EFFECTS OF HIGH NIGHTTIME TEMPERATURE AND ROLE OF PLANT GROWTH REGULATORS ON GROWTH, DEVELOPMENT AND PHYSIOLOGY OF RICE PLANTS

A Dissertation

by

ABDUL RAZACK MOHAMMED

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2009

Major Subject: Molecular and Environmental Plant Sciences

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Approved by:

Co-Chairs of the Committee,	Lee Tarpley
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Committee Members,	William D. Park
	Lloyd T. (Ted) Wilson
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ABSTRACT

Effects of High Nighttime Temperature and Role of Plant Growth Regulators on Growth, Development and Physiology of Rice Plants. (May 2009)

Abdul Razack Mohammed, B.S., Acharya N.G. Ranga Agricultural University; M.S., Mississippi State University

Co-Chairs of Advisory Committee: Dr. Lee Tarpley
Dr. J. Tom Cothren

Seasonally high nighttime temperatures (HNT) along the United States Gulf Coast and in regions of similar climate, during the critical stages of development, could reduce rice yield and quality. To study the effects of HNT on plant physiology, a method for applying a controlled heating treatment to plant canopies was developed using overhead infrared heaters, which are relatively inexpensive and are accurate, precise and reliable in rapidly controlling the temperature. The apparatus successfully maintained air temperatures within the set points $\pm$ 0.5 ^{o}C, and was used for all the experiments. Several experiments were conducted to determine the response of various physiological parameters during and following exposure of rice plants to HNT (32 ^{o}C) or ambient nighttime temperature (ANT) (27 ^{o}C) starting from 2000 h until 0600 h, and with or without plant growth regulator treatments. The plant growth regulator treatments included α-tocopherol (vitamin E), glycine betaine (GB), and salicylic acid (SA), which play different roles in inducing thermo-tolerance in plants.

High nighttime temperature had no effect on plant height, number of tillers and panicles, or rice net leaf photosynthetic rates. However, HNT increased leaf respiration

(dark respiration in the night) (21%) and decreased membrane thermo-stability (60%), pollen germination (20%), spikelet fertility (18% as a % of total spikelets), grain length (2%), and grain width (2%). The HNT also hastened plant development. The combinations of these effects decreased rice yield by 90%. Moreover, under HNT, there were decreases in leaf chlorophyll concentration (7%) and nitrogen concentration (18%). Application of GB and SA increased total antioxidant capacity of the rice plants by 17%, thereby decreasing the leaf respiration rates, increasing membrane thermo-stability, pollen germination, and spikelet fertility, thus increasing the yield. High nighttime temperature decreased leaf starch concentration (14%), grain total nonstructural carbohydrate (TNC) concentration (9%), and grain extractable invertase activity (20%). Vitamin E- or GB-treated plants had greater grain soluble-sugar concentrations, whereas SA-treated plants had greater leaf soluble-sugar concentrations and lower grain TNC concentrations. Invertase activity was shown to be not rate limiting or required for sucrose degradation for starch synthesis in grain of ‘Cocodrie’ rice under short-term high nighttime temperatures exposures during grain filling.

In conclusion, HNT decreased rice yield by increasing plant respiration, rate of crop development, and decreasing membrane thermo-stability, pollen germination, spikelet fertility and grain dimensions. Exogenous application of GB and SA increased yields under HNT, possibly acting through increased antioxidant levels, which might have protected the membranes and enzymes against heat-induced ROS-mediated degradation.

DEDICATION

I would like to dedicate this research to my parents,

MARYAM BEE and ABDUL GHANI MOHAMMED

ACKNOWLEDGEMENTS

I would like to express my gratitude to all the people without whose relentless support this work would not have been possible. I would like to extend a special thank you to the following people:

- Dr. Lee Tarpley, my major professor, for his patience, enthusiastic guidance, invaluable advice, continual encouragement, and financial support throughout the years of study
- Drs. J. Tom Cothren, William D. Park and Lloyd T. (Ted) Wilson, my graduate committee members, for their help and suggestions during the course of my study and research
- Dr. Charles Long with the Texas A&M University System, AgriLife Research and Extension Center for providing me the use of a LiCor 6400
- Dr. Ming-Hsuan Chen and Ms. Janis T. Delgado for their advice and technical assistance with antioxidant analysis
- Mr. James Medley for his technical assistance with leaf nitrogen analysis
- Mr. Robert Freeman for his help with electrical setup and power connections of the infrared heating system
- Mr. Ronnie Porter, Elliott Rounds, Marcus McCabe, Casey Hall, Bo Dixon, Wilbert Moore, Randy Valcin, and Patrick Huff for their great support, encouragement, and friendship during the course of my study

- Texas Rice Belt Warehouse for providing a graduate fellowship and Texas Rice Research Foundation for partial financial support of the research
- Horizon Ag, RiceTec, and the Texas Rice Improvement Association for the provision of seed
- My mother, Maryam Bee, family members, and all my friends for their love, endless encouragement, and unwavering belief in my abilities
- My wife, Huma Firdous Mohammed, for her ever-present cheerfulness, unlimited patience and never-ending support and encouragement that has allowed me to keep things in perspective and bring this project to completion
- GOD, my eternal source of inspiration, encouragement, and strength

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CHAPTER I

INTRODUCTION

Rice (*Oryza sativa* L.) is an important cereal crop grown throughout the world, with about 400 million metric tons of milled rice produced annually, mostly under flooded conditions, and covering 150 Mha (http://www.fas.usda.gov/psd/complete_table /GF-table11-176.htm). In 2008, U.S. rice acreage was 1.19 Mha and U.S. rice production was 9.23 million metric tonnes (http://www.nass.usda.gov/QuickStats/PullDataUS.jsp). The U.S. exports the majority of its produced rice and is one of the top three rice exporting countries in the world behind Thailand and Vietnam (Childs and Livezey, 2006). The value of the exported rice in 2004 was estimated to be $1.1 billion (FAO, 2007). The U.S. Gulf Coast provides a favorable environment for rice production. In Texas, the production of rice in 2004 was 676.1TMT (USDA, 2007) with the highest yields per hectare of the mid-south rice-growing region.

