 600_{-TBA})]$$

$$B = [(Abs 440_{+TBA} - Abs 600_{+TBA}) * 0.0571]$$

$$\text{MDA equivalent (nmol mL}^{-1}\text{)} = (A - B / 157000) * 10^6$$

Experimental design and statistical analysis

A design in factorial arrangement was conducted with eight plants per treatment, where the primary factor was the rice genotypes ('F60', 'LV447', 'FLO2764', 'LV1401', 'CT19021', and 'IR1561') and the secondary factor was the night temperature (24 vs. 30 °C). When significant differences were present, a means comparison test of the treatments was performed using the Tukey test. Data were analyzed using SPSS software (v20.0, IBM Company, USA).

Results and Discussion*Photosynthesis, Carboxylation Efficiency and Intrinsic Water Use Efficiency (WUE_i)*

A high night temperature (30 °C) reduced leaf photosynthesis (P_n) in all the genotypes (Fig. 1). Overall, P_n was reduced by 35 to 40% in all rice genotypes at 30°C. Similar trends were found in the carboxylation efficiency and intrinsic water use efficiency (WUE_i) under a high nighttime temperature (HNT) (Fig. 1). WUE_i showed a greater drop (approximately 75%) in plants under HNT than the control ones. Finally, no differences were found in the internal CO₂ concentration (C_i) (Fig. 1). The adverse effects of HNT on leaf photosynthesis also has been reported in rice (Mohammed *et al.*, 2013) and sorghum (Prasad and Djanaguiraman, 2011). The decrease in P_n under HNT can be associated with a reduction in stomatal conductance (Mohammed *et al.*, 2013) and alterations in plant carbohydrate content because of the lower hexose and sucrose concentrations in heat-stressed leaves (Loka and Oosterhuis, 2016).

Plant respiration

Differences were also observed between night temperatures on total plant respiration in all rice genotypes (Fig. 2). In general, total plant respiration was higher when the night temperature increased, especially in the 'CT19021', 'FLO2764', 'IR1561', 'LV1401' and 'LV447' genotypes. In this regard, 'LV1401' plants showed the largest increase (approximately 75%) in the total plant respiration rate. Furthermore, 'F60' plants did not show changes in two different night temperatures, suggesting an acclimatization response in this genotype. Mohammed and Tarpley (2009) and Peraudeau *et al.* (2015a) also reported a 30% increase in night respiration during nighttime temperatures (≥ 27 °C). Plant respiration is the primary source of energy, fueling growth and maintenance processes in plants, but it can also be seen as a metabolic cost that limits carbon availability for crop productivity (Peraudeau *et al.*, 2015b). In this regard, a higher plant respiration rate in response to HNT is associated with greater carbohydrate consumption, suggesting a role in the demand for ATP or other respiratory products (Turnbull *et al.*, 2002; Peraudeau *et al.*, 2015b). In support of that observation, Loka and Oosterhuis (2016) reported that HNT increased leaf respiration, but they simultaneously observed that ATP levels were markedly decreased. On the other hand, Atkin and Tjoelker (2003) stated that there are two acclimation types of

