

EFFECT OF TWO NOCTURNAL TEMPERATURES ON CHLOROPHYLL FLUORESCENCE PARAMETERS OF FLAG LEAVES AND PANICLE CHARACTERISTICS IN SEVEN RICE (*ORYZA SATIVA* L.) GENOTYPES

OSCAR H. ALVARADO-SANABRIA¹, GABRIEL A. GARCES-VARON² AND HERMANN RESTREPO-DIAZ^{3*}

¹Grupo de Investigaciones Agrícolas (GIA). Facultad de Ciencias Agropecuarias, Universidad Pedagógica y Tecnológica de Colombia

²Federación Nacional de Arroceros, Seccional Saldaña, Carrera 18 N° 23-112, 733570 Saldaña, Colombia

³Departamento de Agronomía, Universidad Nacional de Colombia, Carrera 30 N° 45-03, 111321 Bogotá, Colombia

*Corresponding author's email: hrestreped@unal.edu.co

Abstract

High night temperature periods have reduced rice crop yields in recent years in Colombia. One of the strategies used to address this situation has been selecting tolerant genotypes and identifying physiological variables that serve as selection criteria in breeding programs. The aim of this study was to determine the effect of two-night temperatures (24°C vs. 30°C) on the physiological and agronomic performance of seven rice genotypes (Fedearroz 50 (F50), Fedearroz 60 (F60), IR 1561, FLO 2764, LV447-1, CT19021 and LV1401). At milk growth stage, a group of six plants of each genotype was placed in a growth chamber between 18:00 and 24:00 h at 30°C for a period of eight days (stressed plants). Meanwhile, another group of rice plants sorted by genetic material was kept under normal growth conditions (24°C) in a shade house (Control Plants). Results showed that rice genotype F50 plants showed a lower rate of spikelet fertility compared to the other genotypes studied at 30°C ($p \leq 0.05$). Leaf photosynthetic pigments and chlorophyll α fluorescence parameters (Y(II), Y(NPQ), Y(NO) and Fv/Fm ratio) showed no differences between night temperatures; however, significance differences were found among genotypes. The above results suggest that Y(II), Y(NPQ), Y(NO) and Fv/Fm in rice plants at advanced phenological stages (milk stage) are not an effective tool to quantify the physiological behavior of rice genotypes under a high night temperature condition.

Key words: Photosynthetic pigments, Proline, Spikelet fertility, Maximum efficiency of PSII, Yield components.

Introduction

Climate change has caused an increase in the frequency of high day and nocturnal temperatures worldwide (Porter & Xie, 2014). In Colombia, the annual average temperature has shown a linear increase moving from an average temperature of 21.8°C to 22.3°C between 1980 and 2011 (Ballesteros & Enciso, 2012). This temperature increase has generated negative effects on agricultural production systems such as the reduction of crop yields by about 2% per decade in rice, corn, wheat and soybean crops, observing a more severe effect in tropical regions (Porter & Xie, 2014). In this regard, several authors have reported that the night temperature is an important factor in rice productivity, since nocturnal temperatures above 30°C reduce rice yield due to higher non-viability of pollen lower grain filling, higher percentage of unfilled grains, high plant respiration and low photosynthetic rate (Peng *et al.*, 2004; Mohammed *et al.*, 2013; Mohammed & Tarpley, 2014).

Rice is one of the staple foods with the biggest cultivated area in the world (Anon., 2002) and this crop has covered approximately 500,000 ha during the last decade in Colombia. Fedearroz (2015) has reported that high temperatures have reduced yields in rice-growing areas of Colombia, which has reduced crop productivity. This situation highlights the importance of obtaining tolerant genotypes and developing strategies to minimize the damage caused by this abiotic condition.

