RESEARCH

Impact of Nighttime Temperature on Physiology and Growth of Spring Wheat

P. V. V. Prasad,* S. R. Pisipati, Z. Ristic, U. Bukovnik, and A. K. Fritz

ABSTRACT

Climate models predict greater increases in nighttime temperature in the future. The impacts of high nighttime temperature on wheat (*Triticum aestivum* L.) are not well understood. Objectives of this research were to quantify the impact of high nighttime temperatures during reproductive development on phenology, physiological, vegetative, and yield traits of wheat. Two spring wheat cultivars (Pavon-76 and Seri-82) were grown at optimum temperatures (day/night, 24/14°C; 16/8 h light/dark photoperiod) from sowing to booting. Thereafter, plants were exposed to four different nighttime temperatures (14, 17, 20, 23°C) until maturity. The daytime temperature was 24°C across all treatments. There were significant influences of high nighttime temperatures on physiological, growth, and yield traits, but no cultivar or cultivar by temperature interactions were observed. High nighttime temperatures (>14°C) decreased photosynthesis after 14 d of stress. Grain yields linearly decreased with increasing nighttime temperatures, leading to lower harvest indices at 20 and 23°C. High nighttime temperature (≥20°C) decreased spikelet fertility, grains per spike, and grain size. Compared to the control (14°C), grain filling duration was decreased by 3 and 7 d at night temperatures of 20 and 23°C, respectively. High nighttime temperature increased the expression of chloroplast protein synthesis elongation factor in both cultivars suggesting possible involvement of this protein in plant response to stress.

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Abbreviations: EF-Tu, chloroplast protein synthesis elongation factor; O, constant fluorescence; P, peak of variable fluorescence.

High temperature stress is an important yield limiting factor in both spring and winter wheat (*Triticum aestivum* L.). At the present rates of greenhouse gas emissions and population growth, it is expected that mean surface air temperatures will increase in the range of 1.8 to 5.8°C by the end of this century (Intergovernmental Panel on Climate Change, 2007). It is predicted that future climates will not only be associated with an increase in mean temperatures (Easterling et al., 1997) but also with an increase in the frequency of episodes of high temperatures (Wheeler et al., 2000). In addition, climate models foresee that there will be a relatively greater increase in nighttime temperatures as compared to daytime temperatures. Over the past century global daily minimum temperatures increased more than twice compared to increases in daily maximum temperatures (Easterling et al., 1997). Recent studies have shown that historical yields of rice (*Oryza sativa* L.; Peng et al., 2004) and wheat (Lobell et al., 2005) were strongly correlated with minimum (nighttime) temperatures, rather than daytime maximum temperatures. Decreasing rice yields in the Philippines were related to increasing nighttime temperatures (Peng et al., 2004), and increasing wheat yields in Mexico were related to decreasing nighttime temperatures (Lobell et al., 2005).

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The influence of high temperatures on growth and development of wheat and other crops is well documented (Porter and Gawith, 1999; Wheeler et al., 2000). High temperatures damage photosynthetic membranes (thylakoids) and cause chlorophyll loss (Al-Khatib and Paulsen, 1984), decrease leaf photosynthetic rate, increase embryo abortion (Saini et al., 1983), lower grain number, and decrease grain filling duration and rates (Wardlaw and Moncur, 1995; Wheeler et al., 1996; Ferris et al., 1998; Prasad et al., 2006a) resulting in lower grain yield (Wardlaw et al., 1989; Stone and Nicolas, 1994; Wheeler et al., 1996; Ferris et al., 1998; Gibson and Paulsen, 1999). At the molecular level, high temperatures adversely affect cell metabolism (Berry and Björkman, 1980; Levitt, 1980) and cause changes in the pattern of protein synthesis (Lindquist, 1986; Vierling, 1991; Larkindale et al., 2005). Supra-optimal temperatures suppress the synthesis of the normal complement of cellular proteins and at the same time induce the synthesis and accumulation of many new proteins including heat shock proteins (Vierling, 1991; Feder and Hofmann, 1999), Rubisco activase (Law and Crafts-Brandner, 2001), chloroplast glyceraldehyde 3-phosphate dehydrogenase, and chloroplast protein synthesis elongation factor (EF-Tu; Bhadula et al., 2001).

Most studies on the effects of high temperatures on crop plants have not differentiated between the day- and nighttime temperature regimes. Crop development and growth rates and duration of critical phases can be differently sensitive to minimum temperatures and maximum temperatures (Lobell and Ortiz-Monasterio, 2007). In rice, for example, high nighttime temperatures are more detrimental to grain growth than high daytime temperatures (Morita et al., 2002). Also, a modeling study has shown that in Yanqui Valley of Mexico, historical yields were strongly correlated with nighttime minimum temperatures but not daytime maximum temperatures (Lobell and Ortiz-Monasterio, 2007). It has been suggested that better understanding of the plant responses to high nighttime temperatures is needed to better quantify and reduce uncertainties in climate change impact assessments, because anthropogenic climate change is characterized by greater increases in nighttime minimum temperatures than daytime maximum temperatures (Lobell and Ortiz-Monasterio, 2007). There is very little information on the influence of different nighttime temperatures (at constant daytime temperature) during reproductive stages of wheat on various physiological, growth, developmental, and yield processes.

The objective of this study was to investigate the effects of different nighttime temperatures (14, 17, 21, and 23°C) imposed during reproductive phase (flag leaf emergence to maturity) on phenology, leaf photosynthesis, seed-set, dry matter production, and grain yield of two spring wheat cultivars under fully irrigated conditions. In addition, we also investigated the effects of high nighttime temperatures on the relative levels of chloroplast EF-Tu. This protein has been implicated in plant response to high temperature (Bhadula et al., 2001; Ristic et al., 2004; Rao et al., 2004). A recent study has shown that the relative levels of EF-Tu increase in winter wheat during the daytime episodes of heat stress (Ristic et al., 2008).

MATERIALS AND METHODS

This study was performed in controlled environment facilities at the Department of Agronomy at Kansas State University, Manhattan, KS. Experiments were conducted in fall of 2006 (Experiment 1; replication 1) to determine the effects of high nighttime temperature during reproductive development of two spring wheat cultivars on physiology, growth and yield, and relative levels of chloroplast EF-Tu. The experiment was repeated with the same treatment structure with new randomization in spring of 2007 (Experiment 2; replication 2).

Plant Husbandry and Growth Conditions

Seeds of two spring wheat cultivars Seri-82 and Pavon-76 were treated with the fungicide Captan (*N*-trichloromethylthio-4-cyclohexene-1,2-dicarboximide; Bayer Crop Science, Research Triangle Park, NC) as a precautionary measure against seed-borne diseases. Four seeds were sown by hand at a depth of 2 cm in pots (pot diameter at the top and the bottom was 21 and 16 cm, respectively; pot depth was 20 cm). Rooting medium was comprised of potting soil (Metro Mix 200, Hummert International, Topeka, KS). Four indoor growth chambers (Conviron, Model E15, Winnipeg, MB, Canada) were used for this study. There were 20 pots of each cultivar with a total of 40 pots per growth chamber. The pots were randomly arranged within each growth chamber. After emergence, plants were thinned to three plants per pot which were maintained until maturity. All four growth chambers were maintained at a day/night temperature regime of 24/14°C from sowing to the beginning of booting stage (50% of the plants at growth stage Feekes 10.0 [Large, 1954]). Thereafter, growth chambers were set at four different nighttime temperatures of 14, 17, 20, and 23°C (one temperature regime per growth chamber) from booting to harvest maturity. The treatment temperatures were randomly allocated to each chamber. The daytime temperature was maintained at 24°C in all chambers. Both daytime and nighttime temperatures were held for 10 h with a 2-h transition period. The photoperiod was 16 h and photon flux density (400–700 nm) provided by cool fluorescent lamps was 940 μmol m^{-2} s^{-1} when measured 15-cm away from the lamps and 370 μmol m^{-2} s^{-1} when measured at canopy level. Relative humidity in the chambers was set at 85%. Air temperature, relative humidity, and light level were continuously monitored at 20-min intervals in all growth chambers throughout the experiment. Pots were monitored and watered daily to keep the soil moisture at field capacity and to avoid possible water stress. To minimize or avoid dehydration, pots were kept in trays containing ~1-cm-deep water at all times. Similar growth and temperature conditions and management practices were maintained in both experiments.

Data Collection

At the start of the temperature treatments five pots of each cultivar were randomly tagged in all growth chambers. All physiological,

growth, and yield traits were collected from the plants in tagged pots. Duration to flag leaf appearance, flowering, seed-set, and physiological maturity were measured in all treatments. In addition, biomass and grain yield data were collected at maturity from plants in five pots and were used for comparison.

Physiological Traits

All physiological traits were measured on attached fully expanded flag leaves of five tagged leaves (from five different pots) of each cultivar from all treatments. Chlorophyll content and stability of photosynthetic (thylakoid) membranes were obtained after 0, 10, 22, and 28 d of treatment in Experiment 1 and after 0, 7, 14, 22, and 30 d of treatment in Experiment 2. A self-calibrating chlorophyll meter (SPAD, Model 502, Spectrum Technologies, Plainfield, IL) was used for chlorophyll measurements. Thylakoid membrane stability was assessed by measuring chlorophyll *a* fluorescence using fluorometer (Model OS 30, OptiScience, Hudson, NH), and by determining the ratio of constant fluorescence (O) to the peak of variable fluorescence (P) (Krause and Weis, 1984). Leaf level gas exchange measurements (photosynthesis, stomatal conductance, and transpiration) and leaf temperatures were measured at 0, 7, 15, and 30 d of treatment in Experiment 1 and at 0, 6, 14, 21, and 42 d of treatment in Experiment 2, using the LI-COR 6400 portable photosynthesis system (LI-COR, Lincoln, NE). Gas exchange measurements were taken at midday at growth temperature and ambient CO_2 conditions. Measurements were conducted on fully expanded flag leaves from five plants. The internal LED light source in the LI-COR 6400 was set at 1600 μmol m^{-2} s^{-1} to have a constant and uniform light across all measurements.