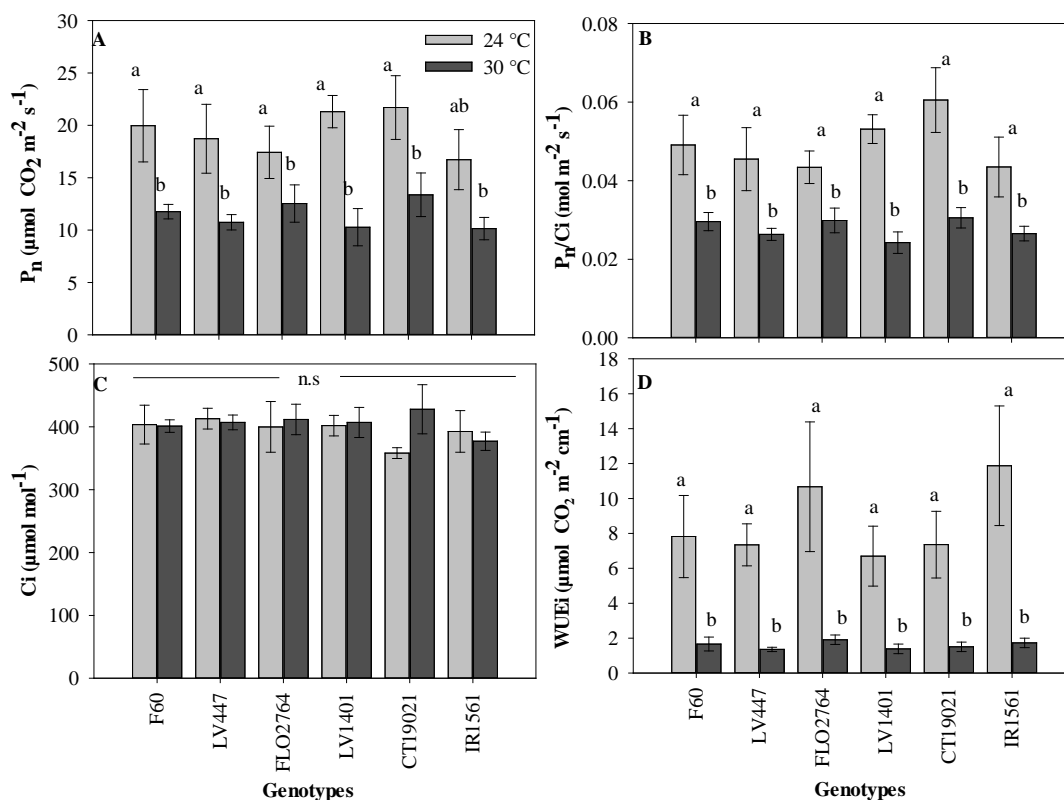


Fig. 1. Effect of two nighttime temperatures (24 vs. 30 °C) on leaf photosynthesis (A), carboxylation efficiency (P_n/C_i) (B), intercellular CO₂ (C_i) (C), and intrinsic water use efficiency (WUE_i) (D) in rice genotypes. Each bar chart represents the mean of 6 plants. Vertical bars represent ± standard error. Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's test

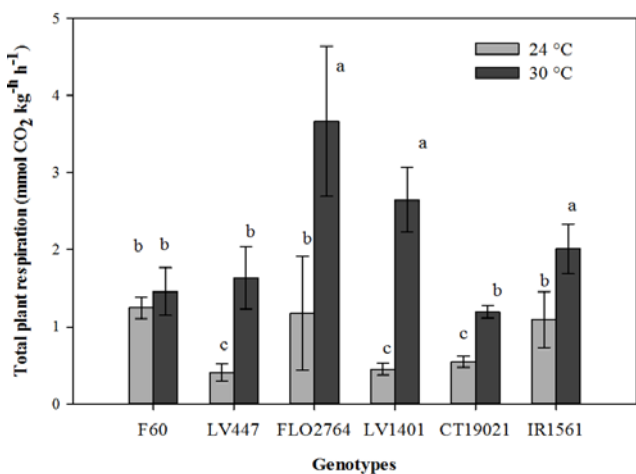


Fig. 2. Effect of two nighttime temperatures (24 vs. 30 °C) on total plant respiration in rice genotypes. Each bar chart represents the mean of 6 plants. Vertical bars represent ± standard error. Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's test

plant respiration to temperature. In Type I, plant respiration is constant at low temperatures, regardless of the growth temperature, and responds to higher temperatures with a lower Q₁₀ if the plants are acclimated to them. In Type II (homeostasis), plant respiration is lower in warm-acclimated plants, regardless of the temperature studied, and Q₁₀ does not necessarily change with the growth temperature. This could

explain the lack of variation in the plant respiration of the 'F60' plants. This genotype might have a Type II strategy under HNT, which suggests that it would have better availability of the redox state of its ubiquinone pool than the other genotypes (Atkin and Tjoelker, 2003).

Growth variables

Distinct differences were found in the leaves, stem, and total dry mass, leaf area and leaf/stem ratio ($P \leq 0.05$) due to genotypes and temperatures (Table 1). HNT reduced approximately 55% of the biomass in leaves (152.72 g), stem (111.43 g) and total (11.43 g) dry weights compared to plants grown under the control conditions (307.04 g in leaves, 313.31 g in stems and 602.36 g in total biomass). Additionally, a lower leaf area was recorded at 30 °C where control plants (24 °C) showed a higher leaf size than HNT plants (66.64 cm vs. 33.64 cm, respectively). Regarding genotypes, 'F60' seedlings had the greatest biomass accumulation and leaf area compared to other genotypes. However, the 'LV1401' genotype seedlings had the highest specific leaf area. In general, a lower plant biomass in rice plants subjected to 30°C could be due to a balance between photosynthesis and night respiration in rice plants (Cheng *et al.*, 2009; Mohammed *et al.*, 2013). HNT causes a lower photosynthetic rate and increased respiration, and it can adversely affect membrane stability, causing a lower biomass accumulation (Peng *et al.*, 2004). Similar observations were obtained by Zhang *et al.* (2013), where HNT (27.4 °C) caused a lower biomass accumulation due to impaired crop growth rate. On the other hand, the differences in growth parameters among genotypes may be primarily attributed to varietal

Table 1. Effect of two nighttime temperatures (24 vs. 30 °C) on growth parameters in rice genotypes

Treatment/ Parameter	Total Dry Mass (mg)	Leaves Dry Mass (mg)	Stem Dry Mass (mg)	Leaf/Stem Ratio (mg mg ⁻¹)	Leaf Area (cm ²)	Specific Leaf Mass (mg cm ⁻¹)
Genotype						
F60	521.23 a ^z	281.55 a	239.67	1.28 a	61.08 a	4.67 bc
LV447	477.98 ab	254.62 ab	223.27	1.22 a	57.51 ab	4.45 c
FLO2764	378.25 b	201.39 bc	176.85	1.22 a	45.16 bc	4.55 bc
LV1401	361.62 b	173.65 c	187.96	1.04 b	33.00 c	5.22 a
CT19021	395.17 ab	210.79 bc	184.38	1.24 a	50.02 ab	4.21 c
IR1561	477.29 ab	242.94 ab	234.85	1.17 ab	48.63 abc	5.03 ab
Significance	***y	***	NS	***	***	***
Temperature (°C)						
24	620.36	307.04	313.31	0.99	66.64	4.74
30	254.15	152.72	111.43	1.36	33.64	5.66
Significance	***	***	***	***	***	NS
Temperature*Genotype						
Significance	NS	NS	NS	NS	NS	NS

^z Means with different letters represent statistically significant differences according to Tukey's test ($p \leq 0.05$)

^y N.S. and *** represent Non Significance and Significance at $P \leq 0.001$, respectively.

Table 2. Effect of two nighttime temperatures (24 vs. 30 °C) on leaf photosynthetic pigments and proline content in rice genotypes

Parameter	CAR (mg g ⁻¹)	Chl a (mg g ⁻¹)	Chl b (mg g ⁻¹)	Chl Total (mg g ⁻¹)	Chl a/b	Chl/CAR	Proline (μmol g ⁻¹)
Genotype							
F60	0.84	2.60	0.91ab ^z	3.46b	2.88b	4.34	0.22
LV447	0.98	3.31	1.08ab	4.40ab	3.05ab	4.73	0.25
FLO2764	1.09	3.80	1.14ab	4.78ab	3.43a	4.39	0.25
LV1401	0.78	2.77	0.90b	3.67ab	3.11ab	4.75	0.22
CT19021	1.10	3.98	1.41a	5.39a	2.90b	4.97	0.23
IR1561	0.98	3.07	1.01ab	4.08ab	3.04ab	4.22	0.29
Significance	NS	NS	*	*	*	NS	NS
Temperature (°C)							
24	0.85	3.0	1.0	3.99	3.03	4.77	0.22
30	1.10	3.5	1.15	4.66	3.03	4.30	0.26
Significance	**y	NS	NS	*	NS	**	NS
Genotype*Temperature							
Significance	NS	NS	NS	NS	NS	NS	NS