One of the strategies to address the negative effects of high temperatures on cereals yield is the selection of tolerant genotypes (Araus *et al.*, 2008). In this regard, breeding programs currently use physiological traits in order to increase the efficiency in the genes selection

(Reynolds *et al.*, 2009; Jha *et al.*, 2014). For example, chlorophyll α fluorescence parameters and leaf gas exchange properties have been used to select wheat genotypes that can tolerate high radiation and temperature (Monneveux *et al.*, 2003; Sayed, 2003). Additionally, the characterization of genotypes tolerant to high night temperatures takes into account biochemical variables such as leaf photosynthetic pigment content (chlorophylls and carotenoids), compatible osmolytes (glycine betaine, proline and soluble sugars) and cell membrane integrity (Malondialdehyde) (Bita & Gerats, 2013). In rice, it has been reported that tolerant genotypes show little variation on photosynthesis, respiration, electron transport, chlorophyll content and quantum yield of photosystem II under high night temperatures (Glaubitz *et al.*, 2014; Mohammed & Tarpley, 2014). Therefore, these variables have been suggested as tools to select genotypes with possible tolerance to different abiotic stress conditions (Sayed, 2003; Araus *et al.*, 2008).

The use of some physiological and biochemical parameters such as leaf photosynthetic rate and proline production have become important in the characterization of genotypes under temperature stress in Colombia in recent years (Restrepo-Diaz & Garcés-Varon, 2013; Sánchez-Reinoso *et al.*, 2014). However, the use of physiological parameters (chlorophyll fluorescence parameters, lipid peroxidation, leaf gas exchange) for the selection of genotypes with characteristics of tolerance to abiotic stresses as future parents in rice breeding programs is still scarce at national level. Therefore, the objective of this study was to evaluate the usefulness of chlorophyll α fluorescence parameters (Y(II), Y(NPQ), Y(NO) and Fv/Fm ratio), panicle characteristics (grain size, grain number, spikelet fertility) and some biochemical tests

(malondialdehyde, proline content and leaf photosynthetic pigments) in seven rice genotypes (two commercial varieties and 5 lines) used in breeding programs to assess the effect of two night temperatures (24°C vs. 30°C).

Materials and Methods

Growing conditions: An experiment was carried out under shade house conditions at Las Lagunas research center of the National Rice Growers Association (Fedearroz) located in Saldaña (Colombia) (3°54'46" N; 74°59'7" W) between September 2014 and January 2015. Six plants of each genotype were planted in plastic trays with 9L capacity filled with sandy soil. Each plant was fertilized with 221 mg N (157 kg ha⁻¹), 15mg P (54 kg ha⁻¹), 215mg K (142 kg ha⁻¹), 71mg S (13 Kg ha⁻¹) and 23mg Zn (12 Kg ha⁻¹). The environmental conditions throughout the experiment were as follows: average daytime temperature of 33°C, average night temperature of 24°C, relative humidity of 77% and a natural photoperiod of 12 hours. The above values were recorded by a weather station (Davis Vantage Pro 2 Plus, NSW, AUS) located in the area of experiment.

Treatments: In this study, two commercial rice cultivars susceptible to heat stress conditions were used (Fedearroz 50 and 60 (F50 and F60)) (Restrepo-Diaz & Garcés-Varon, 2013; Sánchez-Reinoso *et al.*, 2014). In addition, five genotypes used by the National Rice Growers Association in their breeding programs (IR 1561, FLO 2764, LV447-1, CT19021, LV1401) were selected. These genotypes were chosen because they performed well under high day and night temperatures. In this study, 12 plants per genotype were used. Treatments consisted of in dividing each genotype into two groups of six plants each. The first group (control plants) was always located in the shade house at a night time ambient temperature (~24°C). The second group of rice plants was placed in a growth chamber (KBW-400, Binder, Germany) at 30°C between 18:00 and 24:00h for a period of 8 days (stressed plants). At day nine after stress, plants were returned to the shade house and physiological and biochemical response variables were estimated. In addition, plants were exposed to 30°C at milk grain phase. Due to the differences in plant growth cycle between genotypes, plants were placed on different dates into the growth chamber: 'IR 1561' and 'F50' plants on December 23rd, 2014; 'LV447-1' and 'FLO 2764' plants on December 30th, 2014; 'LV1401' and 'CT19021' plants on January 6th, 2015 and 'F60' plants on January 15th, 2015.

Yield components: Panicles were harvested at the end of growing cycle in each genotype. Then, the following yield components were determined in each panicle: filled grain mass, total number of grains, number of unfilled grains, number of filled grains, fertility percentage (expressed as the ratio of filled grains and the total number of breeding sites) and average grain mass.