There is a general consensus that average global temperatures will rise by 1-6 ^{o}C in the next 100 years (Sariento and Quere, 1996; Hansen et al., 1999). Much of this increase in average daily temperature is projected to be due to increase in nighttime temperature (Alward et al., 1999). Nighttime temperatures are expected to increase at a faster rate than daytime temperatures due to less radiant heat loss because of increased cloudiness (Alward et al., 1999).

This dissertation follows the style of Crop Science.

High temperatures are a major constraint to crop productivity, especially when temperature extremes coincide with critical stages of plant development (McWilliam, 1980). Peng et al. (2004) attributed year-to-year variation in rice grain yield to nighttime temperature. High nighttime temperature (HNT) significantly reduced yields in many crop species (Thomas and Raper, 1978; Seddigh and Jolliff, 1984; Gibson and Mullen, 1996; Cantarero et al., 1999; Morita et al., 2005) by increasing respiration rate (Frantz et al., 2004), decreasing sugar and starch content (Turnbull et al., 2002), suppressing floral bud development (Ahmed and Hall, 1993), and by causing male sterility and low pollen viability (Warrag and Hall, 1984). Another effect seen as part of a reduced crop yield in response to high nighttime temperature is decreased antioxidant enzyme activities (Xu and Huang, 2004). Under normal physiological conditions, the toxic effects of reactive oxygen species (ROS) are minimized by enzymatic and non-enzymatic antioxidants (Kreiner et al., 2002). When plants were subjected to stress conditions, oxidant levels overwhelmed the antioxidant levels leading to cell damage (Kreiner et al., 2002). The production of ROS [oxide radical (O_2^-), H_2O_2, and the hydroxyl radical (-OH)] as a result of heat stress needs to be evaluated because these ROS affect many physiological processes in plants.

Plants exposed to environmental stress, such as heat, cold, drought and salinity, produce ROS, which damage macromolecules and cell membranes (Zhang and Kirkham, 1996). Oxygen is potentially toxic to all organisms because metabolism and environmental stresses generate ROS from the oxygen. Angiosperms possess several enzymatic and non-enzymatic scavenging systems to minimize deleterious effects of

ROS. These include lipid-soluble antioxidants (e.g. α-tocopherol and β-carotene), water-soluble reactants (e.g. ascorbic acid and glutathione), and enzymatic antioxidants (e.g. superoxide dismutase, catalase, and enzymes of the ascorbate and glutathione cycle) (Zhang and Kirkham, 1996). Glycine betaine (GB) and salicylic acid (SA) are synthesized in the plants and play important, but different, roles in preventing oxidative damage to the membranes (Bowler et al., 1992; Demiral and Turkan, 2004).

The α-tocopherol (vitamin E) is a small molecule that is synthesized in the plant, mainly concentrated in plastids, and is one of the most effective single-oxygen quenchers (Foyer, 1993). Glycine betaine is an important osmoprotectant and accumulates under stress conditions in some plants (Bohnert and Jensen, 1996). It is an amino-acid derivative that is naturally synthesized and accumulated in many plant families, including the Gramineae (Demiral and Turkan, 2004). It enhances tolerance to high temperatures by protecting some enzymes against heat-induced inactivation (Paleg et al., 1981). Previous studies indicated that GB is particularly effective in protecting photosystem II against heat-induced inactivation (Allakhverdiev et al., 1996). A variety of plants under various stress conditions show improvement in growth, survival, and stress tolerance due to exogenous application of GB (Harinasut et al., 1996; Rajasekaran et al., 1997; Diaz-Zorita et al., 2001). Salicylic acid is a naturally occurring phenolic compound involved in plant growth and development and is also associated with thermo-tolerance in plants. Plants pre-treated with SA show increased thermo-tolerance (Larkindale and Knight, 2002). Salicylic acid also plays an essential role in preventing oxidative damage in plants by detoxifying superoxide radicals (Bowler et al., 1992).

Moreover, there is evidence that SA can alter antioxidant capacity in plants (Chen et al., 1997; Fodor et al., 1997; Rao et al., 1997). Exogenous application of SA improved plant tolerance to heat (Dat et al., 1998) and this improved heat tolerance was associated with its protection against oxidative damage (Larkindale and Huang, 2004).

The research presented herein addresses the responses of rice to high nighttime temperatures and the effects of plant growth regulators (vitamin E, SA and GB). The plant growth regulators used in this study are defined as exogenously applied chemicals that have profound effects on plant growth, development or physiology at low concentrations. The first section deals with the description, setup, and performance of an infrared heating system, which was used in the studies to impose HNT. The second section compares the respiration rates, thermal membrane stability, and total antioxidant capacities of rice plants grown under elevated nighttime temperatures, with and without the application of plant growth regulators. The third section deals with the effects of HNT and plant growth regulators on rice growth, development, and physiology. The fourth section deals with the effects of HNT and plant growth regulators on productive tillers, spikelet fertility, grain characteristics, and yield. The final section deals with changes in sugar and starch concentrations along with invertase activity in leaves and panicles as affected by HNT during early grain-fill (EGF) stage.

CHAPTER II

INSTRUMENTATION ENABLING STUDY OF PLANT PHYSIOLOGICAL RESPONSE TO ELEVATED NIGHTTIME TEMPERATURE

Introduction

Global climate warming can affect production of crops and plants in the natural environment. Increased nighttime temperatures have been implicated in decreased crop yields throughout the world and are predicted to warm more than the daytime temperatures in the future (Houghton et al., 2001). The effects of high nighttime temperatures (HNT) include, for example, increased intervals of HNT during sensitive reproductive stages, which results in poor seed set and decreased grain-filling duration.