^z Means with different letters represent statistically significant differences according to Tukey's test ($p \leq 0.05$)

^y NS, and*,** represent Non significance and significance at $P \leq 0.05$ or $P \leq 0.01$, respectively.

differences, since ANOVA did not show differences in the interaction (Zhang *et al.*, 2013; Garcés-Varon and Restrepo-Díaz, 2015).

Chlorophyll fluorescence and leaf photosynthetic pigments

Additionally, distinct differences were found between factors (nighttime temperatures and genotypes) on the F_v/F_m ratio and leaf photosynthetic pigments (chlorophyll and carotenoids). A lower F_v/F_m ratio was found in rice plants at 30 °C than 24 °C (0.79 vs. 0.74, respectively) (Fig. 3). Regarding genotypes, 'FLO2764', 'LV1401' and 'IR1561' plants showed a higher ratio at the end of the experiment than the others (data not shown). A drop in PSII efficiency (F_v/F_m ratio) may be due to damage in the PSII proteins, usually D1 and/or impairments of the repair system of this protein (Gururani *et al.*, 2015). On the other hand, HNT caused an increase in leaf carotenoid and chlorophyll content, but a lower chlorophyll/carotenoid ratio was observed. Moreover, differences were found in leaf chlorophyll b, total content and chlorophyll a/b ratio among genotypes, where 'FLO2764' plants showed the highest contents (Table 2). In general, HNT mainly caused a higher leaf carotenoid content and a lower chlorophyll/carotenoid ratio. A higher carotenoid content is a tolerance mechanism under heat stress conditions because carotenoids can dissipate energy in the form of heat through

the xanthophyll cycle or can act as antioxidants (Jahns and Holzwarth, 2012). A lower ratio also indicates an acclimatization response to high temperatures (Wahid *et al.*, 2007).

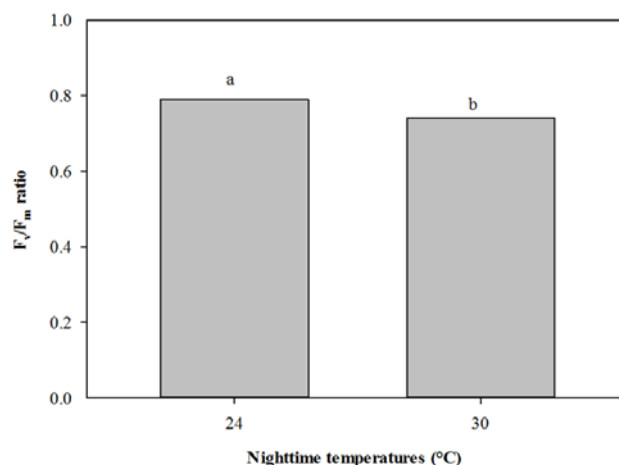


Fig. 3. Effect of two nighttime temperatures (24 and 30 °C) on F_v/F_m ratio readings in rice leaves. Vertical bars represent \pm standard error. Means followed by different letter are significantly different at $p \leq 0.05$ according to Tukey's test