Fluorescence parameters: Chlorophyll α fluorescence parameters were determined in the flag leaf at the end of treatments between 09:00 and 13:00 h in each genotype. The chlorophyll fluorescence parameters were estimated

with a MINI-PAM modulated fluorometer (HeinzWalz, Effeltrich, Germany). Leaves were kept in the dark for 20 minutes before measuring Fv/Fm. The minimum fluorescence (Fo) was measured with a light pulse modulated to less than 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and the maximum fluorescence (Fm) was induced by a pulse of 0.8s at an intensity of 3000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The potential quantum yield of PSII (Fv/Fm) was determined as follows: $Fv/Fm = (Fm - Fo)/Fm$. To achieve the photosynthesis baseline state (Fs'), the samples were subjected to a 900 $\mu\text{mol m}^{-2} \text{s}^{-1}$ actinic light and then, they were subjected to a second saturation pulse (3000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 0.8s) to obtain the maximum fluorescence in adapted light state (Fm'). Fo' was calculated by the Oxborough & Baker (1997) formula: $Fo' = 1/(1/Fo - 1/Fm + 1/Fm')$.

With the above variables and according to Brestic & Zivcak (2013), the actual quantum yield of photosystem II ($Y(II) = Fm' - F'/Fm'$), the quantum yield of the dissipation of non-regulated energy ($Y(NO) = 1/(NPQ + 1 + qL(Fm/Fo - 1))$), and the quantum yield of the energy dissipation ($Y(NPQ) = 1 - Y(II) - Y(NO)$) were estimated.

Leaf chlorophyll and carotenoid content: After determining chlorophyll fluorescence parameters, flag leaves of each panicle were cut and stored under refrigeration for up to 12 hours. Then, samples were macerated using liquid nitrogen and stored at -80°C until the respective determinations were performed. Leaf samples of 50 mg were macerated in a mortar with 5 mL of 80% acetone and then the sample was centrifuged at 8000 rpm for 5 minutes. After that, the absorbance readings at wavelengths of 663nm, 647nm and 470 were performed and chlorophyll a, b and carotenoid content (mg g⁻¹ fresh weight) were determined according to Lichtenthaler (1987).

Proline and malondialdehyde (MDA): Flag leaf samples stored at -80°C were also used to estimate proline content following the technique by Bates *et al.*, (1973): i) 100 mg plant tissue were homogenized in 5 mL of 3% sulfosalicylic acid; ii) samples were centrifuged at 6000 rpm for 30 min; iii) 1 mL of supernatant was extracted, mixed with 1 mL of acid-ninhydrin and 1 mL of glacial acetic acid, and vortexed for 1 minute in a falcon tube; iv) the samples were subjected to a water bath at 98°C for one hour and then the reaction was quenched using iced water; v) 3 mL of toluene were added to each sample and stirred vigorously; vi) the upper phase of the sample was collected and absorbance at 520 nm was determined. Proline content was calculated using a calibration curve and the following formula:

$$\frac{\mu\text{molProline}}{\text{g fresh weight}} = \frac{\left[\frac{\left(\frac{\mu\text{gProline}}{\text{mL}} \times \text{mLToluene} \right)}{115.5 \mu\text{g}} \right]}{\left[\frac{\text{g sample}}{5} \right]}$$

MDA content was determined following Hodges *et al.*, (1999) modified protocol: i) 100mg of plant material were homogenized in 2 mL of 0.1% trichloroacetic acid;

ii) samples were centrifuged at 8000 rpm for 15 minutes; iii) 1 mL of supernatant was extracted and mixed with 2 mL of 20% trichloroacetic acid and 2 mL of thiobarbituric acid (+TBA), in a test tube, apart, 1 mL was mixed with 2 mL of 20% trichloroacetic acid (-TBA); iv) both tubes were vortexed for one minute and subjected to a water bath at 95°C for 30 minutes; v) the reaction was quenched in an ice bed and the absorbances were read at 440, 532 and 600nm; vi) MDA content was determined as follows:

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

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

$$Equivalent\ MDA\ (nmol\ mL^{-1}) = \left(\frac{A - B}{157000} \right) * 10^6$$

Statistical analysis

For the statistical analysis, a factorial design was used, where the first factor corresponded to the night temperature (24°C vs. 30°C) and the second factor to the genotype ('F50', 'F60', 'IR 1561', 'FLO 2764', 'LV447-1', 'CT19021' and 'LV1401'), for a total of 14 treatments (each one with 6 experimental units represented by a plant). When differences were observed, the Tukey post hoc test was performed to compare means. Data analysis was carried out with SPSS (v20.0, IBM Company, USA) statistical software.