The provision of precise and accurate control of night-time temperatures experienced by test plants was the primary reason for developing the infrared heating apparatus. Short-term deviations of tissue temperature have been shown to affect plant function, often with effects carried beyond the period of exposure. For example, sublethal heat shock from short-term tissue temperature increases in otherwise well-controlled infrared heating studies can induce the synthesis of heat shock proteins and other physiological changes (Sun et al., 2002). Another plant-physiological feature that is easy to inadvertently alter when warming test plants is vapor pressure deficit. This can lead to decreased leaf water potential, which can trigger alterations to plants similar to those observed in sublethal heat shock, for example the synthesis of heat shock proteins (Sun et al., 2002). One means to alter the vapor pressure deficit is to alter the absolute

humidity. Kimball (2005) predicts absolute humidity will not change with global warming, suggesting the ability to maintain absolute humidities while altering temperature is a prerequisite for a heating system designed to study plant physiological response to elevated temperature.

Plant physiological experimentation employs both square-wave manipulation of environmental variables (temperature, UVB radiation) as well as ambient +/- some proportion or degree of the quantity of an environmental factor such as temperature, e.g. average seasonal temperature + x °C (Reddy et al., 2001). To facilitate the implementation of the above-mentioned techniques, the inclusion of computer-based data acquisition and control via the internet is highly desirable. Current apparatuses used to study the effects of high nighttime temperatures are limited in ability to carefully control the elevated temperature. Greenhouses, growth chambers, phytotrons, open-top chambers (OTC), and naturally-lit plant growth chambers (known as Soil-Plant-Atmosphere-Research (SPAR) units) are often used in controlled environmental studies. Greenhouses generally have higher humidity, lower wind speed, and lower light intensity compared to outside environments. Moreover, greenhouse coverings typically transmit two-thirds to three-fourths of the available sunlight (Allen et al., 1992). In artificially lit growth chambers, the temperature is well controlled; however, plants are subjected to an artificial light environment. The phytotron has similar light conditions as that of artificially lit growth chambers and also has smaller rooting volumes, which might restrict the partitioning of carbohydrates to roots (Thomas and Strain, 1991). The OTC requires a high flow rate of air in and out of the OTC to control the temperature

and the humidity (Allen et al., 1992). A number of studies have reported higher daytime and nighttime temperatures in OTCs compared to neighboring unenclosed areas (Fangmeier et al., 1986; Adaros et al., 1989). The SPAR units are one of the best in controlling environmental variables (Reddy et al., 2001); however, the cost and lack of mobility of the units makes them site-specific. In many of the above-mentioned facilities, light and temperature are difficult to control and, as a result, these facilities poorly simulate the natural environment (Tingey et al., 1996). In contrast, an infrared heating system can be employed in ways that do not alter other light intensity, humidity, and wind speed, while precisely controlling temperature.

The use of infrared heating for study of plant- and ecosystem response to global warming has increased during the last 15 years. Harte and Shaw (1995) and Harte et al. (1995) conducted a long-term study of the effect of added heat to plants in a montane community. Infrared radiation warms the vegetation similarly to that of normal solar heating and is energetically efficient because it heats the vegetation directly without having to overcome boundary layer resistance if the air were to be heated first (Kimball, 2005). An improvement in infrared heater control was made by Nijs et al. (1996), who varied the heat output to maintain a constant 2.5 ^{o}C difference in canopy temperature compared to the control plots.

The Free Air Temperature Increase (FATI), as coined by Nijs et al. (1996), is based on modulated infrared radiation and increases temperature in a controlled fashioned, without enclosing the plants. More recent reports on the use of infrared heating systems for ecosystem warming include Luo et al. (2001), Shaw et al. (2002),

Wan et al. (2002), Noormets et al. (2004) and Kimball et al. (2008). All the above-mentioned studies have primarily used infrared heating to study the effect of warming of plant population with the intent to estimate possible ecosystem effects of global warming. In contrast, we sought to develop an infrared-based system to study plant physiological responses to high temperature. This chapter describes the controlling capabilities of the presented infrared heating system, and provides results that illustrate successful application of the apparatus in the study of plant physiological response to high nighttime temperature.

Materials and Methods

Infrared Heaters

The infrared heaters, purchased from Omega (RAD 3113 BV/208, OMEGA Engineering, Inc. Stamford, Connecticut, USA) are housed in rigid aluminum that is 77.8 cm in length and 9.4 cm in width. The aluminum housing is equipped with interlocking connectors, mounting clamp, conduit connector, polished aluminum reflector, and single radiant (RAD) elements. The single RAD element is a rod-shaped heating element (1cm diameter and 57.8 cm long) mounted at the focal point of the polished aluminum reflector. The working voltage of the heating element is 120 volts and has a power of 1100 watts. The operating wavelengths of the infrared heaters are well above 1200 nm, and the infrared heater output is negligible below 1200 nm (Omega, 2008). Hence, there is no significant emission of photo-morphogenic wavelengths (wavelengths below 780 nm). Stranded, insulated, nickel-plated copper wire is used for connecting the heaters to the power controllers. For protection of the infrared heaters and personnel, a grill (GR-3, OMEGA Engineering, Inc. Stamford, Connecticut, USA) is provided for each infrared heater. A detailed description of infrared heaters is provided in 'The Electric Heaters Handbook' (Omega, 2008). Kimball et al. (2008) have previously described ways to weather proof similar infrared heaters.

Power Semi-conductor Controllers

A silicon controlled rectifier (SCR) power controller is an output device used for fast heat switching, to control variable resistance heaters and to switch higher amperage

electric heaters. In the setup, the infrared heaters are controlled using power semi-conductor controllers (SCR71P-208-030-S60, OMEGA Engineering, Inc. Stamford, Connecticut, USA) to enable proportioning heating action instead of on/off action. These power controllers are single-phase models, 208 volt, 30 amp with 60-s soft-start option. These power controllers use 'phase-angled fired' proportional control, which eliminates thermal shock and extends the working life of the heating elements. The 'phase-angled fired' proportional SCR control also provides a smooth, rapid (in milliseconds) and controlled heating process. This is potentially advantageous for vegetation warming studies because it provides a nearly continuous adjustment of the heater output thus minimizing the risk of prolonged or intense heating of the plant material with unexpected deviations away from the set point. The power controller receives a 4 – 20 mA process output signal from a temperature controller. This signal is processed by the electronics in the power controller to switch the heaters at sub-cycle intervals, resulting in a smooth radiation output. A detailed description of power semi-conductor controllers is provided in 'The Electric Heaters Handbook' (Omega, 2008).

i-Series Temperature Controllers

The basic function of a temperature controller is to compare the actual temperature with the set temperature and produce an output which will maintain that set temperature. The i-Series temperature controllers (CNi16D53-C24, OMEGA Engineering, Inc. Stamford, Connecticut, USA) used in the present setup include extremely accurate digital panel meters and single loop autotuned proportional integral derivative (PID) control mode controllers that are simple to configure and use, while

providing tremendous options including direct connectivity to an Ethernet network with the ability to serve Web pages over a LAN or the Internet. The i-Series temperature controllers are Deutsche Industrial Norm (DIN) compatible for easy incorporation into industrial mounting and control systems. The instrument utilizes Chip On Board (COB) and Surface Mount Technology (SMT) assembly techniques and automation and provides ability to program and set the temperatures and alarms. The presented system uses 1/16 DIN Omega-series controllers with dual display, analog output, 0 to 10 Vdc (0-20 mA), at 500 ohm max, and relay. The embedded internet and serial communication interface allows remote Data Acquisition and Control (DAC) and flexibility in programming via OLE (object Linking and Embedding) for Process Control (OPC) software on remote PCs. The i-Series temperature controller has both RS-232 and RS-485 serial communication interface, which allows multiple temperature controllers to be connected through a single industrial server. A detailed description and applications of i-Series temperature controllers are provided in 'The Electric Heaters Handbook' (Omega, 2008).

i-Server

The temperature controllers are connected to an i-Server (EIS-2-RJ, OMEGA Engineering, Inc. Stamford, Connecticut, USA) using a RJ45 serial port. The RS-485 interface standard used for connecting the temperature controllers to the i-Server provides distances up to 1200 m, data rates up to 10 Mbps, up to 32 line drivers on the line, and up to 32 line receivers on the same line (Park et al., 2003, pp.35-83), so is

convenient for system expansion. The i-Server takes a dynamically assigned Internet Protocol (IP) address from a Dynamic Host Configuration Protocol (DHCP) server on the network. This DHCP client capability is a valuable and unique feature of the i-Server that makes it extremely easy and simple to use on almost any Ethernet network. The i-Server connects to an Ethernet network with a standard RJ45 connector. In addition, the i-Server can be used to create a virtual tunnel on an Ethernet/Internet network simulating a local point-to-point serial connection between a serial device and a PC. The serial devices will function over the Ethernet network or the Internet as if they were connected directly to a PC. The COM port on the i-Server simulates a local COM port on the PC. The i-Series temperature controllers and i-Servers connected to an Ethernet network or the Internet makes it possible to monitor and control a process from any remote place. A detailed description of the i-Server and its applications are provided in 'The Electric Heaters Handbook' (Omega, 2008).

OLE for Process Control (OPC) Server Software

The OPC servers are hardware drivers that are written to a common standard. The OPC compliant programs (OPC Clients) are available for Distributed Control System, Supervisory Control and Data Acquisition and Human Machine Interface. Previously, each software or application developer was required to write a custom interface to exchange data with hardware field devices. The OPC eliminates this requirement by defining a common, high performance interface. The OPC specification is a non-proprietary technical specification that defines a set of standard interfaces based

upon Microsoft's OLE/COM technology. A complete description and specifications of OPC servers and OPC clients are available at the OPC Website (http://opcfoundation.org/).

Thermocouples

The temperature input reading can be provided through thermocouple, RTD, or process voltage/current. Thermocouples were used in the presented system to provide flexibility, i.e., use of infrared (non-contact) thermocouple, rapid response air temperature thermocouple, or hypodermic needle-type (internal temperature of desired plant part) thermocouple as desired. In the presented system, the temperature controllers receive input from the rapid response air thermocouples (GTMQSS-040E-12, OMEGA Engineering, Inc. Stamford, Connecticut, USA), which were attached to the temperature controllers by thermocouple wire (304-T-MO-032, OMEGA Engineering, Inc. Stamford, Connecticut, USA). The thermocouples are low noise thermocouple probes with type 'T' grounded-junction probe with a Teflon-insulated extension wire and subminiature male connector termination. The thermocouple wire is also a type 'T' wire, MgO insulation, 0.03" cable and the sheath material of the wire is 304 stainless steel (Omegaclad thermocouple wire, The Electric Heaters Handbook, Omega, 2008).

Setup and Working of Infrared Heating System

The thermocouple is attached to the i-Series temperature controller by thermocouple wire. The i-Series temperature controller also communicates with the

power controller and i-Server. The i-Series temperature controller communicates with the power controller by electrical wire connections and with the i-Server through an RS-485 interface via a RJ45 serial port. The power controllers are connected to the infrared heaters by stranded, insulated, nickel-plated copper wire. The i-Server communicates with the Ethernet/Internet via a RJ45 serial port. The OLE software is installed on a PC connected via the Ethernet/Internet. The temperature can be set at predetermined set points using i-Series temperature controllers, which can be accessed from a remote distance through a PC via the internet and i-Server. Sophisticated temperature regimes can be applied through use of the OLE software, as can data acquisition.

Air temperatures can be set at predetermined set points using i-Series temperature controllers. When the temperature is below the set point, as determined by the readings from the thermocouples, a signal from an i-Series temperature controller is sent to a power controller, which in turn controls the heater output to maintain the temperature very near the set point.

Plant Culture and Temperature Treatments

Three experiments were conducted in the greenhouse at the Texas A&M University System, AgriLife Research and Extension Center at Beaumont, Texas, USA. 'Cocodrie', a commonly grown U.S. rice cultivar of tropical japonica background, was used in all the experiments. The average ambient nighttime (between 2000 h to 0600 h) temperature during the reproductive period of the rice growing season at the location varied between 26 to 28 ^{o}C (Fig. 2.1A). Hence, the ambient and elevated nighttime

temperatures were set at 27 ^{o}C and 32 ^{o}C (ambient plus 5 ^{o}C), respectively. This is a fairly large temperature difference relative to most vegetation warming studies and is also a square-wave treatment requiring the maintenance of a constant temperature over long periods of time, thus challenged the heating system's ability for accurate and precise heating without causing plant physiological artifacts.

Plants were grown in 3-L pots that were placed in a square wooden box (0.84 m^2), 10 pots per box. The boxes were lined with black plastic (thickness = 0.15 mm; FILM-GARD, Minneapolis) that served as a water reservoir. Pots were filled with a clay soil (fine montmorillonite and thermic Entic Pelludert (Chen et al., 1989) that is common to rice farms in the area. At 20 days after emergence (DAE), the boxes were filled with water to approximately 3 cm above the top of the soil in each pot. A reflective foam cover (Cellofoam Sheathing / Underlayment, Cellofoam North America, Inc., Conyers, Georgia, USA) was placed over the water surface to prevent direct infrared heating of water. A three-way split application of nitrogen was used as described by Mohammed et al. (2007). Nitrogen was applied in the form of urea and ammonium sulfate, and phosphorus in the form of P_20_5. At planting, urea-N was applied at the rate of 112.3 kg ha^{-1} along with 45.4 kg ha^{-1} phosphorus (P_20_5). The second and third nitrogen fertilizations (both 44.9 kg ha^{-1} nitrogen in the form of ammonium sulfate) were applied 20 DAE and at the panicle-differentiation stage.

Plants were subjected to nighttime temperature through the use of the nearly continuously controlled infrared heaters, which were positioned 1.0 m above the topmost part of the plants. This involved controlled heating of small unenclosed areas of the

greenhouse. Air temperatures were controlled at predetermined set points (27 ^{o}C and 32 ^{o}C). The nighttime temperatures were imposed from 2000 h until 0600 h starting from 20 days after emergence until harvest. The assignment of heat treatment to location in the greenhouse was random within each experiment. Nighttime temperature and humidity were independently monitored using standalone sensor/loggers (HOBOs, Onset Computer Corporation, Bourne, Massachusetts, USA) in both the ambient and the high nighttime temperature treatments. The HOBOs were placed at 0.75 m, 1 m and 1.25 m below the infrared heaters to measure the temperature at different levels below the heaters. In experiment-I, half of the plants under infrared heating were exposed to a wind velocity of 8.05 kilometers (km) per hour using industrial fans with speed controls (Super Fan, Mobile Air Circulator, Air Vent Inc., Dallas, Texas, USA). Wind speed was measured using a wind speed meter (Brunton, Atmospheric Data Center, ADC^{TM} • WIND^{TM}, The Brunton Company, Riverton, Wyoming, USA).

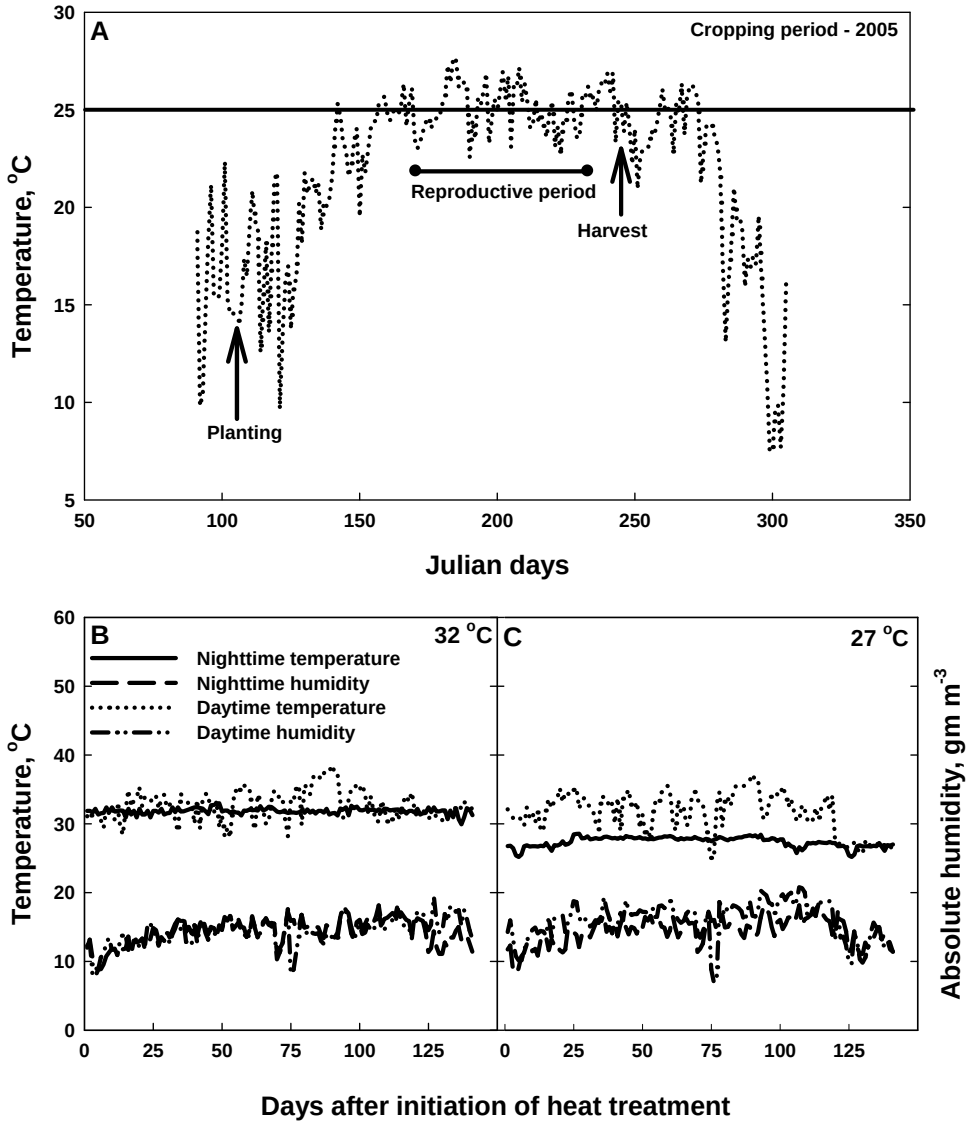

Figure 2.1. Average nighttime temperatures (between 2000–0600 h) at the Texas A&M University System, AgriLife Research and Extension Center at Beaumont during the cropping period (A) and average nighttime temperatures imposed during the course of the experiment using infrared heaters as well as average daytime temperature during the same period (B, C). In panels B and C, average daytime and nighttime humidity are also shown under two temperature regimes (B, C).

Results and Discussion

The infrared heaters provided accurate nighttime (2000–0600 h) temperatures during the cropping season (emergence to harvest). The average nighttime temperatures were 27.3 and 31.8 ^{o}C for the ambient (27 ^{o}C) and ambient + 5 ^{o}C (32 ^{o}C) temperature treatments, respectively (Fig. 2.1B, C), indicating the ability of the described apparatus to maintain a large temperature differential for an unenclosed space. For most of the time of heat exposure (82%), nighttime temperature was held within 0.5 ^{o}C for the 32 ^{o}C treatment and the minimum and maximum recorded temperatures for the 32 ^{o}C treatment were 30.0 and 32.9 ^{o}C. Similar results are reported in previous studies (Nijs et al., 1996; Kimball et al., 2008), except these studies reported short episodes of tissue temperature increases up to 14 °C above set point. We did not observe any "thermal shock" type rise in tissue temperature, which can be attributed to the combination of (1) the smooth, very rapid modulation of heater output provided through the use of the phase-angled fired SCR power controllers with the autotuned PID temperature controllers; which prevented thermal shock not only of the heating elements, but also of the target vegetation; and (2) the use of the fast response, low noise air-sensing thermocouples which were subject to very little temperature buffering of the target and system heating response. Nijs et al. (1997) also reported very little change in the surrounding air temperature, although canopy temperature differences were achieved. A difference between Nijs et al. (1997) and other infrared vegetation warming studies, compared with the present study is our use of air temperature, instead of canopy temperature for controlling system response.

The results suggest that the vapor pressure deficit is not altered to any great extent under the infrared heating (Fig. 2.1B, C). The humidity during the cropping season was 14.3 and 14.4 gm m^{-3} under the high nighttime and ambient nighttime temperature treatments, respectively. Global climate change models predict that absolute humidity will not change with global warming (Kimball, 2005). The ability to maintain the same absolute humidity in the presented study was possibly due to the heating of unenclosed areas along with light, but nearly constant, wind providing some mixing of the air. The accuracy of the infrared heater in maintaining the set temperature greatly decreased with wind speeds above 8.05 km per hour (Fig. 2.2A). At 8.05 km per hour, the infrared heaters were off by 3 ^{o}C. Similar results of decreased infrared heater thermal radiation efficiency with increase in wind speed are reported in previous studies (Kimball 2005; Kimball et al., 2008). However, the decrease in efficiency with wind speed can be estimated (Kimball 2005). In the presented setup, the infrared heaters were able to maintain the set temperatures when mounted 1 m above the canopy (Fig. 2.2B). The increase in mounting distance above the canopy also decreased the ability of the infrared heaters to maintain the set temperatures. Similar results of decrease in the ability to maintain the set temperatures with increase in mounting distance were reported by Kimball (2005).

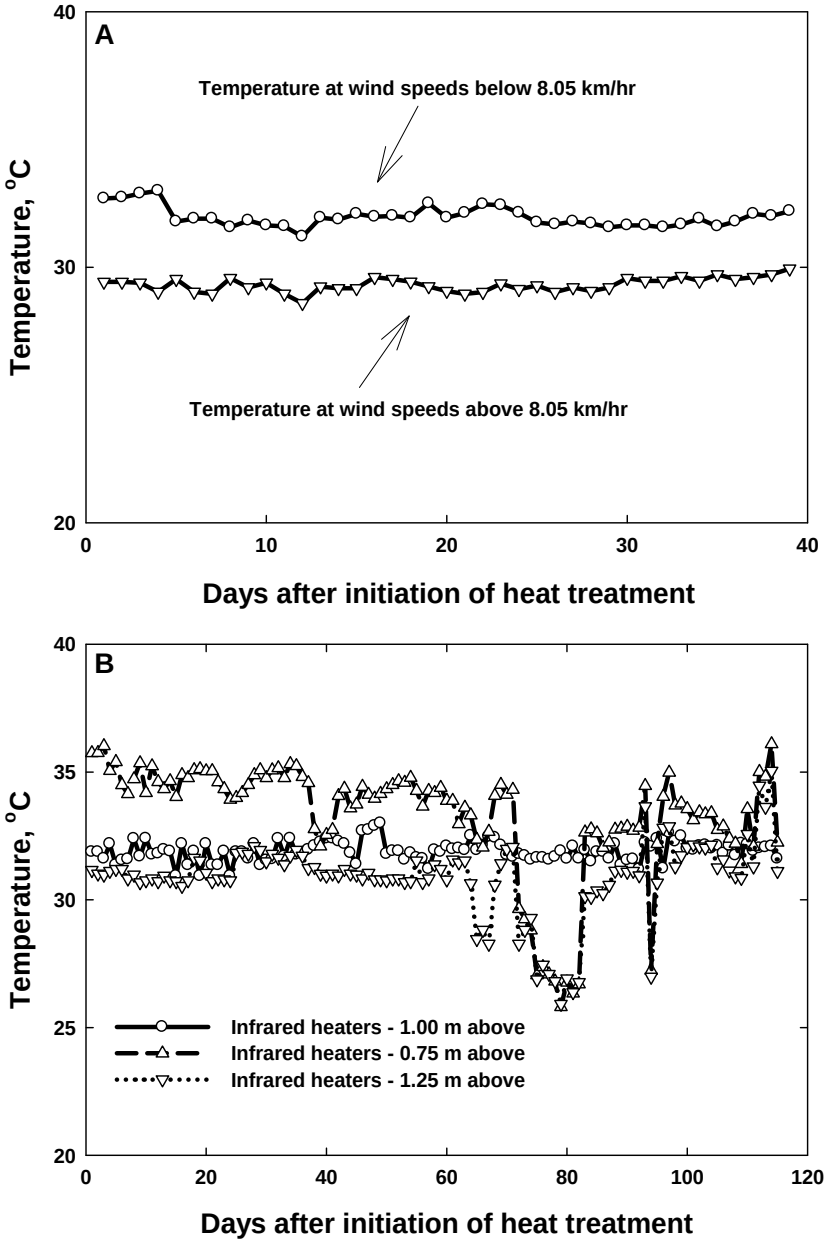

Figure 2.2. Effect of wind and mounting height above the canopy of height of the infrared heaters on the ability to control set temperatures.

Conclusions

The described infrared heating system meets the requirements of a heating system for plant physiology studies in that the elevated temperature can be accurately, precisely and reliably controlled, and can be scaled in replicated study of populations of plants with minimal perturbation of other environmental factors. Changes to physiology that can alter plant tolerance to abiotic stresses, such as “thermal shock” events or unusual alteration to the vapor pressure deficit due to change in the canopy to air temperature or change in the absolute humidity, are avoided. The combination of the lack of effect on other environmental factors and lack of unintended effects on the plant physiology indicate that the presented apparatus is suitable for study of plant physiological response to high nighttime temperature. The infrared heating system was able to maintain constant set-point temperature, provided the heaters were not too high above the vegetation, and provided wind speeds were less than 8.05 km per hour.

CHAPTER III

IMPACT OF HIGH NIGHTTIME TEMPERATURE ON RESPIRATION, MEMBRANE STABILITY, ANTIOXIDANT CAPACITY AND YIELD OF RICE PLANTS

Introduction

Rice (*Oryza sativa* L.), which is the staple food of over half of the world's population (Khush, 1997), is cultivated under a wide range of environments between latitudes 45^{o}N and 40^{o}S (Grist 1986). Global circulation models project that the global temperature is likely to increase by 1.4 to 5.8 °C because of projected increases in concentrations of all greenhouse gases by the end of the 21st century (Houghton et al., 2001). Nighttime temperatures are projected to increase more than daytime temperatures (Alward et al., 1999). Peng et al. (2004) reported an increase in nighttime temperature (increase by 1.13 ^{o}C) over a period of 25 years (1979-2003) at the International Rice Research Institute, Manila, Philippines. A rise in nighttime temperature by 1 ^{o}C can reduce rice grain yield by 10% (Peng et al., 2004). Thus, identifying and developing management practices, such as the application of plant growth regulators to prevent or negate the negative effects of high nighttime temperatures, can be beneficial for worldwide rice production and food stability.

A projected increase in plant respiration in response to climate warming is of serious concern as respiratory processes could consume a larger portion of total photosynthates (Paembonan et al., 1992). Respiration is typically partitioned into the functional components of construction (growth) and of maintenance and ion uptake

(Lambers, 1985; Amthor, 1986). Maintenance respiration is mainly associated with turnover of proteins and lipids and maintenance of ion concentration gradients across membranes (Penning de Vries, 1975) and is the most responsive to environmental changes (Ryan, 1991). High nighttime temperatures (HNT) are considered to be disadvantageous because they can stimulate respiration, thereby affecting yield (Zheng et al., 2002). Increased respiration rates as a result of high temperatures can lead to production of reactive oxygen species (ROS) in many plants (McDonald and Vanlerberghe, 2005). Under normal physiological conditions, the toxic effects of ROS are minimized by enzymic and non-enzymic antioxidants; however, under stress conditions, oxidant levels can overwhelm the antioxidant levels leading to cell damage (Kreiner et al., 2002). Increased cell damage as a result of ROS can decrease membrane thermal stability (MTS), thereby disrupting water, ion, and organic-solute movement across plant membranes, thus affecting carbon production, consumption, transport and accumulation (Christiansen, 1978). A common method of evaluating damage to membranes is by examining MTS, which measures electrolyte leakage from tissues, such as leaves, subjected to stresses, such as drought (Blum and Ebrercon, 1981), heat (Sullivan, 1972) and freezing (Dexter, 1956). Membrane thermal stability was positively associated with yield performance in wheat (*Triticum esculentum* L.) under heat-stressed conditions (Reynolds et al., 1994).

The antioxidant concentration of a plant is closely associated with its stress tolerance in some circumstances (Smirnoff, 1995). The severity of ROS-induced damage depends upon the antioxidant status of the plant. Plants pre-treated with α-tocopherol

(vitamin E), glycine betaine (GB), or salicylic acid (SA), showed induced thermo-tolerance and protection against oxidative damage (Fryer, 1992; Diaz-Zorita et al., 2001; Larkindale and Knight, 2002). The α-tocopherol is one of the most effective single-oxygen quenchers and is a strong antioxidant, whereas GB enhances tolerance to high temperatures by protecting certain enzymes (e.g., RUBISCO and citrate synthase) against heat-induced inactivation (Caldas et al., 1999; Mäkelä et al., 2000). Salicylic acid plays an essential role in preventing oxidative damage in plants by detoxifying superoxide radicals (Bowler et al., 1992) and stabilizing trimers (a trimer is a macromolecular complex formed by three, usually non-covalently bound, macromolecules like proteins or nucleic acids) of heat shock transcription factors (Larkindale and Knight, 2002). Salicylic acid is also involved in calcium signaling (Kawano et al., 1998) and induces thermo-tolerance (Larkindale and Knight, 2002). Despite the importance of antioxidants in stress tolerance, little is known about the response of rice thermo-tolerance to these plant growth regulators (vitamin E, GB and SA).

Our principal objectives in the present study were to determine 1) the effects of high nighttime temperature on respiration, membrane stability, antioxidant capacity and yield of rice plants, and 2) if application of the plant growth regulators (α-tocopherol, glycine betaine and salicylic acid) can negate the negative effects of high nighttime temperatures on rice plants. The plant growth regulators used in this study are defined as exogenously applied chemicals that have profound effects on plant growth, development or physiology at low concentrations.

Materials and Methods

Plant Culture

Three experiments were conducted in the greenhouse at the Texas A&M University System, AgriLife Research and Extension Center at Beaumont, Texas, USA. 'Cocodrie', a commonly grown U.S. rice cultivar, was used in all three experiments. In the first experiment (Exp-I), a set of plants were grown under ambient nighttime temperature (ANT) and another set under HNT. Each set had twelve plants, three plants per plant growth regulator (vitamin E, GB, SA) treatment. In the second (Exp-II) and third (Exp-III) experiments, a set of plants were grown under ANT and another set under HNT. Each set had twenty plants, five plants per plant growth regulator (vitamin E, GB, SA) treatment. Plants were grown in 3-L pots that were placed in a square wooden box (0.84 m^2), 10 pots per box. The boxes were lined with black plastic (thickness = 0.15 mm; FILM-GARD, Minneapolis) that served as a water reservoir. Pots were filled with a clay soil (fine montmorillonite and thermic Entic Pelludert (Chen et al., 1989) that is common to rice farms in the area. At 20 days after emergence (DAE), the boxes were filled with water to approximately 3 cm above the top of the soil in each pot. A foam cover was placed over the water surface to prevent direct infrared heating of water. A three-way split application of nitrogen was used as described by Mohammed et al. (2007). Nitrogen was applied in the form of urea and ammonium sulfate, and phosphorus in the form of P_20_5. At planting, urea-N was applied at the rate of 112.3 kg ha^{-1} along with 45.4 kg ha^{-1} phosphorus (P_20_5). The second and third nitrogen fertilizations (both 44.9 kg ha^{-1} nitrogen in the form of ammonium sulfate) were applied 20 DAE and at the

panicle-differentiation stage. The plants were well-matched in terms of developmental stage, as indicated by tiller development, at the beginning of the heat treatments described below.

Temperature Treatments

The assignment of heat treatment to greenhouse location was random within each experiment. The average nighttime temperatures (between 2000 h to 0600 h) at Beaumont, Texas, USA (Longitude: 94^{o} 16′ 59″ W; Latitude: 30^{o} 4′ 0″ N) during reproductive growth (panicle differentiation stage to harvest) of the rice plants often ranged above 25 ^{o}C (up to 28 ^{o}C) (Fig. 2.1A). Hence, the ambient nighttime temperature (ANT) was set at 27 ^{o}C. The greenhouse was maintained at 27 ^{o}C nighttime temperature and within this, plants of the HNT treatment were subjected to elevated nighttime temperature through the use of nearly continuously controlled (sub-second response) infrared heaters (Chromalox, Ogden, Utah, USA), which were positioned 1.0 m above the topmost part of the plants. In ambient nighttime temperature treatments, dummy heaters were provided to account for shading. The single fixed element radiant process heater is mounted in an aluminum housing (frame). The length and breadth of the frame is 77.8 cm and 9.4 cm respectively. The length of the heating element is 57.8 cm and the diameter is 1cm. The voltage of the heating element is 120 volts. The operating wavelengths of the infrared heaters are well above 1200 nm, and the infrared heater output is negligible below 1200 nm (Omega, 2008). Hence, there was no significant emission of photo-morphogenic wavelengths (wavelengths below 780 nm). The infrared

heaters were controlled using semi-conductor controllers (SCR Power Control, Watlow Electric Manufacturing Company, St. Louis, Missouri, USA) to enable proportioning heating action instead of on/off action. Air temperatures surrounding the plants were controlled at predetermined set points (27 ^{o}C and 32 ^{o}C). When the temperature was below the set point as determined by the readings from the air-temperature thermocouples positioned within a few centimeters of the uppermost parts of the plant, the controller sent a signal to the infrared heaters, which provided short slightly elevated heating events, as needed, to raise the temperature to the desired set point. Air temperatures were monitored and maintained within the set points $\pm$ 0.5 ^{o}C (Fig. 2.1B, C). The nighttime temperature was imposed from 2000 h until 0600 h starting from 20 DAE until harvest. Daytime temperature, nighttime temperature, and humidity were monitored using standalone sensor/loggers (HOBOs, Onset Computer Corporation, Bourne, Massachusetts, USA) in both portions of the study (Fig. 1B, C).

Plant Growth Regulator Treatments

The α-tocopherol (vitamin E), GB and SA were applied at the rate of 100 µL per application to the leaves using a pre-calibrated perfume-bottle sprayer. Each plant was treated three times (on a single day) to enable thorough coverage, prior to imposing heat stress. α-tocopherol was applied at the rate of 58 µmol per plant per spray (i.e., 580 mM); GB at the rate of 182.3 µmole per plant per spray (i.e., 1.823 M), and SA at the rate of 0.1 µmol per plant per spray (i.e., 1mM). Vitamin E and SA were purchased from

Sigma-Aldrich (St. Louis, MO) and GB was supplied by Capstone Food Ingredients (Marion, MA).

Respiration Measurements

Leaf-level respiration was measured using an LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, Nebraska, USA). Respiration was measured on the penultimate leaf during the grain-filling stages. The area of the chamber was 6 cm^2 and the middle portion of the leaf was preferred for measuring respiration rates (dark respiration at night per unit leaf area). While measuring respiration rates, the photosynthetic photon flux density (PPFD), provided by a 6400-02 LED light source, was set at 0 μmol m^{-2} s^{-1} (dark environment). The temperature and CO_2 concentration in the leaf cuvette were set at 25 °C and 360 μmol, respectively. Respiration was measured during the nighttime between 2300 h and 0001 h at boot stage, early grain-fill (EGF) stage and mid-dough (MD) stage.

Membrane Thermal Stability Assay

Leaves were harvested at EGF stage for the membrane thermal stability (MTS) assay. The MTS of leaves was measured using the procedure described by Martineau et al. (1979). Each sample assay consisted of two sets of five leaf discs cut with a 1 cm diameter punch from the penultimate leaves. The sets were placed into two separate test tubes with 10 mL deionized water, after rinsing them three or four times with deionised water. One set of test tubes was submerged in a water bath at 55 ^oC for 20 minutes to a

depth equal to the height of water in the tubes. The other set of test tubes was held at room temperature (25 ^{o}C). After that, both sets of test tubes were incubated at 10 ^{o}C for 12 h. Conductance was measured using a SensION5 Conductivity Meter (Hach Company, