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Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress

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Abstract

Spikelet fertility (seed-set) is an important component of yield that is sensitive to high temperature. The objectives of this research were (a) to quantify the effects of high temperature on spikelet fertility and harvest index of rice; (b) to determine if there were species, ecotype, and/or cultivar differences in response to high temperature; and (c) to understand the reasons for lower and/or differential spikelet fertility and harvest index of rice cultivars at high temperatures. Fourteen rice cultivars of different species (*Oryza sativa* and *Oryza glaberrima*), ecotypes (*indica* and *japonica*) and origin (temperate and tropical) were exposed to ambient and high temperature (ambient + 5 °C) at Gainesville, Florida. High temperature significantly decreased spikelet fertility across all cultivars, but effects varied among cultivars. Based on decreases in spikelet fertility at high temperature, cultivar N-22 was most tolerant, while cultivars L-204, M-202, Labelle, Italica Livorna, WAB-12, CG-14 and CG-17 were highly susceptible and cultivars M-103, S-102, Koshihikari, IR-8 and IR-72 were moderately susceptible to high temperature. There were no clear species or ecotype differences, as some cultivars in each species or within ecotypes of tropical and temperature origin were equally susceptible to high temperature (for example M-202 temperate *japonica*, Labelle tropical *japonica*, CG-14 *O. glaberrima*, and WAB-12 interspecific). Decreased spikelet fertility and cultivar difference at high temperature were due mainly to decreased pollen production and pollen reception (pollen numbers on stigma). Lower spikelet fertility at elevated temperature resulted in fewer filled grains, lower grain weight per panicle, and decreased harvest index. There is a potential for genetic improvement for heat tolerance, thus it is important to screen and identify heat-tolerant cultivars. Spikelet fertility at high temperature can be used as a screening tool for heat tolerance during the reproductive phase.

Keywords: High temperature stress; Heat tolerance; Cultivars; Pollen production; Pollen reception; Spikelet fertility; Harvest index

1. Introduction

Rice (*Oryza sativa* L.) is one of the major cereal crops consumed by humans, with approximately 90%

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of the rice produced and consumed in tropical Asia. General circulation models predict that global mean air temperatures are likely to increase by 1.4–5.8 °C by end of this century depending on changes in greenhouse gas concentrations (IPCC, 2001; Section 9.3.3, p. 555). In addition, climate is expected to be more variable with frequent episodes of stressful temperatures during crop-growing season. Recent studies have shown that annual mean maximum and minimum temperatures have increased by 0.35 and 1.13 °C, respectively, for the period of 1979–2003 at International Rice Research Institute, Manila, Philippines (Peng et al., 2004). Grain yields of rice declined by 10% for each 1 °C increase in minimum temperature during the growing-season (Peng et al., 2004). Previous, studies have shown a 7–8% yield decrease in rice for each 1 °C increase daytime maximum/nighttime minimum in temperature from 28/21 to 34/27 °C (Baker et al., 1992). Most of the rice is currently grown in regions where temperatures are close to the optimal for growth (28/22 °C); therefore, any further increase in mean temperature or episodes of high temperature during sensitive stages may reduce rice yields. Thus, identifying and developing heat-tolerant cultivars will be a vital task for rice breeders to meet the food requirements of growing populations in future climates.

High temperatures during reproductive development are particularly injurious if they occur just before or during anthesis resulting in lower seed-set in most crops including common bean (*Phaseolus vulgaris* L.; Gross and Kigel, 1994; Prasad et al., 2002), peanut (*Arachis hypogaea* L.; Prasad et al., 1999a, 1999b, 2001, 2003), cowpea (*Vigna unguiculata* (L.) Walp; Hall, 1992), tomato (*Lycopersicon esculentum* L; Sato et al., 2000), cotton (*Gossypium hirsutum* L.; Reddy et al., 2000), wheat (*Triticum aestivum* L., Saini and Aspinall, 1982), and rice (Satake and Yoshida, 1978; Mackill et al., 1982). In rice, the reproductive processes that occur within 1 h after anthesis – dehiscence of the anther, shedding of pollen, germination of pollen grains on stigma, and elongation of pollen tubes, are more sensitive to high temperatures and are disrupted at day temperatures above 33 °C (Satake and Yoshida, 1978). Similarly, night temperature ≥29 °C increases susceptibility of rice to sterility with a subsequent reduction in seed-set and grain yield (Ziska et al., 1996; Satake and Yoshida, 1978).

There is no information available to document species or ecotype differences in response to high temperature. Two major subspecies of *Oryza sativa* grown across the globe are: *indica* and *japonica* types. In tropical regions of Africa, *Oryza glaberrima* types are also grown. Studies have shown that intraspecific variation in grain yield exists among cultivars of both *indica* and *japonica* types (Moya et al., 1998; Ziska et al., 1996; Matsui et al., 1997, 2000, 2001a) in response to high temperature. The objectives of the present study were (a) to quantify the effects of high temperature on spikelet fertility and harvest index in rice; (b) to determine if there were species, ecotype, and/or cultivar differences in response to high temperature; and (c) to understand the reasons for lower and/or differential spikelet fertility and harvest index of rice cultivars at high temperatures.

2. Materials and methods

2.1. Environmental conditions

This research was conducted between June and November for two seasons (2001 and 2002), in temperature-gradient greenhouses (TGG) at the Plant and Soil Science Field Teaching Laboratory of the University of Florida and USDA-ARS in Gainesville (29°68′N, 82°27′W), USA.

Four TGGs were located adjacent to each other aligned in a north–south direction. Sinclair et al. (1995) and Fritschi et al. (1999) describe the design, structure and control system of the TGGs. Each TGG was built on a semi-circular galvanized steel framework and covered with ‘Sixlight’ polyethylene telephtalate film (Taiyo Kogyo Co., Tokyo, Japan), which transmits about 90% of the incoming solar photosynthetically active radiation. Each TGG was 27.4 m long, 4.4 m wide and 2.2 m high at the apex.

The soil in the TGG was natural millhopper fine sand (a loamy, siliceous, hyperthermic grossarenic paeudult). Each TGG was divided into four experimental sections along the length of the structure. Each experimental section was 5 m long and 4.4 m wide, and is representative of a temperature treatment. A temperature difference of 5 °C from the inlet end (Section 1) to the outlet end (Section 4) of each

greenhouse was maintained by regulating the rate of unidirectional ventilation relative to energy input from heated air from natural gas heaters located outside the TGG and incident solar radiation (during the daytime). At night and during low solar irradiance periods of the day, heated air was introduced via ducts continuously to a point with overhead paddle fans near the top of the TGG at the beginning of Sections 2–4.

Air temperatures were measured in the center of each section with shielded and aspirated copper constantan thermocouples positioned 0.6 m above the soil surface. Carbon dioxide concentration was at ambient (approximately 370 μmol mol^{-1}) throughout the TGG. Photosynthetically active radiation was measured with a calibrated quantum sensor. The data collection, processing and operation of greenhouses were controlled by system consisting Keithley-Metrabyte hardware (Keithley Instruments, Boston, MA, USA) and FIX DMACS software (Intellution, Norwood, MA, USA). Information from the temperature and solar radiation sensors in each section in all four greenhouses was collected every minute. For our purpose moving averages at hourly intervals over 24 h periods throughout the duration of experiments was calculated. All temperatures reported are mean daytime (07:00–18:00 h Eastern Standard Time, EST)/mean nighttime (18:00–07:00 h EST). All times are reported in EST.

2.2. Cultivar and plant husbandry

Healthy grains of 10 (year 2001) to 14 (year 2002) cultivars representing a range of *japonica* and *indica* types from temperate and tropical ecosystems and *O. glaberrima* types and interspecifics from Africa (Table 1) were sown by hand on 26 June in 2001 and 2002. Grains were sown at a depth of 1–2 cm in five rows (each row was 1.6 m long) at a spacing of 20 cm at ambient temperature and ambient + 5 °C (high temperature) sections (Sections 1 and 4, respectively) in natural field soil of the TGG. Sections 2 and 3 were not used in these experiments. Greenhouses were irrigated in the late afternoon each day to maintain high soil water content. Plants were grown under non-flooded conditions, but with fully saturated soil moisture. Late afternoon irrigations were used to minimize interference with air temperatures during the pollination and seed-set phases. At the time of sowing, plots were fertilized with 60 kg N, 60 kg P and 60 kg K ha^{-1}. Additional top dressings of 60 kg N ha^{-1} each were applied at panicle initiation and at seed filling stages. Plants were healthy

Table 1
Origin, species, ecotype group, subspecies, and grain type of various rice cultivars used during 2001 and 2002

Cultivar	Origin	Species	Ecotype-subspecies	Grain type
Italica Livorna	Italy	*O. sativa*	Temperate *japonica*	Medium
M-103	USA	*O. sativa*	Temperate *japonica*	Medium
M-202	USA	*O. sativa*	Temperate *japonica*	Medium
S-102	USA	*O. sativa*	Temperate *japonica*	Short
Koshihikari	Japan	*O. sativa*	Temperate *japonica*	Short
L-204	USA	*O. sativa*	Tropical *japonica*	Long
Labelle	USA	*O. sativa*	Tropical *japonica*	Long
N-22	India	*O. sativa*	Tropical *indica*	Medium
IR-8	Philippines	*O. sativa*	Tropical *indica*	Long
IR-72	Philippines	*O. sativa*	Tropical *indica*	Long
WAB 450-12-2-BL1-DV1[a,b]	WARDA[d]	Interspecific *sativa* × *glaberrima*.	Interspecific cross WAB 56-104 × CG-14	Long
WAB 450-16-2-BL1-DR4[a,c]	WARDA	Interspecific *sativa* × *glaberrima*	Interspecific cross WAB 56-194 × CG-14	Long
CG-14[a]	WARDA	*O. glaberrima*	African *glaberrima*	Long
CG-17[a]	WARDA	*O. glaberrima*	African *glaberrima*	Long

[a] Cultivars used only in 2002.
[b] Termed as WAB-12 in this manuscript.
[c] Termed as WAB-16 in this manuscript.
[d] West Africa Rice Development Association (African Rice Center), Ivory Coast.

throughout the season and there were no problems of pests or diseases. Plots were kept free of weeds using hoes.

2.3. Phenology

Data on seedling emergence, initial plant population, and time from sowing to boot leaf stage, panicle initiation, panicle emergence, flowering, start of seed (grain) growth, and harvest maturity were noted in all treatments at every 2 d interval during 2001. In 2002, only the time from sowing to flowering and harvest maturity were noted.

2.4. Yield and harvest index

Sub-samples of five random plants were harvested at maturity in 2001, while in 2002, plants from 0.5 m long rows were harvested at maturity to determine harvest index. All plants were separated into vegetative (tillers, leaf blades and stems) and reproductive parts (panicles). Plant parts were oven dried at 65 °C for 3 d and dry weights were recorded. After drying, the panicles were threshed by hand and grain weights were recorded. Harvest index was calculated as a ratio of grain weight to total above ground crop dry weight. As cultivars were grown in randomly assigned rows, and because of differences in plant size and height among cultivars, and smaller sampling size, the data on biomass and yield should not be extrapolated to yields per hectare as in monoculture or in large yield trials.

2.5. Spikelet fertility (seed-set)

At panicle emergence, 12 randomly selected panicles (one each from separate plant) were tagged in each cultivar in all TGG. These tagged panicles were harvested at physiological maturity and data on panicle length, panicle weight, and numbers of filled and unfilled grains per panicle, and grain weight per panicle were recorded. Spikelet fertility was estimated as the ratio of number of filled grains to total number of reproductive sites (florets) and expressed as percentage. Each floret was pressed between the forefinger and thumb to determine if the grain was filled or not. Number of filled grains included both completely and partially filled grains. Dry weights of filled spikelets, unfilled spikelets and rachis were recorded.

2.6. Pollen numbers and pollen viability

At flowering 10 individual florets from 10 different plants were collected from each cultivar in ambient and high temperature treatments. Number of pollen grains per floret, and the number of pollen grains on a stigma (pollen reception) were recorded. For pollen production, the samples were collected early in the morning just before anthesis (07:00–08:00 h EST), while floret samples to count the number of pollen germinating on stigma were collected in late afternoon (13:00–14:00 h EST). The spikelets were selected from the second or third branch from top on the primary rachis. Generally third or fourth spikelets on the branch were used to estimate pollen numbers, number of pollen grains on stigma and pollen viability.