Growth, Dry Matter Production, Yield, and Components of Yield

At the time of anthesis, a total of 10 individual spikes were tagged on plants in five different pots (two spikes in each pot). At maturity, the numbers of filled and unfilled grains were estimated from the tagged spikes for each plant. Data from the two spikes were pooled for further analyses. Individual spikelets were checked for grain by pressing the floret between the thumb and the index finger. Spikelet fertility percentage was estimated as the ratio of spikelets with grain to the total number of spikelets.

At maturity, data on plant height (base to tip of the plant), tiller number, and spike number were recorded on plants from five pots. Plants were hand-harvested by cutting them at the ground level and component part dry weights (leaf + stem and spikes) were recorded. Leaves and stems were dried at 65°C for 7 d. Spikes were dried at 40°C for 10 d and hand-threshed, and grain dry weights were measured and reported on a per plant basis. Data on weight per grain were determined based on grain numbers and dry weights.

All the remaining plants of each cultivar from each treatment (bulk samples) were also harvested, spikes separated and threshed after drying, and data on grain weight and total vegetative dry weight of the samples were recorded after oven-drying for 7 d. Harvest index was estimated as the ratio of grain yield by the total aboveground biomass for both individual plant samples and bulk samples.

Grain Growth Rates and Grain Filling Duration

To estimate individual grain growth rates, spikes flowering on the same day in each cultivar were tagged at the time of flowering in Experiment 2. Thereafter, 5 to 10 kernels were harvested from five different pots every 7 to 10 d and dry weights were measured after oven-drying for 7 d at 62°C (all kernels were measured and individual kernel weight were determined and presented). Duration of grain filling was estimated as time from start of grain growth to time when grains reached maximum grain size. The rate of grain filling was determined by the slope of the regression line for increasing kernel weights.

EF-Tu Analyses

For EF-Tu analysis, samples of leaf tissue were obtained after 25 d of stress from fully expanded flag leaf blades from three randomly selected plants. Samples were collected early in the morning (0700 h) just before the end of nighttime temperatures regime. Collected leaves were immediately frozen in liquid nitrogen and stored at −80°C until further analyses. Chloroplast EF-Tu was analyzed using one-dimensional SDS-PAGE and immunoblotting (Bhadula et al., 2001). Total soluble protein was extracted from the leaf tissue, and protein content was determined using RC DC Protein Assay (BioRad, Hercules, CA). Extracted proteins were separated on 10% polyacrylamide gels. Equal amounts of protein (15 μg per well) were loaded on the gels. Following electrophoresis, proteins were transferred to a PVDF membrane, and blots were probed for EF-Tu using maize (*Zea mays* L.) anti-EF-Tu polyclonal antibody and the ECL immunoblot kit (Ristic et al., 2008). The relative levels of EF-Tu were estimated by determining band volume using Quantity One software (BioRad, Hercules, CA).

Data Analyses

All data were statistically analyzed using PROC MIXED in the SAS software (SAS Institute, 2003) as described by Littell et al. (2006). The experimental design was a randomized complete block with a split plot treatment structure; nighttime temperatures were used as main plots and cultivars were subplots. Individual plants were treated as repeated measures within a cultivar. There were two replications (Experiments 1 and 2) of each combination of temperature and cultivar. Replication, plants, temperature, and variety were used as class variables. Replication and replication × temperature were treated as random effects, and temperature and cultivar as fixed effects. Plants nested within variety were used in the subject statement and a compound symmetry covariance structure provided the best model fit. After PROC MIXED analyses, we used traditional regression analyses to quantify the response of individual traits to temperature, as suggested by Littell et al. (2006). This approach was chosen because (i) for most traits only temperature effects were significant, and the cultivar and interaction between temperature and cultivars were not significant; and (ii) our main objective was to see overall response to temperature rather than comparing specific temperatures. However, standard errors of means for all variables were shown as an estimate of variability. Temperature responses for all traits were tested for linear or curvilinear relationships and tested for their

significance using regression analysis in SAS. For the photosynthesis time series data (days after start of temperature treatment), means were separated using standard errors and *t* test. To determine the seed filling duration, linear-plateau regression analysis was conducted to determine the end of linear phase of seed filling period. Seed filling rates across treatments were compared using regression procedures in SAS.