Results

Yield components (number of unfilled grains, number of filled grains, percentage of fertility and filled grain mass per panicle):

Significant differences were found in the interaction between genotype and temperature on total number of grains, number of filled grains, number of unfilled grains and fertility percentage ($p \leq 0.05$) (Fig. 1). Plants of the genotype F50 showed a higher total number of grains compared to other genotypes subjected to a night temperature of 30°C (Fig. 1A). The genotypes F50, F60 and LV1401 had the highest value of filled grains at both temperature conditions (24 and 30°C) (Fig. 1B). It was observed that most genotypes did not differ regarding the number of unfilled grains except for 'CT19021' plants at 24°C. Meanwhile, 'F50' plants showed the largest number of unfilled grains at the end of the experiment followed by genotype CT19021 at 30°C (Fig. 1C). The above results are corroborated by the percentage of fertility, since this variable was lower in 'F50' and 'CT19021' plants at 30°C (Fig. 1D).

Regarding grain mass, no differences in the interaction between genotypes and night temperatures were obtained. In this context, differences were separately observed in the factors genotype or temperature (Fig. 2). It was observed that an increase in night temperatures favored the filled grain mass in rice plants (Fig. 2A). Moreover, 'F50', 'F60' and 'LV1401' rice plants showed the highest value at the end of the experiment (Fig. 2B).

