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ORIGINAL ARTICLE

Response of rice plants to heat stress during initiation of panicle primordia or grain-filling phases

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Leaf photosynthesis, a major determinant for yield sustainability in rice, is greatly conditioned by high temperature stress during growth. The effect of short-term high temperatures on leaf photosynthesis, stomatal conductance, Fv/Fm, SPAD readings and yield characteristics was studied in two Colombian rice cultivars. Two genotypes, cv. Fedearroz 50 (F50) and cv. Fedearroz 733 (F733) were used in pot experiments with heat stress treatment (Plants were exposed to 40°C for two and half hours for five consecutive days) and natural temperature (control) treatment. Heat treatments were carried out at the initiation of panicle primordial (IP) or grain-filling (GF) phases. The results showed that short-term high temperature stress produced a reduction on the photosynthesis rate in both cultivars either IP or GF phases. Similar trends were found on stomatal conductance in all cases due to high temperatures. Although Fv/Fm and SPAD readings were not affected by high temperatures, these variables diminished significantly among phenological phases. 'F733' rice plants showed higher number spikelet sterility due to heat stress treatments. These results seem to indicate that heat-tolerant cultivars of rice is associated with high levels of photosynthesis rate in leaves.

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Key words: Chlorophyll fluorescence, Indica rice, stomatal conductance, photosynthesis, heat stress

Rice is one of the most important food crops in Colombia, where occupies a total area of 420.721 ha, with a production of 2.262.055 tons in 2010 (Fedearroz, 2011). Currently, global climate change has caused detrimental effects on yield physiology of rice because of the rise in atmospheric temperatures (Fageria, 2007; Jagadish *et al*., 2007). Likewise, environmental situations such as El Niño phenomenon or prolonged period of time without rains (dry season) can also cause severe conditions of heat stress in rice-growing areas at certain periods of the year in Colombia (Castilla *et al*., 2010). Photosynthesis is one of the most susceptible processes to heat stress among the physiological processes in plants (Yin *et al*., 2010). Photosynthesis of rice leaves are considerably reduced at temperatures higher than 35°C (Taniyama *et al*., 1988). Heat stress can also affect mechanisms such as rubisco activation state, maximal efficiency of PSII photochemistry (Fv/Fm), the actual PSII efficiency in the light-adapted state and/or non-photochemical quenching in rice leaves (Yin *et al*., 2010). On the other hand, some adverse environmental factors such as drought, low solar radiation, N deficiency, low or high temperatures in the initiation of panicle primordial and/or spikelet filling stage can increase spikelet sterility and consequently grain yield (Fageria, 2007).

In Colombia, rice is presently grown in regions where temperatures are far to the optimal for growth (28/22°C); hence, it is common to find episodes of high temperature during sensitive stages could reduce yields (Castilla *et al*., 2010). Several reports concerning with the physiological responses of rice leaves to heat stress have been published among cultivars of both indica and japonica types (Cao *et al*., 2009; Taniyama *et al*., 1988; Yang and Heilman, 1993; Yin *et al*., 2010). However, the most of these studies are mainly performed in temperate or subtropical regions. The information about photosynthesis studies of rice cultivars in tropical regions, especially, Latin America is scarce. In addition, little is known about the physiological behavior of Colombian cultivars; a better knowledge on the susceptibility and/or tolerance to heat stress would contribute to improve breeding programs. For these reasons, the aim of this work was to study the effects of short-term high temperature stress during initiation of panicle primordia or grain-filling stages on physiological mechanisms such as chlorophyll fluorescence, stomatal conductance and photosynthesis.

MATERIALS AND METHODS

This research was conducted between June and October of 2010 at "Centro de Investigación Las Lagunas" Experimental farm in Saldaña, Colombia. Two indica rice cultivars (cv. Fedearroz 50 (F50) and cv. Fedearroz 733 (F733)) were used. Both cultivars are widely cultivated by Colombian rice growers. Plants were placed outdoors and cultivated into 2 L pots containing soil as substrate. The soil was sandy loam that contained organic matter at 2%. Each plastic pot contained two rice plants. Each pot was provided with 1g N, 0.3g P_2O_5, and 0.7g K_2O as a top dressing about 45 days before heading. The plants were irrigated in excess twice a day, early morning and late afternoon, to keep the soil saturated. The average day-time temperature (06h00-18h00) was ~29.5°C. The average night-time temperature (18h00-06h00) was~25.6°C. Relative humidity was 68.1% in the day time and 85.3% at night. The maximal irradiance at noon was about 1516 W m^{-2}. The experiment lasted 120 days.

Both cultivars were split into three treatments: a) plants without heat stress (Control), b) plants under heat stress at the initiation of panicle primordia (IP), and c) plants under heat stress at grain-filling (GF). Sun-lit phytotron (KBW-400, Binder, Germany) was used for short-term high temperature stress. Plants were exposed to 40°C for two and half hours (09h30-12h00) for five consecutive days to achieve short-term high temperature stress. Then, plants were returned back to outdoors.

Measurements of net photosynthetic gas exchange (Pn) and stomatal conductance (g_s) were performed on a fully expanded attached leaf of rice seedlings by a portable photosynthesis system (LI-6200; LICOR, Inc., Lincoln, NE) using a 250-cm^3 cuvette, and a leaf-porometer (SC – 1 Decagon Devices, Inc, Pullman, WA), respectively. Two sets of 3-days measurements [27-29 July and 10-12

September (referred to as "IP" and "GF", respectively)] were carried out between 09h00 and 13h00. During all gas exchange measurements, conditions within the cuvette were: PAR greater than 800 µmol m^{-2} s^{-1}, leaf temperature 27 ± 5°C, and leaf to air water vapor pressure difference 1.8 ± 0.5 kPa.

Leaf chlorophyll fluorescence measurements were carried out at 31 July (IP), and 13 September (GF) using a continuous excitation chlorophyll fluorescence analyzer (Handy PEA; Hansatech Instruments, Kings Lynn, UK). Leaves were acclimated to the dark using light weight leaf clips for at least 20 min before measurements were taken. Baseline (F_0) and maximum (Fm) fluorescence were measured and variable (Fv = Fm – F_0) fluorescence and the ratio of variable fluorescence to maximum fluorescence (Fv/Fm) ratio were calculated from these data. Leaf absorbance readings were also taken using a chlorophyll meter (SPAD-502; Minolta, Ramsey, NJ) as a nondestructive tool for estimating leaf Chlorophyll (Markwell *et al*., 1995). SPAD readings were also recorded at 31 July (IP), and 13 September (GF).

Means and standard errors were obtained for each data. Data were subjected to analysis of variance by using a factorial design. All percentage values were transformed using the arcsine transformation before analysis. Where a significant *F*-test was observed, mean separation among treatments was obtained by Tukey's test. Data were analyzed using Statistix (version 8.0; Analytical Software, Tallahassee, FL, US).

RESULTS AND DISCUSSION

Photosynthetic rates of two Colombian rice cultivars were examined at two different stages of crop growth (IP and GF) under short-term high temperature stress and the results are summarized in the Figure 1A and B. Figure 1 shows that significant differences were obtained in the triple interaction cultivar x stage x temperature. Short-term high temperature stress caused a reduction on the photosynthesis rate in both cultivars either IP or GF phases. At IP stage, 'F50' rice plants showed a higher reduction on photosynthesis net when plants were exposed to high temperatures, as compared to 'F733' (Figure 1A). Heat stress decreased the photosynthesis by~35% in 'F50' rice plants; whereas, CO_2 assimilation rate diminished by~20% in 'F733'. At GF stage, Pn was lower than in IP stage in all cases (Figure 1B). Photosynthesis significantly decreased when plants were under high temperatures for five consecutive days in both cultivars. 'F50' and 'F733' rice plants showed a reduction on photosynthesis net by ~38% and ~20%, respectively, as compared to control plants.

Cao *et al*. (2009) also found that high temperatures during vegetative and early grain filling phases caused a diminishing on photosynthetic rate of flag leaf in different rice's genotypes. Regarding stomatal conductance (g_s), similar trends than in measurements of photosynthesis net were obtained (Figure 2).

In general, g_s was also seriously affected by short-term high temperature stress. The measurements revealed varietal differences on g_s due to two temperature levels in both stages. For instance, when rice plants exposed to high temperature for 5 days, g_s decreased by ~20% in cv. Fedearroz 50 and ~ 30% in cv. Fedearroz 733 at IP stage and by ~ 40% in cv. Fedearroz 50 and ~ 50% in cv. Fedearroz 733 at GF phase. A significant correlation between g_s and Pn was also obtained for the pooled data from all treatments combinations (r = 0.86, P < 0.01; Figure 3). Lowest photosynthesis and stomatal conductance data were observed when plants were exposed to short-term heat stress at grain filling stage in both cultivars. We examined the effects of high temperatures on Fv/Fm, SPAD readings, spikelet per panicle and spikelet sterility in two cultivars at two different phenological stages. Table 1 shows that there was not significant differences in triple interaction cultivar x stage x temperature on Fv/Fm, SPAD readings, spikelet per panicle and spikelet sterility. Even though Fv/Fm and SPAD readings were not affected by high temperatures, these variables diminished significantly among phenological phases. The decrease of photosynthesis, stomatal conductance, Fv/Fm, and SPAD values during grain-filling is not surprise since leaf senesce as well as plant canopy was in progress. In contrast to above results, studies performed by Yin *et al*. (2010) found that Fv/Fm decreased significantly at temperatures higher than 35°C in cv.9311 rice leaves at maximum tillering. Nevertheless, recent works have showed that heat stress does not cause serious PSII damage, but it can inhibit the repair of PSII (Allakhverdiev *et al*., 2008, Sharkey, 2005). Despite the fact that differences were not found in the triple interaction on the number of spikelet per panicle, however; there were varietal differences on the number of spikelet per panicle. 'F733' had a higher number than 'F50'. Symptoms of rice grain rot were observed in the heat-treated plants (data not shown). This work showed that the reduced yield characteristics of Colombian cultivars under high temperatures may also be caused by lower photosynthesis rate, since CO_2 assimilation rate is vital for yield sustainability because of it contributes in the spikelet number determination (Fageria, 2007; Ishii 1995). Cao *et al*. (2009) also concluded that the relative high yield in heat-tolerant genotypes of rice is associated with high levels of photosynthesis rate in leaves. In consequence, these results seem to confirm field observations where 'F50' rice plants under heat stress either IP or GF phases have showed a higher susceptibility, causing a lack of response on yield characteristics.

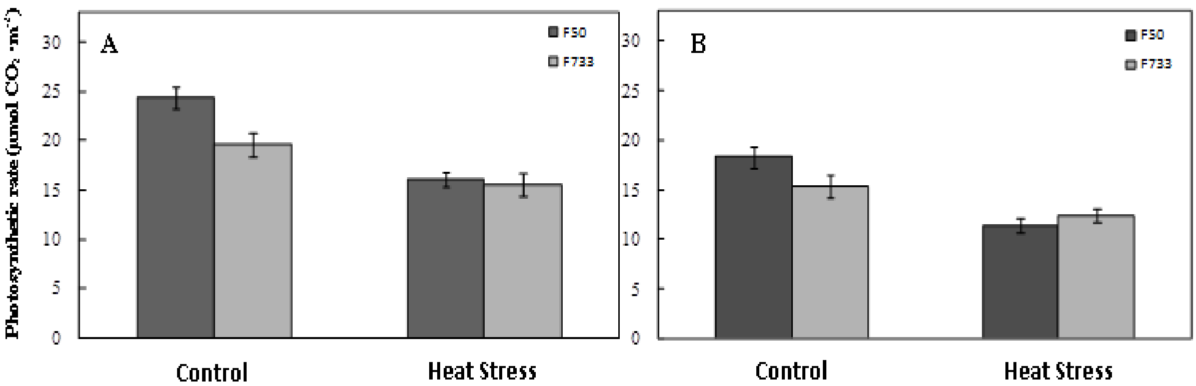

Figure 1 Effect of short-term high temperature stress on leaf photosynthesis of cv. Fedearroz 50 (■ F50), and cv. Fedearroz 733 (□ F733) during initiation of panicle primordial (A) or grain-filling (B) phases. Each bar chart represents the mean of four values. Vertical bars represent ± S.E.

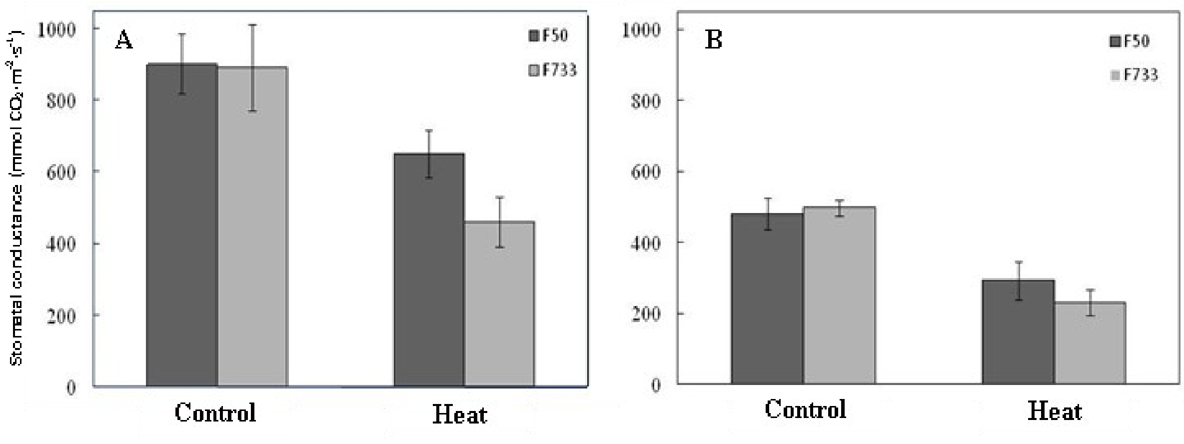

Figure 2 Effect of short-term high temperature stress on stomatal conductance of cv. Fedearroz 50 (■ F50), and cv. Fedearroz 733 (□ F733) during initiation of panicle primordial (A) or grain-filling (B) phases under short-term high temperature stress. Each bar chart represents the mean of four values. Vertical bars represent ± S.E.

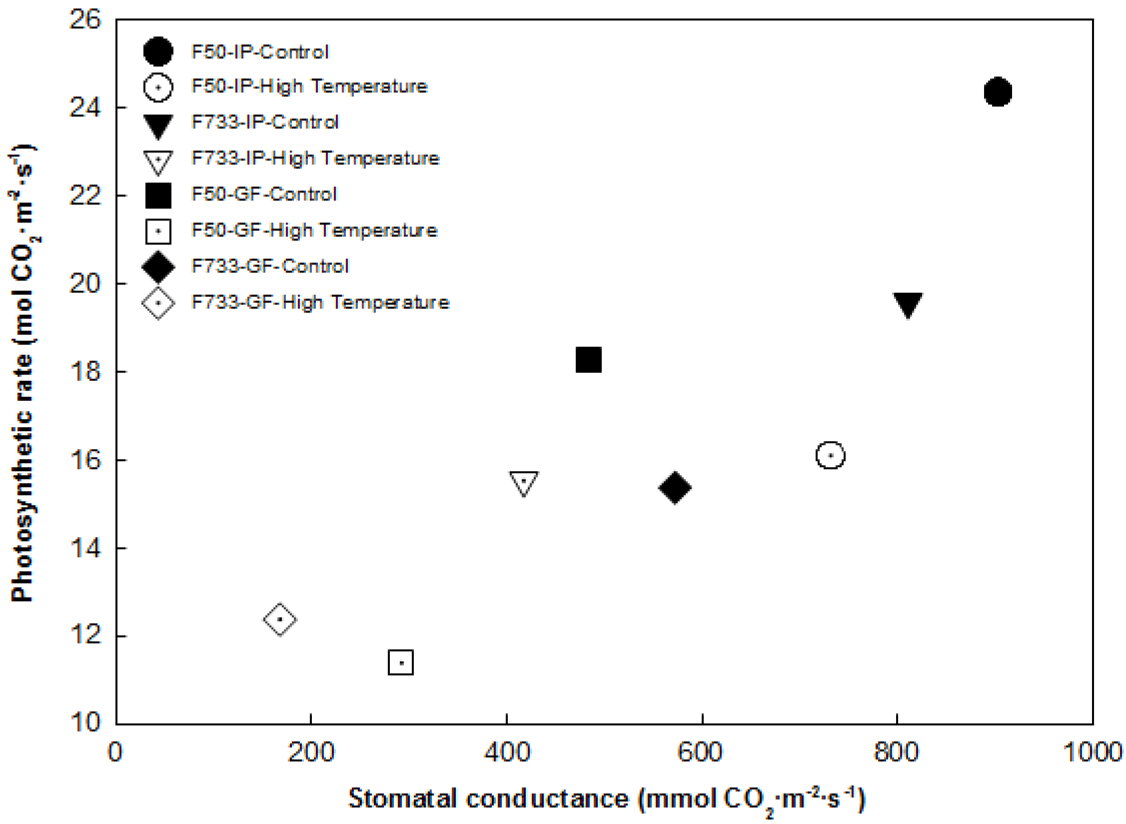

Figure 3 Relationship between stomatal conductance and leaf photosynthesis across cultivars and phenological phases. Each point represents the mean of four values.

Table 1. Effects of short-term high temperature stress during initiation of panicle primordial or grain-filling phases on Fv/Fm, SPAD readings, Spikelts per panicle and spikelet sterility of two Colombian rice cultivars (Fedearroz 50 (F50) and Fedearroz 733 (F733))

Cultivar	Stage	Temperature	Fv/Fm	SPAD	Spikelets per panicle	Spikelet sterility (%)
F50	IP	Control	0.84 a	40.33 a	291.13 a	43.84 b
		Heat stress	0.82 a	42.05 a	297.88 a	42.19 b
	GF	Control	0.77 b	34.88 b	291.13 a	43.84 b
		Heat stress	0.79 b	31.99 c	299.88 a	45.18 b
F733	IP	Control	0.84 a	41.45 a	361.25 b	22.15 a
		Heat stress	0.82 a	41.15 a	308.00 b	45.68 b
	GF	Control	0.78 b	34.75 b	361.25 b	22.15 a
		Heat stress	0.78 b	33.62 bc	375.88 b	54.53 b
Analysis of variance						
Cultivar			N.S.	N.S.	***	N.S.
Stage			***	***	N.S.	***
Temperature			N.S.	N.S.	N.S.	**
Stage x temp			**	**	N.S.	N.S.
Stage x cultivar			N.S.	N.S.	N.S.	N.S.
Temp x cultivar			N.S.	N.S.	N.S.	***
Cult x Temp x Stage			N.S.	N.S.	N.S.	N.S.
C.V. (%)			2.35	4.30	14.83	18.61

Ns, **, and ***, non-significant differences or significant differences at P <0.01, or 0.001, respectively.

In summary, high-temperature stress during the initiation of panicle primordia or grain-filling stages had a profound effect on CO_2 assimilation of rice leaves mainly because of low stomatal conductance, especially, in 'F50' rice plants. In addition, the effects of short-term high temperature stress on photosynthesis and grain filling varied among genotypes and the developmental stages of plant when exposed to the stress.

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