RESULTS

Average nighttime temperatures during reproductive development in Experiment 1 were 14.2 (±0.6), 17.3 (±0.3), 19.8 (±0.3), and 23.2 (±0.9)°C. The temperatures in Experiment 2 were ±0.6 of the target temperatures. Relative humidity during daytime and nighttime were similar across all temperature regimes at 78 ± 9% for the entire season.

There were significant effects of temperature, but not of cultivar or interaction between temperature and cultivar on most traits (Table 1), unless specifically indicated. The temperature responses were best described by linear relationships. Linear lines were shown for traits when linear regressions were significant. Curved lines were shown to indicate the significance of curvilinear relationships.

Table 1. Probability values of main effects of temperature (T), cultivar (C), and T × C interaction on various physiological, growth, and yield traits.

Traits	T	C	T × C
Leaf photosynthesis	0.03	0.13	0.28
Stomatal conductance	0.05	0.11	0.17
Chlorophyll fluorescence	0.02	0.14	0.13
Chlorophyll content	0.05	0.15	0.25
Plant height	0.67	0.27	0.55
Tiller number	0.67	0.89	0.35
Ear number	0.50	0.51	0.54
Vegetative dry weight	0.01	0.86	0.88
Total dry weight	<0.0001	0.72	0.45
Grain dry weight	0.0013	0.15	0.02
Grain numbers	0.005	0.05	0.14
Harvest index	<0.0001	0.32	0.02
Seed-set	<0.0001	0.64	0.43
Total sites	0.018	0.31	0.57
Grain size	0.0008	0.0002	0.10

Phenology

Durations to flag leaf emergence in cultivars Seri-82 and Pavon-76 were similar (55 and 57 d after emergence). There was a significant influence of nighttime temperature on various phenological stages during reproductive development. The interaction between temperature and cultivar was not significant. Durations of various phenological stages were similar at 14 and 17°C (about 56, 63, 72, and 112 d for flag leaf appearance, anthesis, seed-set, and physiological maturity, respectively). However, relative to 14°C, exposure to 20°C nighttime temperature decreased duration to anthesis by 1 d, seed-set by 2 d, and physiological maturity by 4 d. Further increase in nighttime temperature to 23°C decreased duration to flowering, seed-set, and physiological maturity by 2, 4, and 10 d, respectively.

Physiological Traits

There were no significant differences between cultivars or interaction between cultivar and temperature on various physiological traits (Table 1). The responses at 14, 17, and 20°C were similar, but exposure to 23°C significantly decreased most traits when averaged across all measurements. Leaf temperatures were not significantly influenced by nighttime temperatures throughout reproductive development. Overall, when averaged across all measurement times during the stress period there were strong negative effects of elevated nighttime temperature >20°C on chlorophyll content and leaf photosynthetic rates (Fig. 1) and increases in O/P ratio of chlorophyll *a* fluorescence, which is indicative of greater damage to thylakoid membranes. Stomatal conductance was similar across 14, 17, and 20°C, however, exposure to 23°C slightly increased conductance by 10% (not shown).

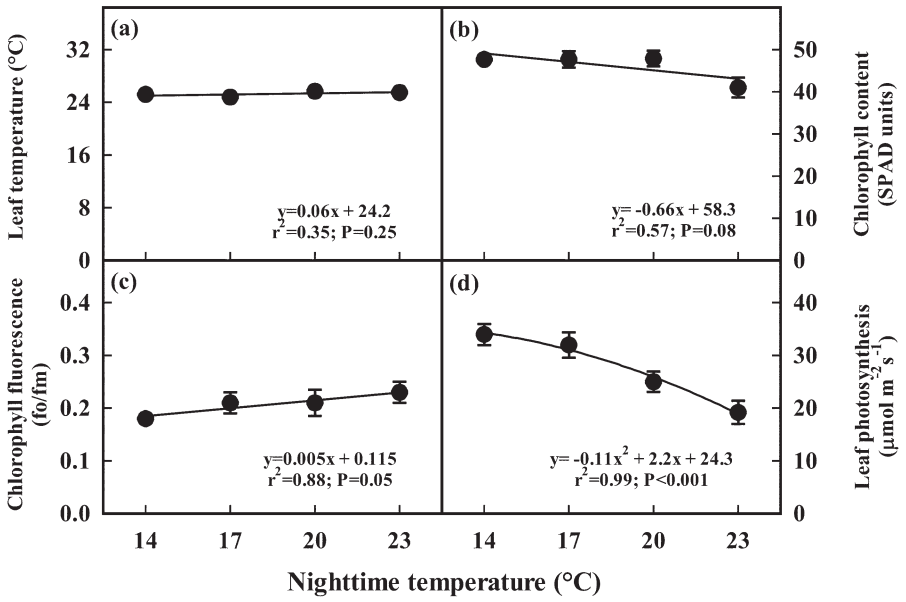

Figure 1. Impact of nighttime temperature on (a) leaf temperature; (b) chlorophyll content; (c) chlorophyll *a* fluorescence (as measured by the ratio of constant fluorescence to the peak of variable fluorescence, fo/fm); and (d) leaf photosynthetic rates. Data are averaged across two cultivars (Seri-82 and Pavon-76) and seven measurements taken at about 7- to 10-d intervals after the start of temperature treatments, two replications and five repeated measures. Each datum indicates mean value (n = 140) and vertical bars denote standard errors.