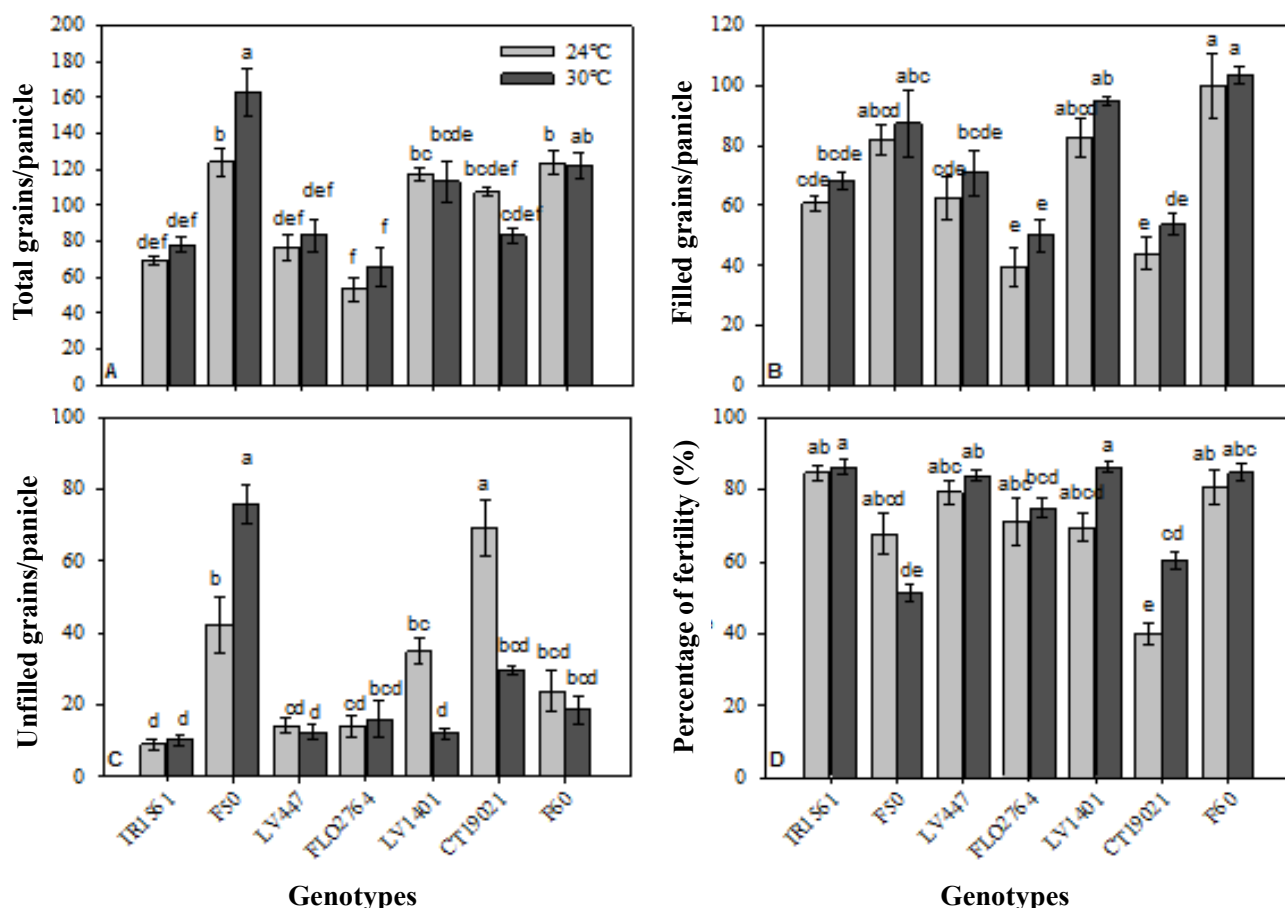


Fig. 1. Total number of grains (A), filled grains (B), unfilled grains (C), and fertility percentage (D) in panicles of seven rice genotypes under two nighttime temperatures (24°C vs. 30°C). Data represent the average of 6 ± EE replicates and significant differences were found at $p \leq 0.05$ according to the ANOVA.

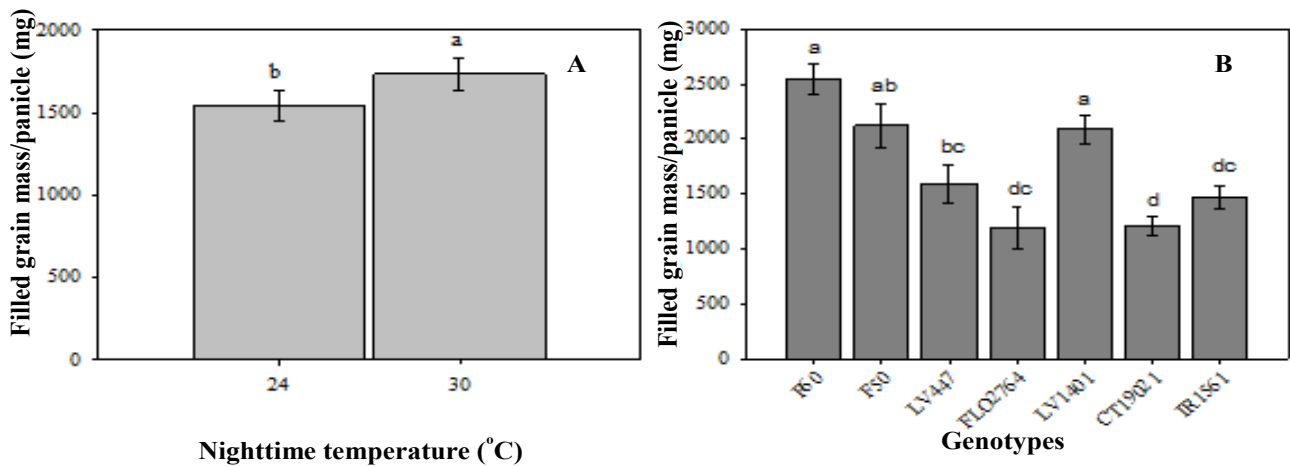


Fig. 2. Filled grain mass of rice panicles under two night temperatures (A) and in seven genotypes (B). Data represent the average of 6 \pm EE replicates and significant differences were found at $p \leq 0.05$ according to the ANOVA.

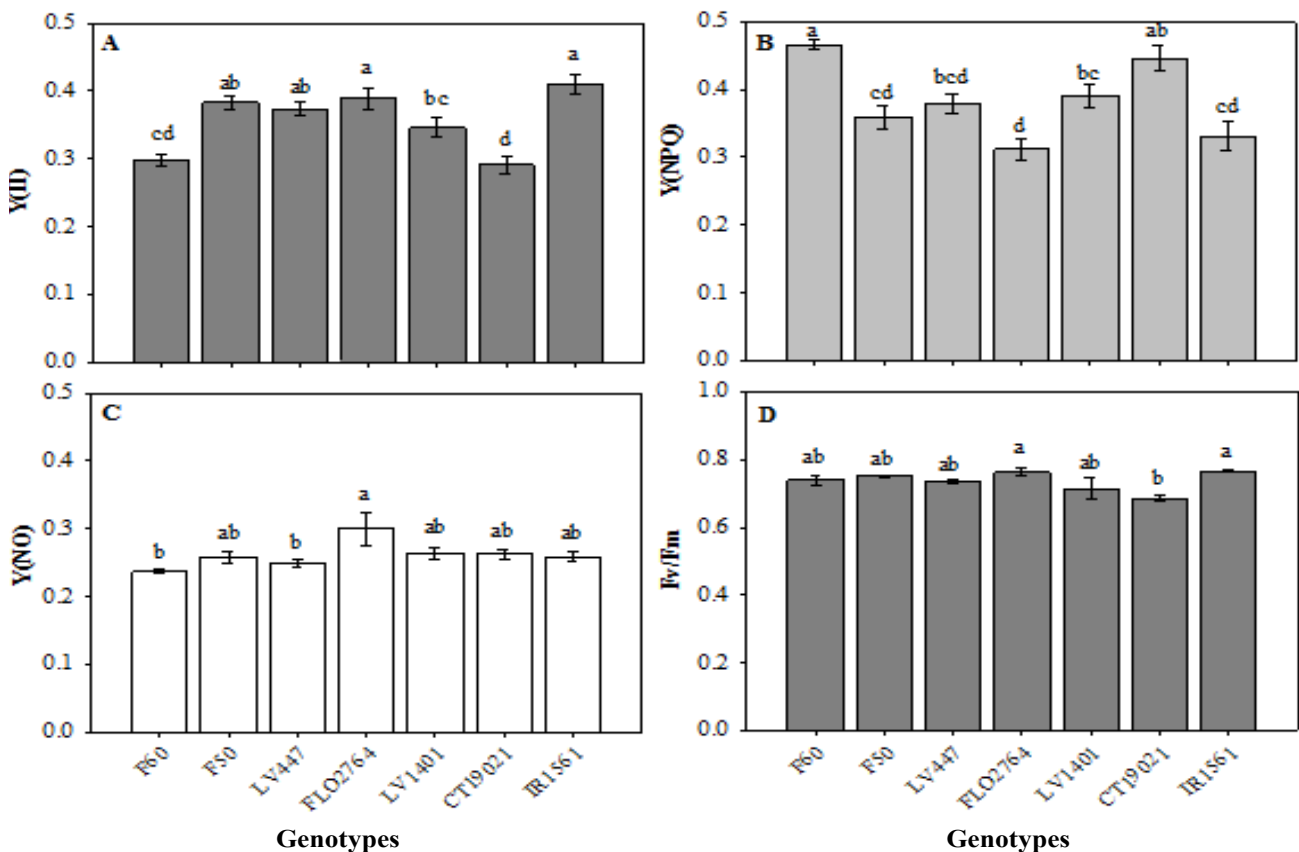


Fig. 3. Actual quantum yield of photosystem II (Y(II))(A), controlled non-photochemical dissipation (Y(NPQ)) (B), uncontrolled non-photochemical dissipation (Y(NO))(C) and potential quantum yield of PSII (Fv/Fm)(D) in flag leaves of seven genotypes of rice plants. Data represent the average of 6 \pm EE replicates and significant differences were found at $p \leq 0.05$ according to the ANOVA.

Chlorophyll *a* fluorescence parameters: Differences were only observed on Y(II), Y(NPQ) and Y(NO) among rice genotypes (Fig. 3). The higher Y(II) was found in genotypes IR 1561, F50, LV447 and FLO 2764 (Fig. 3A). However, an opposite effect was observed in Y(NPQ) where the highest values were presented in genotypes F50 and CT 19021 (Fig. 3B). Furthermore, 'FLO 2764' plants were the ones that had more uncontrolled non-photochemical dissipation (Y(NO)) (Fig. 3C). Finally, the different genotypes were within the optimal range (0.7-0.8) of the Fv/Fm ratio (Fig. 3D).

Biochemical tests (leaf photosynthetic pigments, MDA and proline): Only ($p \leq 0.05$) differences on the leaf chlorophyll and carotenoid contents were observed among the different studied genotypes (Table 1). In general, chlorophyll a, b and carotenoid contents were lower in 'F60' plants compared to the other genotypes. When leaf photosynthetic pigments were studied using the chlorophyll/ carotenoids ratio, it was observed that 'FLO 2764' plants had the highest ratio. Regarding MDA, 'F60' plants also had the lowest flag leaf lipid peroxidation compared to the other genotypes. Finally, no differences on proline content between temperatures or genotypes were found (Table 2).

Table 1. Summary of analysis of variance of the effect of two nighttime temperatures on the physiological and biochemical behavior of seven rice genotypes.

	Abbrev	Source of variation		
		Genotype	Temperature	Genotype x Temperature
Chlorophyll a	Chl a	***	NS	NS
Chlorophyll b	Chl b	***	NS	NS
Carotenoids	CAR	***	NS	NS
Chlorophyll/Carotenoids ratio	Chl/CAR	***	NS	NS
Proline	Pro	NS	NS	NS
Malondialdehyde	MDA	*	NS	NS
Actual quantum yield of photosystem II	Y(II)	***	NS	NS
Non photochemical quenching	Y (NPQ)	***	NS	NS
Uncontrolled non-photochemical dissipation	Y (NO)	*	NS	NS
Maximum efficiency of PSII	Fv/Fm	**	*	NS
Filled grain mass per panicle	FGP	***	*	NS
Number of total grains	NTG	***	NS	***
Number of unfilled grains	NUG	***	*	***
Number of filled grains	NFG	***	*	*
Percentage of fertility	PF	***	***	*

*, and *** significantly different at 0.05 and 0.001 probability levels, respectively. NS, not significant at $\alpha = 0.05$

Table 2. Leaf Photosynthetic pigment content (Chlorophylls and Carotenoids), Malondialdehyde (MDA) and proline in flag leaves of seven rice genotypes under two different night temperatures (24°C vs. 30°C).

Parameter	CAR (mg g ⁻¹)	Chla (mg g ⁻¹)	Chlb (mg g ⁻¹)	Chl/CAR (x+c)	MDA (nmol g ⁻¹)	Proline (μ mol g ⁻¹)
Genotype						
F60	0.33c ^z	0.95b	0.38b	4.24b	32.11b	25.38
F50	0.75b	2.13ab	0.84ab	3.96b	40.81ab	38.64
LV447	1.16a	2.99a	1.10a	3.50b	78.17a	47.98
FLO2764	0.81ab	3.03a	1.30a	5.43a	66.14ab	40.99
LV1401	0.84ab	2.57a	0.93a	4.13b	58.11ab	43.36
CT19021	0.84ab	2.43a	0.99a	4.18b	67.04ab	58.67
IR1561	1.01ab	3.16a	1.25a	4.34b	43.05ab	29.09
Significance ^y	*	*	*	*	*	NS
Temperature (°C)						
24	0.84	2.50	0.97	4.11	53.82	38.99
30	0.79	2.37	0.93	4.28	59.84	46.65
Significance	NS	NS	NS	NS	NS	NS
Temperature*Genotype						
Significance	NS	NS	NS	NS	NS	NS

^z Within each column and for each factor, different letters after the means indicate significant differences according to Tukey test or ANOVA ($p \leq 0.05$)

^y N.S. and *: not significant and significant at $p \leq 0.05$

Discussion

A night temperature of 30°C reduced the fertility percentage mainly in 'F50' plants by approximately 30% compared to genotypes F60, LV1401 and IR1561 (Fig. 1). A plausible explanation for the different responses among the genotypes studied in this research may be due to the fact that the varieties have different acclimatization mechanisms under this stress condition (Jagadish *et al.*, 2010). In this regard, Mohammed & Tarpley (2009a) stated that a high number of sterile spikelets might be

caused by an increase in the maintenance respiration during the grain filling stage. Similarly, Shi *et al.*, (2013) mentioned that the differences in the fertility rate among genotypes might be due to the fact that cultivars susceptible to high night temperatures could show reduced nitrogen and non-structural carbohydrate translocation from leaves and stems to panicles. In addition, a lower expression of heat shock proteins (FKBP type), or calcium signaling proteins (calmodulins and kinases) are involved in genotype susceptibility (mainly at the grain filling stage). Regarding filled grain mass,

commercial cultivars (F50 and F60) and genotype LV1401 plants showed the higher grain mass and this might be mainly due to the fact that these genotypes produced a greater number of grains per panicle. There is a direct relationship between the number of grains produced and the mass of the grains produced per panicle (Mohammed & Tarpley, 2011).

Furthermore, no differences among the various indices of energy dissipation (Y(II), Fv/Fm and NPQ) on rice plants subjected to 24°C or 30°C were obtained. This behavior has also been reported by Mohammed & Tarpley (2014) and Glaubitz *et al.*, (2014), who also found no differences in these indexes when different rice genotypes were exposed to high night temperatures ($\geq 28^\circ\text{C}$). Likewise, the Y(NO) index has been defined as an uncontrolled and harmful form of energy dissipation (Brestic & Zivcak, 2013). Therefore, Y(NO) can help to understand that genotype FLO 2764 (Y(NO)= 0.3) may show some damage in the PSII system due to the area conditions where this research was conducted compared to 'LV447' and 'F60' (Y(NO)= 0.25 and 0.24, respectively). This allows us to infer that 'LV447' and 'F60' plants can dissipate the energy better in a controlled manner, either through photosynthesis and/or photorespiration Y(II) or in the form of heat (Y(NPQ)). Regarding the Fv/Fm ratio, this indicates the maximum amount of energy that the plant is able to use in photosynthesis and that generally decreases under a stress condition (Baker, 2008). In general, the Fv/Fm values of the different genotypes were around their optimum (0.7-0.8) in the present study. A lower value of the Fv/Fm ratio does not necessarily mean damage to the PSII due to a stressful condition, but may be specific to the genotype or leaf senescence (Lim *et al.*, 2007; Panda & Sarkar, 2013).

The main function of chlorophylls and carotenoids is to collect and transmit light energy to transform later it into chemical energy (Taiz & Zeiger, 2006). Similarly, the concentration of these pigments generally decreases under stressful conditions (Mohammed & Tarpley, 2009b) or during leaf senescence (Hörtensteiner, 2006). In this study, no changes occurred in the concentration of these photosynthetic pigments when the night temperature was increased; however, differences between genotypes were observed. In this regard, Mohammed & Tarpley (2014) did not find any differences in the concentration of chlorophyll a, b and carotenoids in two rice genotypes under two different night temperature conditions. Nevertheless, Dong *et al.*, (2014) found that the concentration of chlorophyll a and b decreased when two rice materials were subjected to high night temperature (28°C) and concluded that obtaining differences might depend on the moment in which the evaluation is carried out. In our case, the lack of differences in the concentration of photosynthetic pigments due to night time temperature treatments and the differences between genotypes might be due to the fact that the evaluation of this variable was performed at the advanced stage of maturity when there is greater leaf senescence (Restrepo & Garcés, 2013). Regarding the other biochemical variables, the high night temperature (30°C) did not cause an increase in lipid peroxidation or proline production (osmolyte involved in cell homeostasis). Similar results were obtained by Rizhsky *et al.*, (2004), Shah *et al.*,

(2011) and Szabados & Saviouré (2010) who reported that no increases in lipid peroxidation and proline accumulation were observed under high night temperature conditions. Finally, 'F60' plants showed lower lipid peroxidation compared to the other genotypes.

In summary, 'F50' rice plants had the lowest fertility rate when exposed to 30°C compared to the other genotypes. Furthermore, differences were only found in the chlorophyll fluorescence indices and pigment production among the different genotypes studied. Also, a high night temperature did not produce oxidative stress (represented as MDA production) in the studied genotypes. Finally, the above results suggested that the fluorescence parameters (Y(II), Y(NPQ), Y(NO) and Fv/Fm) in advanced phenological stages (milky grain stage) were not an effective tool to quantify the physiological behavior of rice genotypes under high night temperature conditions.

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