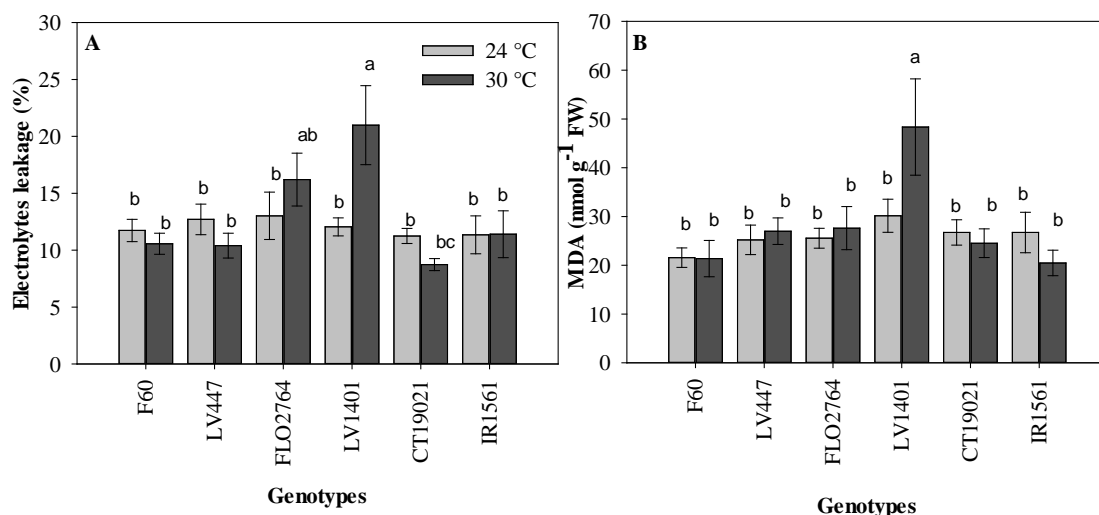


Fig. 4. Effect of two nighttime temperatures (24 and 30 °C) on electrolyte leakage (A) and MDA content (B) in rice genotypes. Each bar chart represents the mean of 6 plants. Vertical bars represent \pm standard error. Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's test

Electrolyte leakage, MDA, proline and photosynthetic pigments

Differences were only found ($P \leq 0.05$) in the interaction between genotype vs. night temperatures on MDA and electrolyte leakage in rice leaves (Fig. 4). At 30 °C, 'LV1401' seedlings had the highest membrane lipid peroxidation. Similar trends were also found for electrolyte leakage. A higher MDA content is often used as an indicator of oxidative damage (Sanchez-Reinoso *et al.*, 2014). In this aspect, an increased MDA content in rice leaves under HNT indicates that the production of ROS is greater than in the control plants (Shah *et al.*, 2011). Similar observations, where HNT affects membrane stability in susceptible genotypes, were also reported in wheat, sorghum and soybean (Prasad *et al.*, 2008, Prasad and Djanaguiraman 2011, Djanaguiraman *et al.*, 2013). On the other hand, Mohammed and Tarpley (2009) reported that susceptible rice genotypes under HNT showed a low total antioxidant capacity, resulting in higher electrolyte leakage. The previous statement could help explain the fact that 'LV1401' seedlings showed higher membrane damage in our experiment. Finally, no differences in proline content among genotypes and/or temperatures were found (Table 2). The lack of differences on leaf proline levels under HNT can be associated with low oxidative damage in almost all genotypes, since this amino acid has been recognized as a multi-functional molecule under abiotic stresses, showing a role as an antioxidant (Kishor and Sreenivasulu, 2014).

Conclusions

In summary, physiological traits, such as photosynthesis and respiration, as well as biochemical markers (MDA content), helped to expand our knowledge of the tolerance mechanism to heat stress in rice genotypes grown in tropical regions. Based on our results, we can conclude that a high night temperature (30 °C) may cause a decrease in the leaf photosynthesis rate and increased respiration of susceptible genotypes. Biochemical traits, such as MDA and leaf chlorophyll/carotenoid ratio, can also be considered for screening tolerant genotypes since these parameters suggest

oxidative damage under HNT. Finally, our results also suggest that 'F60' plants might represent a genotype that shows acclimatization to high night temperature conditions, and they can be cultivated in regions where periods of extremely high night temperatures can be expected.

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