Effects of temperature over time varied with greater decreases in leaf photosynthesis as plants senesce (Fig. 2). Temperature effects were significant after 14 d of exposure (Fig. 2). The decreases in leaf photosynthesis were greater at higher nighttime temperatures (23°C) when compared to 14, 17, or 20°C. The time series responses of temperature on chlorophyll content and chlorophyll *a* fluorescence were similar to that of leaf photosynthetic rates with greater decreases in chlorophyll content and increase in thylakoid membrane damage with longer exposure to high temperatures (not shown).

Growth and Yield Traits

Plant height and tiller numbers were not significantly influenced by high nighttime temperatures (Table 1; Fig. 3a). Vegetative dry weights were significantly influenced by high nighttime temperature and the response was best described by curvilinear relationship (Fig. 3b). Vegetative growth was not affected as nighttime temperature increased from 14 to 20°C, but further increase to 23°C significantly decreased vegetative dry weights. Grain yield was linearly decreased (0.25 g plant^{-1} °C^{-1}; about 5% °C^{-1}) as temperature increased from 14 to 23°C (Fig. 3c). Harvest index at nighttime temperatures of 14 and 17°C was similar, while further increase in temperature to 20 and 23°C decreased harvest index by 5 and 10% when compared to 14°C (Fig. 3d). There was no influence of cultivar or temperature by cultivar interaction on any of the growth or yield traits (Table 1).

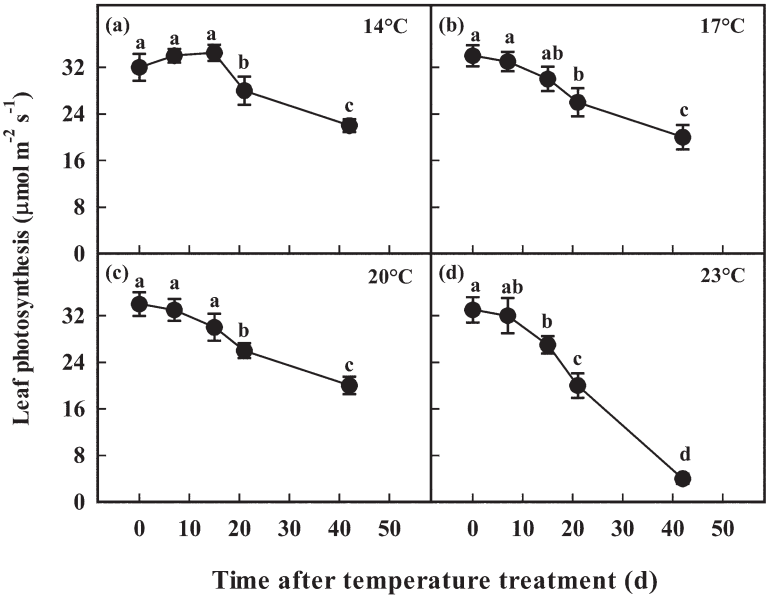

Figure 2. Time series data on leaf photosynthetic rates measured in Experiment 2 during the reproductive development at nighttime temperature regimes of (a) 14°C; (b) 17°C; (c) 20°C; and (d) 23°C. Data are averaged across two cultivars (Seri-82 and Pavon-76). Each datum indicates mean value (n = 10) and vertical bars denote standard errors. Same letters on mean values indicates no significant difference and different letters indicate significant difference.

Spikelet Fertility, Grain Numbers, and Grain Size

Spikelet fertility was significantly influenced by nighttime temperatures (Table 1; Fig. 4a). There were no significant differences in spikelet fertility at 14 and 17°C; however, exposure to 20 and 23°C significantly decreased spikelet fertility by 18 and 26%, respectively. Overall, the response of spikelet fertility to temperature was best described by linear decreases with increasing nighttime temperatures. Grain numbers per spike linearly decreased with increasing temperatures from 14 to 23°C (Fig. 4b). Similarly, total reproductive sites per spike were also significantly and linearly decreased with increasing nighttime temperatures above 14°C (Fig. 4c). The response of individual kernel weights was best described by a curvilinear relationship (Fig. 4d). There was no difference in individual grain size at nighttime temperatures of 14 to 17°C (about 36 mg kernel^{-1}). However, further increase in nighttime temperature to 20 and 23°C decreased grain size to 33 and 28 mg kernel^{-1}, respectively.

Grain Filling Rate and Grain Filling Duration

Individual kernel growth rate and grain filling duration were influenced by nighttime temperature but not by cultivar or by interaction between cultivar and temperature. Increase in nighttime temperature from 14 to 17°C did not affect grain filling rate or grain filling duration (Fig. 5). Further increase in temperatures to 20 and 23°C decreased grain filling durations by 3 and 7 d, respectively, but had no significant influence on rate of grain filling.

EF-Tu Expression

Immunoblot analysis of protein extracts from flag leaf tissue collected 1 h before the end of night period (0700 h) revealed the effects of high nighttime temperatures on the relative levels of EF-Tu (Fig. 6). At 14°C, the relative amounts of EF-Tu were low. However, increasing temperature from 14 to 20°C, resulted in over two- and fivefold increases in cultivars Seri-82 and Pavon-76, respectively.

DISCUSSION

This study clearly showed that increasing nighttime temperature from 14 to 23°C decreased grain yield of wheat. Most of the physiological and yield responses were best described by linear responses; however, some showed curvilinear responses (vegetative weights and grain size). This study also showed that the physiological processes such as leaf photosynthesis and stomatal conductance, which were measured during daylight hours when daytime temperature was the same in all treatment chambers (23°C), were also decreased in

plants exposed to higher nighttime temperatures. These differential responses of leaf photosynthesis and stomatal conductance were not related to differences in relative humidity which was similar (85 ± 8%) in all temperatures at the time of measurements. These responses may be due to indirect effects of high nighttime temperature on senescence as suggested by faster development indicated by smaller duration to seed-set and physiological maturity and also due to faster loss of chlorophyll content (SPAD reading) and damage to thylakoid membranes. Exposure to high temperature stress decreases chlorophyll synthesis and loss of chlorophyll (Tewari and Tripathy, 1998). Loss of chlorophyll is usually attributed to membrane damage (Ristic et al., 2008) and/or leaf senescence (Al-Khatib and Paulsen, 1984). Exposure to high nighttime temperature increased O/P ratio of chlorophyll *a* fluorescence indicating damage to thylakoid membrane damage (Krause and Weis, 1984); the higher the increase in O/P, the greater the damage (Krause and Weis, 1984; Ristic et al., 2008).

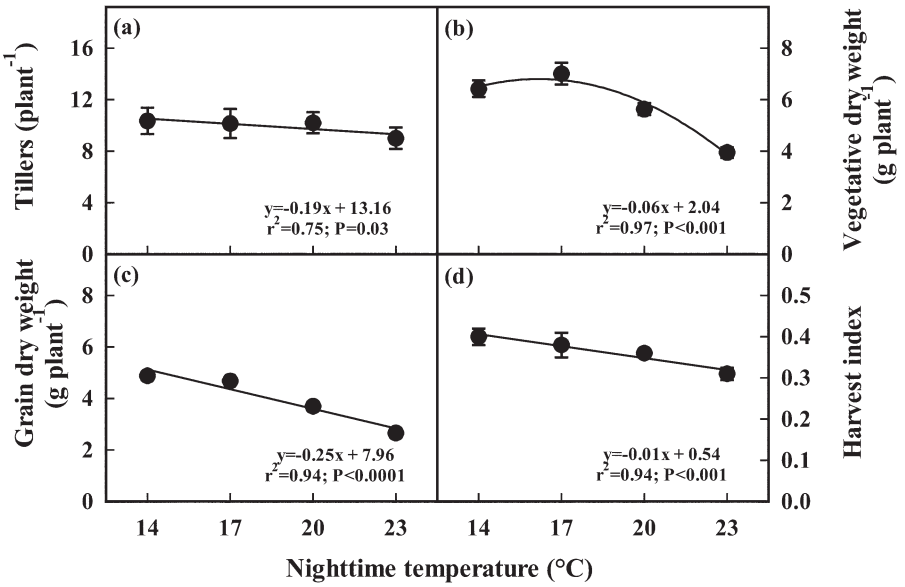

Figure 3. Impact of nighttime temperature on (a) number of tillers; (b) vegetative dry weight; (c) grain dry weight; and (d) harvest index. Data are averaged across two cultivars (Seri-82 and Pavon-76), two replications and five repeated measures (five pots). Each datum indicates mean value (n = 20) and vertical bars denote standard errors.

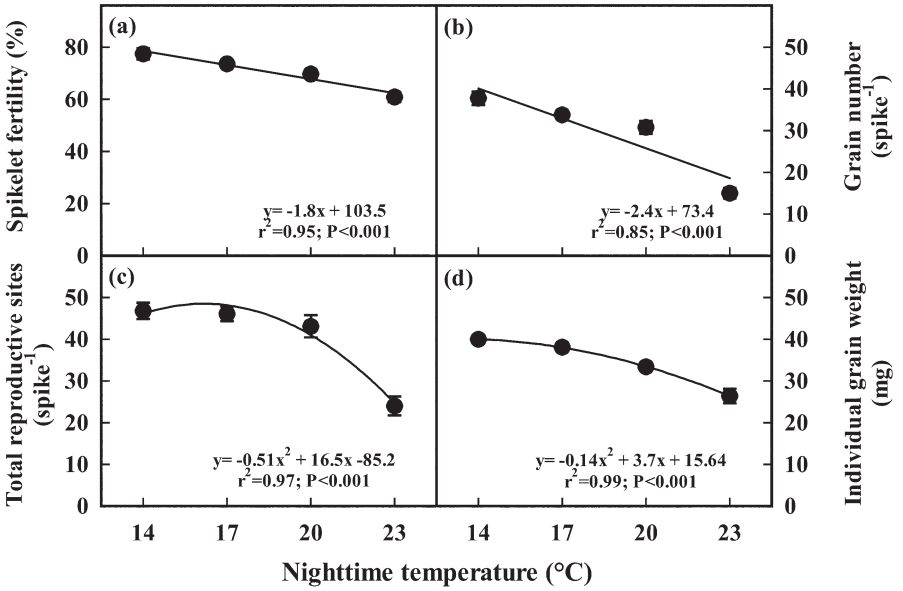

Figure 4. Impact of nighttime temperature on (a) spikelet fertility; (b) number of grains; (c) number of potential reproductive sites; and (d) individual grain weight (grain size). Data are averaged across two cultivars (Seri-82 and Pavon-76), two replications and five repeated measures (five pots). Each datum indicates mean value (n = 20) and vertical bars denote standard errors.

High nighttime temperature decreased grain numbers per spike. Lower grain numbers were a result of lower spikelet fertility and lower number of potential sites (total reproductive sites per spike). High growth temperatures are known to influence reproductive potential and reproductive processes in wheat (Saini et al., 1983) and other crop species (peanut [*Arachis hypogaea* L.] Prasad et al., 2003; sorghum [*Sorghum bicolor* (L.) Moench], Prasad et al., 2006a; and rice, Prasad et al., 2006b). Furthermore, studies have suggested that the negative effects of nighttime temperatures on seed-set are greater than those of daytime temperatures (Mutters and Hall, 1992; Ahmed et al., 1993). In cowpea [*Vigna unguiculata* (L.) Walp], larger decreases in pod-set were observed when plants were exposed to high temperature stress during the second half (later 6 h) of the 12-h night period than the first half (early 6 h) (Mutters and Hall 1992). Similarly, peanut flowers were more sensitive to high daytime temperature stress during the first 6 h of daytime than the last 6 h of the day (Prasad et al., 2000). These responses were mostly related to timing of anthesis, pollen shed, pollen germination, pollen tube growth, and fertilization. The differential responses of daytime versus nighttime temperature on reproductive traits particularly those related to anthesis timing, pollen viability, pollen tube growth, stigma viability, fertilization, and spikelet fertility of wheat are not well understood and need further investigation.

Grain size was highly sensitive to increases in nighttime temperature and was decreased above 17°C. Final grain size (kernel weight) is determined as a product of the rate and the duration of grain growth. In this study, high nighttime temperature (20°C) slightly increased

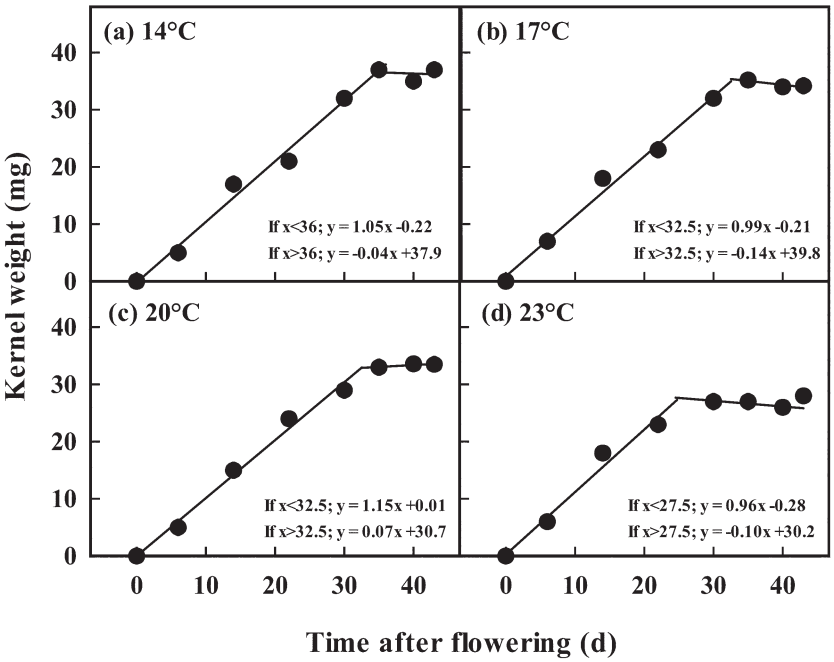

Figure 5. Time series data on individual kernel weights measured during the reproductive development at nighttime temperature regimes of (a) 14°C; (b) 17°C; (c) 20°C; and (d) 23°C. Data are averaged across two cultivars (Seri-82 and Pavon-76) and five repeated measures (five pots). Each datum indicates mean value (n = 10) and vertical bars denote standard errors.

grain growth rate in the early stages of grain growth, but decreased duration of grain growth resulting in smaller grain size. This is in agreement with previous studies which suggested that increase in grain filling rate does not compensate loss of duration, thus resulting in smaller grain size and grain yields (Shpiler and Blum, 1986; Tashiro and Wardlaw, 1991). In our study, high nighttime temperature of 23°C decreased both grain filling rate and grain filling duration. Decreased grain size could be due to decreased grain filling rates or grain filling duration or to decreases in endosperm cell numbers or cell size under high nighttime temperatures. Studies on rice suggest that high nighttime temperature decreased both grain filling rate and grain filling duration (Morita et al., 2005). In wheat, higher temperatures (30°C compared to 20°C) during grain filling period decreased endosperm sizes (Hoshikawa, 1962). Wardlaw (1970) also reported that the high temperatures (27/22°C compared to 15/10°C) during the first 5 d following anthesis did not decrease final endosperm cell numbers. In rice, it was suggested that the main factor causing the decrease in average grain weight under high nighttime temperatures was due mainly to smaller endosperm size and decreased grain filling duration (Morita et al., 2005).

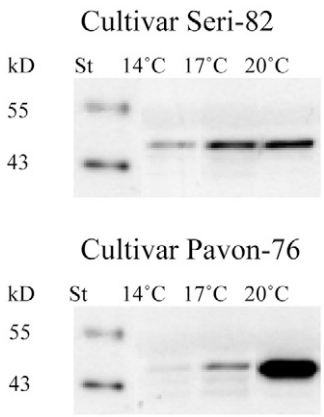

Figure 6. Impact of nighttime temperature on relative levels of chloroplast protein synthesis factor (EF-Tu) in two cultivars (Seri-82 and Pavon-76) of spring wheat. St, protein standard (Cruz Marker, Santa Cruz Biotechnology, Santa Cruz, CA). Equal amounts of protein (15 μg) were loaded in each lane.

High nighttime temperature increased expression of chloroplast EF-Tu in both cultivars (Fig. 6). Increased levels of this protein were seen at temperature (20°C) where photosynthetic rates were decreased and thylakoid membranes damaged. It may be possible that increased accumulation of EF-Tu under high night temperature conditions is related to plant response to supra-optimal temperature. Chloroplast EF-Tu plays a key role in polypeptide elongation during the translational phase of protein synthesis (Riis et al., 1990). It is possible that increases in the level of this protein may enhance the overall efficiency of protein synthesis which, in turn, may have an impact on plant response or tolerance to high temperatures (Moriarty et al., 2002). Further studies are needed to investigate the mechanism or mechanisms of action of EF-Tu and the possible relation to high-night temperatures.

The presented responses to high nighttime temperature were specific to the two chosen cultivars (Seri-82 and Pavon-76) which are popular spring wheat cultivars in various parts of the world. Furthermore, our preliminary research suggested that under high daytime temperature stress conditions, cultivar Seri-82 showed greater tolerance when compared to cultivar Pavon-76. Studies on high temperature tolerances to season-long and short-term changes in day and night temperatures have shown cultivar differences in wheat (Ristic et al., 2008; Al-Khatib and Paulsen, 1990) and other crops such as rice (Prasad et al., 2006b), peanut (Craufurd et al., 2003), cowpea (Ismail and Hall, 1998), and cotton (*Gossypium hirsutum* L.; Kakani et al., 2005). Differential cultivar responses to night temperature were shown in cowpea (Ismail and Hall, 1998). Therefore, it is possible that crop cultivars may also respond differently to changes in nighttime temperatures. Further investigations with a larger number of wheat cultivars on responses to high nighttime temperatures and screening cultivars tolerance may be useful to determine implications of nighttime temperature changes on wheat production.

In conclusion, this study showed that increasing nighttime temperature from 14 to 23°C decreased photosynthesis, seed-set, grain filling duration, and grain yield in both spring wheat cultivars (Seri-82 and Pavon-76). High nighttime temperatures (20°C) also increased expression of chloroplast EF-Tu. This research highlights the importance of high nighttime temperatures in determining the responses of wheat and possibly other crops to climate change.

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