The numbers of pollen grains on surface of stigma (pollen reception) were determined on 10 spikelets from 10 different plants. The spikelets were fixed in 50% ethanol soon after collection and stigma from these spikelets were excised on a glass slide and stained with 1% iodine potassium iodide (IKI) solution. Afterwards the numbers of pollen grains stained with IKI were counted.

Pollen viability was estimated using 1% IKI stain. Pollen grains stained uniformly were considered viable. For pollen viability, 10 anthers from 10 different plants were collected early in the morning before anthesis, and anthers were opened with a needle and pollen grains were immediately brushed on glass slide and covered with a drop of IKI. Pollen viability was estimated as the ratio of number of stained pollen to total number of pollen grains and expressed as percentage.

2.7. Leaf photosynthesis

Photosynthetic rate of individual attached flag leaves was measured with an LI-6200 Portable Photosynthesis System (LI-COR, Lincoln, NE, USA) during the period of anthesis (4 September 2001). In cultivars IR-8 and IR-72, anthesis was late therefore, photosynthetic rates were measured once on top fully expanded leaves along with measurements on other cultivars, and again on flag leaf during period of anthesis. Data reported here is at same physiological stage (seed-set) for all cultivars. Observations were taken during midday between 10:00 and 13:00 h when photosynthetic photon flux density was between 1600

and 1800 μmol m^{-2} s^{-1}. Three observations were taken from three different leaves. Leaves used for photosynthetic measurements were marked and their areas and dry weights recorded. Photosynthetic rates were expressed on a leaf area basis.

2.8. Leaf membrane thermal stability

Leaf membrane thermal stability was measured by an electrolytic leakage technique (Agarie et al., 1995) during the period of anthesis. Fully expanded flag leaves of each cultivar were selected from three different plants at ambient temperature treatments, each taken from different TGG. The cut leaves were immediately placed into a plastic bag lined with moistened filter paper and transported to the laboratory in a cold box. Thereafter, midribs were removed and leaves were thoroughly washed with de-ionized water and completed hydrated by soaking in de-ionized water for 2 h in a refrigerator. Then, each leaf was cut into 1 cm circular sections and six sections put into each vial containing 30 ml of de-ionized water. Half of the vials were used as controls (kept at room temperature, 25 °C) and the other vials were subjected to heat treatment (45 °C for 12 h) in a hot water bath. After the treatment period, both control and heat-treated samples were kept refrigerated at 5 °C for 12 h. Thereafter, the samples were brought to room temperature and conductivity readings of the aqueous phase were taken at 25 °C using an electrical conductivity meter. The samples were then autoclaved for 15 min (120 °C and 0.10 MPa). After the samples cooled to the room temperature a second conductivity reading of the aqueous phase was taken at 25 °C. Leaf cell membrane thermal stability (CMTS) was estimated using the following equation: CMTS (%) = $(1 - (T_1/T_2))/(1 - (C_1/C_2))100$, where T and C refer to conductivity in the control and heat-treated samples and subscripts 1 and 2 refer to conductance before and after autoclaving, respectively. Membrane thermal stability was expressed as relative injury (RI) using the following equation: RI (%) = 100 − CMTS.

2.9. Time-of-day of anthesis

The time-of-day of anthesis was determined for all cultivars in 2002. Anthesis timing was checked for 3 days starting from 06:00 to 13:00 h in three different greenhouses under ambient temperatures at hourly intervals. Data from all greenhouses were not collected on a single day due to time constraint in moving between greenhouses. The spikelets were considered open when anthers protruded from the glumes. In addition, anthers were tapped to check if anther dehiscence had occurred. There was no anthesis prior to 06:00 h for any cultivar.

2.10. Data analyses

The data of various traits were analyzed as a split plot design with temperatures (two ambient and ambient + 5 °C) as main-plot and cultivars (10 or 14) as sub-plots, with four replications (greenhouses) using analysis of variance techniques in SAS (SAS, 2003). Data on leaf photosynthesis, cell membrane thermostability and time-of-day of anthesis was analyzed as a completely randomized design with three replications.

3. Results

3.1. Temperature and phenology

Mean seasonal daytime (07:00–18:00 h)/nighttime (18:00–07:00 h) temperatures from emergence to harvest were 28.3/21.3 and 33.1/27.3 °C at ambient (AT) and ambient + 5 °C (high temperature, HT), respectively, in 2001; and 28.9/22.7 and 32.9/26.1 °C, respectively in 2002. Mean daytime and mean nighttime temperatures in ambient and high (ambient + 5 °C) temperature treatments throughout the growing season are shown in Fig. 1. High temperature decreased duration from emergence to appearance of flag leaf by about 2–3 d and duration to physiological maturity by about 6–8 d on average across all cultivars. The mean cultivar durations from emergence to flowering (when 50% of plants flowered) were delayed by 2.2 d at high temperature in 2001 but advanced by 2.5 d in 2002 (Table 2). This is because the mean daytime/mean nighttime temperatures from sowing to panicle emergence in high temperature treatment was about 2 °C greater in 2001 compared to 2002 (36/29 °C versus 34/27 °C), which could have exceeded the optimum temperature for panicle emergence for rice. In both years cultivars IR-

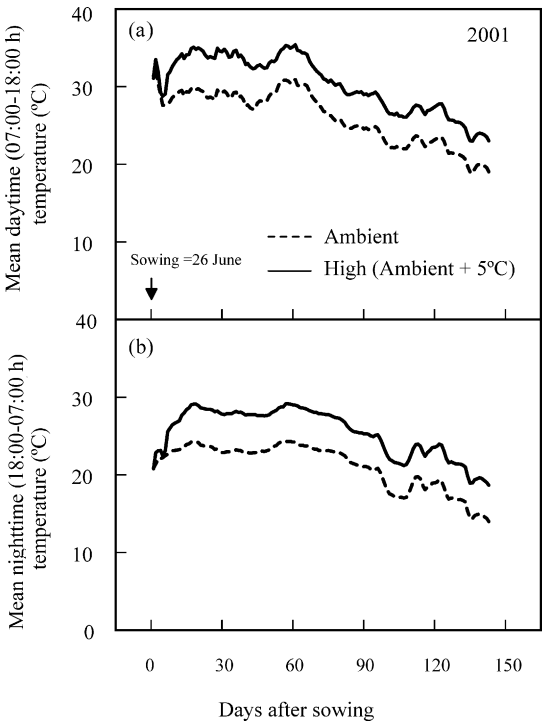

Fig. 1. Daily (a) mean daytime (07:00–18:00 h EST) and (b) mean nighttime (18:00–07:00 h EST) temperatures in ambient and high (ambient + 5 °C) temperature treatments from sowing to harvest maturity during the crop-growing season in 2001.

72 and IR-8 flowered very late in the season (longer duration from emergence to flowering). The mean daytime/mean nighttime temperatures during the period from 10 d before flowering to 5 d after anthesis (15 d periods) during 2001 and 2002 are given for each cultivar in Table 2. In both years, most cultivars flowered while mean daytime/mean nighttime temperatures during the 15 d periods were >34/27 °C in the high temperature treatment. Cultivars IR-8 and IR-72 were the exceptions; they flowered late in 2001 and were exposed to relatively cooler temperatures. In 2002, although cultivars IR-8 and IR-72 flowered late, they were exposed to higher temperatures, almost on par with other cultivars (Table 2).

3.2. Yield and harvest index

There were no significant effects of temperature (T) on vegetative biomass in both years (Table 3). The interaction between temperature and cultivar (T × C) was significant in 2001 as high temperature significantly decreased vegetative biomass in cultivars Labelle and IR-8, but not in other cultivars. High temperature significantly ($P < 0.001$) decreased grain yield in both years and the responses varied with cultivars. The negative effects (% decrease from

Table 2
Duration from sowing to 50% anthesis and mean daytime/mean nighttime temperature during booting and flowering (i.e. 10 d before to 5 d after anthesis) at ambient (AT) and high (ambient + 5 °C, HT) temperature treatments for different cultivars during 2001 and 2002

Cultivar	Duration to anthesis (d)				Temperature (day/night °C) during booting and flowering			
	2001		2002		2001		2002	
	AT	HT	AT	HT	AT	HT	AT	HT
L-204	65	72	67	65	31.1/23.7	33.9/27.9	29.6/23.9	34.1/27.6
M-202	64	66	66	63	31.0/23.6	34.9/27.7	29.6/24.0	33.7/27.4
Labelle	70	72	69	67	30.2/23.4	34.7/27.4	29.5/23.8	34.2/27.6
Italica Livorna	60	58	58	57	32.3/24.4	37.2/29.1	29.4/24.9	34.0/27.6
S-102	64	70	62	59	31.8/23.9	35.0/27.7	29.6/24.0	33.7/27.7
Koshihikari	64	68	63	61	31.4/23.8	35.4/28.3	29.6/24.0	33.8/27.6
M-103	60	69	65	62	31.3/24.0	35.0/27.8	29.6/24.0	34.0/27.6
N-22	65	62	66	64	30.9/23.6	35.3/27.9	29.5/23.9	34.0/27.6
WAB-12	–[a]	–	83	80	–	–	30.3/23.9	34.8/27.3
WAB-16	–	–	89	79	–	–	30.1/23.8	34.8/27.3
CG-14	–	–	82	79	–	–	30.1/23.8	34.6/27.2
CG-17	–	–	80	77	–	–	30.6/24.0	34.8/27.3
IR-8	95	90	100	97	26.5/20.6	31.8/25.5	29.1/23.1	33.2/26.8
IR-72	97	94	92	93	26.7/21.2	31.5/26.1	30.3/23.2	34.5/27.1

Mean seasonal day/night temperatures from emergence to harvest were 28.3/21.3 and 33.1/27.3 °C at AT and HT, respectively, in 2001 and 28.9/22.7 and 32.9/26.2 °C, respectively in 2002.

[a] Data not available.

Table 3
Effect of ambient (AT) and high temperature (ambient + 5 °C, HT) on vegetative biomass and grain yield of different rice cultivars during 2001 and 2002

Cultivar (C)	2001						2002					
	Vegetative biomass (g plant^{-1})			Grain yield (g plant^{-1})			Vegetative biomass (g m^{-1})			Grain yield (g m^{-1})		
	AT	HT	% decrease from AT	AT	HT	% decrease from AT	AT	HT	% decrease from AT	AT	HT	% decrease from AT
L-204	11.9	12.3	0[b]	11.9	1.3	89.1	80.4	72.9	9.3	84.3	12.0	85.8
M-202	12.0	10.6	11.7	13.6	1.9	86.0	174.6	159.4	8.7	106.4	22.5	78.9
Labelle	22.7	12.3	45.8	21.8	4.5	79.4	135.7	93.9	30.8	87.1	4.4	94.9
Italica Livorna	8.7	11.6	0	12.5	1.4	88.8	103.8	91.2	12.1	82.6	20.9	74.7
S-102	9.2	9.6	0	15.3	1.6	89.5	81.0	64.2	20.7	107.2	41.1	61.7
Koshihikari	9.1	9.9	0	12.3	2.7	78.0	92.8	106.0	0	63.5	16.4	74.2
M-103	8.7	9.3	0	12.2	4.4	63.9	104.7	112.5	0	74.2	16.5	77.8
N-22	17.4	23.8	0	17.7	9.2	48.0	206.1	262.4	0	111.8	130.2	7.7
WAB-12	–[a]	–	–	–	–	–	258.0	232.5	9.9	31.8	12.1	61.9
WAB-16	–	–	–	–	–	–	191.0	140.9	26.2	80.9	29.2	63.9
CG-14	–	–	–	–	–	–	384.0	278.5	27.5	160.2	21.6	86.5
CG-17	–	–	–	–	–	–	311.5	269.0	13.6	111.2	10.1	90.9
IR-8	17.7	11.2	36.7	8.2	6.2	24.4	215.5	218.5	0	88.4	45.8	48.2
IR-72	15.9	12.6	20.8	8.4	5.5	34.5	183.0	240.0	0	53.6	24.2	54.9
Mean	13.4	12.6		13.4	3.9		180.1	167.3		88.8	27.1	
LSD (0.05) T	NS			0.8			NS			10.2		
LSD (0.05) T × C	1.8			1.8			NS			27.1		

Data are the mean of four replications.
[a] Data not available.
[b] Negative values are shown as 0.

ambient) of high temperature on grain yields were lowest in cultivars N-22 (48 and 8% in 2001 and 2002, respectively), IR-8 (25 and 48%), IR-72 (35 and 55%). In all other cultivars decreases in grain yields were >60% in both years.

The negative effects of high temperature on grain yields were much greater than on biomass, leading to significantly lower harvest index at high temperatures. Harvest index was significantly ($P < 0.001$) decreased across all cultivars on average from 0.47 to 0.20 during 2001 and from 0.33 to 0.13 during 2002 (Table 4). The decrease in harvest index was less in cultivars N-22 (43 and 16% in 2001 and 2002, respectively), and WAB-16 (42% in 2002) compared to all other cultivars where the decrease in harvest index ranged between 45 and 88%.

3.3. Spikelet fertility (seed-set)

There were significant (all $P < 0.001$) effects of temperature, cultivar and interaction between temperature and cultivar on spikelet fertility during both years (Table 4). On average across all cultivars, high temperatures decreased spikelet fertility from 74 to 38% during 2001 and from 76 to 37% during 2002. The negative effects (% decrease from ambient) of high temperature on spikelet fertility were greatest in cultivar L-204 (86 and 76% in 2001 and 2002, respectively) followed by M-202 (77 and 70%), Labelle (71 and 67%) and Italica Livorna (71 and 65%) and thus classified as highly susceptible to high temperature. The smallest percent decreases were in cultivar N-22 (25 and 9%) indicating that it was the most tolerant to high temperature stress. Cultivars M-103 (45 and 20%) and S-102 (50 and 38%), Koshihikari (50 and 64%) were intermediate. The two interspecifics WAB-12 and WAB-16 and the two *O. glaberrima* types CG-14 and CG-17 were also susceptible to high temperatures and spikelet fertility was decreased by 78, 44, 63 and 65%, respectively (Table 4). During 2001, cultivars IR-8 and IR-72 had cooler temperature during anthesis, therefore spikelet fertility was not decreased. While in 2002 temperatures were comparatively higher during anthesis, thus, spikelet fertility of cultivars IR-8 and IR-72 was decreased by 14 and 35%, respectively. It was also reflected in greater decreases in grain yield of cultivars IR-8 and IR-72 in 2002 when compared to

Table 4
Effect of ambient (AT) and high temperature (ambient + 5 °C, HT) on harvest index and spikelet fertility of different rice cultivars during 2001 and 2002

Cultivar (C)	2001						2002					
	Harvest index			Spikelet fertility (%)			Harvest index			Spikelet fertility (%)		
	AT	HT	% decrease from AT	AT	HT	% decrease from AT	AT	HT	% decrease from AT	AT	HT	% decrease from AT
L-204[a]	0.45	0.09	80.0	66.4	9.1	86.3	0.49	0.13	73.5	83.3	19.7	76.4
M-202[a]	0.50	0.12	76.0	76.1	17.5	77.0	0.35	0.10	71.4	91.3	27.5	69.9
Labelle[a]	0.47	0.19	59.6	79.3	22.9	71.1	0.37	0.05	86.5	83.7	27.3	67.4
Italica Livorna[a]	0.52	0.08	84.6	69.6	20.3	70.8	0.44	0.16	63.6	90.5	31.4	65.3
S-102[b]	0.59	0.12	79.1	78.8	39.5	49.9	0.53	0.29	45.3	89.1	55.7	37.5
Koshihikari[b]	0.54	0.21	61.1	79.6	40.4	49.2	0.39	0.13	66.7	87.9	31.7	63.9
M-103[b]	0.56	0.27	51.8	85.2	45.7	46.4	0.39	0.10	74.4	74.9	59.9	20.0
N-22[c]	0.49	0.28	42.9	92.5	69.1	25.3	0.32	0.27	15.6	89.4	81.1	9.3
WAB-12[a]	–[d]	–	–	–	–	–	0.11	0.04	63.6	45.7	10.2	77.7
WAB-16[b]	–	–	–	–	–	–	0.26	0.15	42.3	79.8	44.8	43.9
CG-14[a]	–	–	–	–	–	–	0.27	0.06	77.8	67.8	25.1	63.0
CG-17[a]	–	–	–	–	–	–	0.24	0.03	87.5	63.7	22.6	64.5
IR-8[b]	0.27	0.33	0[e]	57.2	53.7	6.1	0.21	0.08	61.9	52.4	45.3	13.5
IR-72[b]	0.32	0.29	9.4	56.7	60.4	0	0.29	0.17	41.4	66.2	43.4	34.4
Mean	0.47	0.20		74.2	37.9		0.33	0.13		76.3	37.0	
LSD (0.05) T	0.02			7.0			0.04			5.4		
LSD (0.05) T × C	0.04			15.8			0.09			14.4		

Data are the mean of 48 observations (12 panicles × 4 replications).
[a] Cultivars were classified as highly susceptible when decreases in spikelet fertility and/or harvest index due to high temperature was >66%.
[b] Cultivars were classified as moderately susceptible when decreases in spikelet fertility and/or harvest index due to high temperature was 34–66%.
[c] Cultivars were classified as tolerant when decreases in spikelet fertility and/or harvest index due to high temperature was >33%.
[d] Data not available.
[e] Negative values are shown as 0.

2001. As the harvest index of cultivars M-103, S-102, Koshihikari and IR-8 was decreased by >60%, these cultivars were classified as moderately susceptible to high temperatures.

The effects of temperature, cultivar, and interaction between temperature and cultivar (all $P < 0.001$) on number of filled grains and grain weights per panicle (Table 5) were similar to those obtained on spikelet fertility. The negative effects of high temperature on number of filled grains and grain weight per panicle were small in cultivar N-22 when compared to other cultivars.

3.4. Pollen production and pollen viability

There were significant (all $P < 0.001$) effects of temperature, cultivar and interaction between temperature and cultivar on pollen production and number of pollen grains on the stigma (Table 6). On average, high temperature decreased pollen production by 51% and number of pollen grains on stigma by 43%. There were differential responses to high temperature among cultivars for pollen production. Decrease in pollen production due to high temperature was larger in cultivar M-202 (87%) followed by L-204 (78%), Italica Livorna (74%), Labelle (61%), S-102 (54%) and M-103 (49%) while the effects were smaller in cultivar N-22 (26%). Similarly, the number of pollen grains on the stigma surface was lower at high temperatures and effects varied among cultivars. These effects were similar to those on pollen production with largest negative effects of high temperature on cultivar M-202 (78%) followed by L-204 (72%) and Labelle (65%), while smallest effects were on cultivar N-22 (20%) and S-102 (27%). As mentioned earlier cultivars IR-8 and IR-72 were exposed to relatively cooler temperatures at the time of anthesis during 2001, therefore the effects on pollen were smaller. There were strong positive correlation between pollen production and

Table 5
Effect of ambient (AT) and high temperature (ambient + 5 °C, HT) on filled grains and grain weight of different rice cultivars during 2001 and 2002

Cultivar (C)	2001						2002					
	Filled grains (number panicle^{-1})			Grain weight (g panicle^{-1})			Filled grains (number panicle^{-1})			Grain weight (g panicle^{-1})		
	AT	HT	% decrease from AT	AT	HT	% decrease from AT	AT	HT	% decrease from AT	AT	HT	% decrease from AT
L-204	78.6	5.7	92.7	1.95	0.14	92.8	59.7	13.4	77.6	1.59	0.34	78.6
M-202	94.8	15.7	83.4	2.40	0.38	84.2	82.5	19.4	76.5	2.10	0.37	82.4
Labelle	214.3	39.6	81.5	3.46	0.64	81.5	139.3	39.8	71.4	2.64	0.66	75.0
Italica Livorna	48.9	11.7	76.1	1.29	0.28	78.3	42.2	13.0	69.2	1.32	0.30	77.3
S-102	69.9	22.3	68.1	2.08	0.60	71.2	64.3	30.4	52.7	1.74	0.75	56.9
Koshihikari	65.2	24.2	64.8	1.44	0.49	66.0	53.4	14.4	73.0	1.24	0.26	79.0
M-103	83.5	28.2	66.2	1.85	0.60	67.6	51.0	37.8	25.9	1.11	0.75	32.4
N-22	127.3	84.8	33.4	2.07	1.51	27.1	84.7	93.2	0	1.49	1.58	0
WAB-12	–[a]	–	–	–	–	–	56.5	11.6	79.5	1.23	0.24	80.5
WAB-16	–	–	–	–	–	–	100.1	47.5	52.5	2.33	1.15	50.6
CG-14	–	–	–	–	–	–	90.1	27.4	69.6	2.04	0.99	51.5
CG-17	–	–	–	–	–	–	55.9	20.5	63.3	1.22	0.31	74.6
IR-8	60.0	62.1	0[b]	1.43	1.47	0	66.9	39.3	41.3	1.30	1.10	15.4
IR-72	77.3	68.4	11.5	1.55	1.37	11.6	58.4	51.1	12.5	1.37	1.51	0
Mean	92.0	36.3		1.95	0.75		71.8	32.8		1.62	0.74	
LSD (0.05) T	10.3			0.23			6.0			0.14		
LSD (0.05) T × C	23.1			0.51			16.0			0.38		

Data are the mean of 48 observations (12 panicles × 4 replications).
[a] Data not available.
[b] Negative values are shown as 0.

number of pollen grains on the stigma ($r^2 = 0.76$; $n = 20$; $P < 0.01$).

High temperature decreased pollen viability from 91 to 75%, when averaged across all cultivars (Table 6). There were no significant interactions between temperature and cultivars on pollen viability. However, there were significant differences among cultivars with Labelle and Koshihikari having

Table 6
Effect of ambient (AT) and high temperature (ambient + 5 °C, HT) on pollen production, pollen reception (pollen shed), pollen viability and spikelet fertility of different rice cultivars during 2001

Cultivar (C)	Pollen production (number anther^{-1})			Pollen reception (number stigma^{-1})			Pollen viability (%)		
	AT	HT	% decrease from AT	AT	HT	% decrease from AT	AT	HT	% decrease from AT
L-204	945	211	77.7	14.3	4.0	72.1	91.6	68.7	25.0
M-202	1177	151	87.2	17.6	3.8	78.4	91.7	73.8	19.5
Labelle	1496	591	60.5	17.1	6.0	64.9	76.8	60.2	21.6
Italica Livorna	1174	306	73.9	21.7	8.8	59.4	96.2	71.8	25.4
S-102	1109	513	53.7	17.4	12.6	27.6	93.1	69.4	13.0
Koshihikari	1322	430	67.5	18.1	10.8	40.3	79.7	62.2	22.0
M-103	965	499	48.3	17.4	10.4	40.2	98.8	79.6	19.4
N-22	1380	1028	25.5	18.9	15.1	20.1	95.4	87.7	8.1
IR-8	1466	1234	15.8	17.2	15.3	11.1	93.6	89.6	4.3
IR-72	1311	1101	16.1	14.9	12.7	14.7	91.6	86.4	5.7
Mean	1235	607		17.5	10.0		90.9	74.9	
LSD (0.05) T	84			1.3			4.3		
LSD (0.05) T × C	187			2.8			NS		

Data are the mean of 10 observations.

significantly lower pollen viability compared to all other cultivars. The pollen staining technique with IKI indicates only the formation of starch in pollen grains, thus indicates potentially viable pollen. However, it does not mean that the pollen will germinate on the stigma, grow pollen tube and fertilize the egg.

3.5. Leaf photosynthesis

There were significant effects of temperature ($P = 0.001$) and cultivar ($P < 0.001$) but no interaction ($P = 0.197$) between temperature and cultivar on flag leaf photosynthetic rate (Table 7). On average across all cultivars, high temperature decreased leaf photosynthetic rates by only 14%. Among cultivars Italica Livorna, N-22 and IR-72 had significantly lower photosynthetic rates (about 23 μmol m^{-2} s^{-1}) compared to all other cultivars (about 30 μmol m^{-2} s^{-1}) when averaged across the two temperature treatments.

3.6. Leaf membrane thermal stability

The relative injury caused due to high temperature stress as a result of flag leaf electrolyte leakage for different cultivars ranged between 44 and 56%. There were slight but significant ($P = 0.023$) differences among cultivars (Table 7). The relative injury in cultivars Italica Livorna, M-103 and IR-72 were on par with each other but greater than other cultivars. Cultivars Koshihikari, N-22 and Labelle had the lowest relative injury.

3.7. Time-of-day of anthesis

Time-of-day of anthesis varied among species and cultivars (Table 7). There was a large variability in time-of-day of anthesis during different days; therefore the earliest and the latest anthesis timing recorded for the cultivars are shown. For cultivars of *O. glaberrima* (cultivars CG-14 and CG-17) and interspecifics (WAB-12 and WAB-16) anthesis occurred early during the day between 07:00 and 08:30 h compared to *O. sativa* cultivars which flowered later during the day between 09:00 and 12:30 h. Among *O. sativa*, cultivars IR-8, IR-72 and N-22, the latest time-of-day of anthesis was earlier (10:00 h) than other cultivars such as L-204, M-202 and Koshihikari (10:30 h), and Italica Livorna, S-102, and M-103 (11:00 h) and Labelle (12:30 h).

Table 7
Effect of ambient (AT) and high temperature (ambient + 5 °C, HT) on leaf photosynthetic rates of different rice cultivars at anthesis

Cultivar (C)	Leaf photosynthesis (μmol CO_2 m^{-2} s^{-1})		Relative injury (%)	Time-of-day of flowering (h) (earliest–latest)
	AT	HT	HT	AT
L-204	30.0	27.0	47.3	09:00–10:30
M-202	33.7	27.4	48.9	09:30–10:30
Labelle	39.8	28.6	44.1	09:30–12:30
Italica Livorna	22.4	22.5	55.6	10:00–11:00
S-102	28.8	33.8	47.8	09:30–11:00
Koshihikari	34.0	28.4	46.1	09:00–10:30
M-103	28.9	29.9	53.1	09:00–11:00
N-22	24.1	21.4	45.3	09:00–10:00
WAB-12	–[a]	–	–	07:00–07:30
WAB-16	–	–	–	07:30–08:30
CG-14	–	–	–	08:00–08:30
CG-17	–	–	–	07:00–08:30
IR-8	35.3	24.3	47.6	09:30–10:00
IR-72	27.4	20.1	50.7	09:30–10:00
Mean	29.9	25.8	48.7	–
LSD (0.05) T	1.9		–	
LSD (0.05) T × C	NS		6.63[b]	

Relative injury (100 − cell membrane thermostability) and time-of-day (h, EST) of anthesis in different rice cultivars. Data are the mean of three samples or observations.

[a] Data not available.

[b] LSD is for effects of cultivar.

4. Discussion

High temperature of 5 °C above the ambient air temperatures in Gainesville, Florida, significantly decreased spikelet fertility and grain yield of various rice cultivars. The mean daytime/mean nighttime air temperatures during the crop-growing season in our experiments were 28/22 °C, which are similar to those in many regions where rice is an important component of cropping system and culture (for example in tropical Asia, West Africa and southern United States). Thus, it is likely that under future climate change rice production of current cultivars will decrease in many regions where rice is presently cultivated and where current temperatures are at or above optimum.

There were cultivar differences in response to high temperature. Differential cultivar responses to high temperatures were attributed to differences in spikelet fertility and harvest index. Although in our study the most tolerant cultivar N-22 was an *indica* type and the most susceptible cultivar, L-204, was a *japonica* type, we cannot generalize that *indica* types are more tolerant than *japonica* types to high temperatures. Similarly, temperate or tropical origin, or species differences, did not necessarily reflect in tolerance, as cultivars in *O. glaberrima* type (CG-14 and CG-17), or interspecific line (WAB-12) or tropical *japonica* (Labelle) or temperate *japonica* (L-204) were equally susceptible and had poor spikelet fertility at high temperature. Previous research in controlled environments showed no difference in response of tropical *indica* (IR-72) and temperate *japonica* (M-103) in terms of high temperature tolerance (Snyder, 2000). Yield losses due to high temperatures were similar and the ceiling daytime maximum/nighttime minimum temperatures (seed yield = zero) were 40/30 °C for both cultivars (Snyder, 2000). Matsui et al. (2001a) showed variation in Japanese cultivars in response to high temperature stress during daytime (10:00–16:00 h). While, Baker (2004) emphasized that southern US rice cultivars Cocodrie, Cypress, and Jefferson were equally sensitive and all of them did not produce any seed when exposed to a constant temperature of 36 °C during the day and night. Severe effects of seed-set in studies of Baker (2004) could be due to extremely high night temperatures. Most of these studies (Snyder, 2000; Matsui et al., 2001a; Baker, 2004) and results presented here evaluated only a limited number of cultivars. Further research with more numbers of cultivars from each species and ecotype is needed to document species differences and/or influence of place of origin on high temperature tolerance in rice.

The main effects of high temperature stress during flowering in our study were (a) decreased pollen production; and (b) indehiscence of anthers resulting in poor pollen shed and decreased number of pollen grains intercepted by the stigma. Although not documented in our study poor pollen germination after reaching the stigma might have restricted seed-set. Physiologically lower pollen production at elevated temperature may be attributed to impaired cell division of microspore mother cells (Takeoka et al., 1992). Whereas high temperatures at and soon after anthesis can result in poor anther dehiscence, poor pollen germination and retarded pollen tube growth. All of these can cause poor spikelet fertility. Overall, in our study there were strong positive correlations between spikelet fertility and pollen production ($r^2 = 0.71$; $n = 20$; $P < 0.01$; Fig. 2a) and pollen reception ($r^2 = 0.84$; $n = 20$; $P < 0.01$; Fig. 2b).

Satake and Yoshida (1978) suggested that in cultivar N-22, the dehiscence of anther begins soon after the glumes open and is completed when the anthers are still situated inside the glumes on short filaments, thus pollen grains of N-22 could be easily shed on to stigma at that time. Matsui et al. (2001b) observed that the heat-tolerant cultivar Nipponbare, which had higher spikelet fertility had well developed cavities in anthers and thick locule walls which enabled easy rupture of the septa in response to swelling of pollen. This mechanism resulted in better anther dehiscence and pollen shed of heat-tolerant cultivars. Zheng and Mackill (1982) suggested that heat-tolerant cultivars have better anther dehiscence and shed more pollen grains on stigma. Morphological characters such as stigma hyperplasia (i.e. proliferation of female organs or tissues including multiple stigmas) and stamen hypoplasia (i.e. decreased number of stamens or abnormal stamens), can result in failure of pollination (contact of pollen with stigma) and can affect fertilization (Takeoka et al., 1991). Study of morphological and structural traits in cultivars with differential sensitivity to high temperature may provide better understanding of heat tolerance in rice.

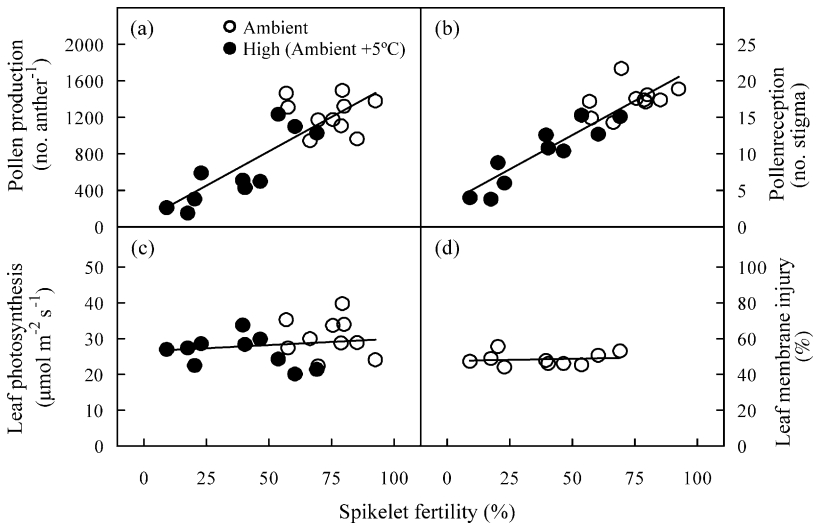

Fig. 2. Relationship between spikelet fertility and (a) pollen production; (b) pollen reception by stigma; (c) flag leaf photosynthesis; and (d) flag leaf membrane injury measured by electrolyte leakage. Open symbols are values from plants grown at ambient temperature and solid symbols are values from plants grown at high temperature (ambient + 5.0 °C), except (d) where spikelet fertility at high temperature is regressed against relative injury of leaf samples taken from ambient temperature. Regression equations: (a) $y = 15.01x + 76.96$; $r^2 = 0.71$; $n = 20$; (b) $y = 0.19x + 3.17$; $r^2 = 0.84$; $n = 20$; (c) $y = 0.035x + 26.44$; $r^2 = 0.03$; $n = 20$; (d) $y = 0.023x + 47.63$; $r^2 = 0.02$; $n = 10$.

The decreased spikelet fertility and seed yields were not a result of decreased photosynthesis at high temperature. There were no correlations between spikelet fertility and leaf photosynthesis ($r^2 = 0.03$; $n = 20$; Fig. 2c). There were no interactions between cultivar and temperature effects on leaf photosynthesis, yet there were highly significant and variable effects of high temperature on spikelet fertility and yield of rice cultivars. The heat tolerance or susceptibility of cultivars during reproductive growth was not related to cell membrane thermostability of mature flag leaves ($r^2 = 0.02$; $n = 10$; Fig. 2d). Although tolerant cultivar N-22 had higher membrane thermostability (low relative injury; 45%), it was on par with that of susceptible cultivars such as L-204 (47%) and M-202 (49%). However, the susceptible cultivar Italica Livorna had slightly higher relative injury (56%). Thus, the membrane thermostability of leaves does not always correlate with high temperature tolerance at reproductive phase with respect to spikelet fertility, however, it might give some indication of susceptibility. Similar observations were made previously on peanut (Kakani et al., 2002; Craufurd et al., 2003).

Our observations on the time-of-day for flower opening (anthesis) and pollen shed showed cultivar differences (Table 7). Despite early flowering (07:00 and 08:30 h) in African cultivars of *O. glaberrima* (CG-14 and CG-17) and interspecifics (WAB-12 and WAB-16) the spikelet fertility was decreased by high temperatures. Due to large variability in the time-of-day of anthesis during different days, further research over the entire flowering period is necessary to establish accurate time-of-day of anthesis for these cultivars. In addition, effect of high temperature on any potential changes in the time-of-day of anthesis needs attention. The time-of-day of anthesis during the day is important due to the fact that spikelet sterility is induced by high temperature during or soon after anthesis (1–3 h after anthesis in rice, Satake and Yoshida, 1978; 1–6 h after anthesis in peanut, Prasad et al., 2000) but not after fertilization is completed. In our experiment during the anthesis phase, air temperatures rapidly increased after sunrise and reached the critical temperatures of >35 °C by 09:00 h. This timing and rise in high temperatures are typical of tropical climates. Therefore, shifting time-of-day of anthesis to early hours of the morning will help plants to escape high temperature stress during processes of pollen shed, pollination and fertilization, thus can minimize the sterility caused by

high temperatures. Sheehy et al. (2001) showed a large variation in time-of-day of flowering among rice cultivars. It has been suggested that there is a potential for genetic improvement to advance flowering to an earlier time-of-day in present high yielding cultivars (Nishiyama and Blanco, 1980). A search for cultivars with earlier time-of-day of flowering among *O. sativa* would be useful for developing cultivars to avoid high temperature stress at critical stages of flower development (anthesis, pollen shed, pollen tube growth and fertilization).

Lower harvest index at elevated temperatures was due mainly to lower grain yield caused by decreased spikelet fertility. The decrease in spikelet fertility and differential response of cultivars at high temperature was mainly associated with impaired (decreased) pollen production and pollen shed (pollen reception by stigma). The tolerant cultivar N-22 had smallest decreases in spikelet fertility, grain yield and harvest index at elevated temperature, while susceptible cultivars (such as L-204 and M-202) had larger decreases in harvest index. In general, harvest index is highly correlated with grain yield, which in turn is mainly related to spikelet fertility, a function of number of percent filled grains. The fact that spikelet fertility was consistent for both years, and that it can be estimated easily by relatively small samples of panicles, indicates that spikelet fertility could be adopted as a screening tool for identifying high temperature (heat) tolerant cultivars. There is a potential for developing high temperature tolerant rice cultivars, thus efforts must continue towards cultivar screening and identifying tolerance. Breeding for high temperature tolerance and heritability of traits related to tolerance during reproductive period needs attention.

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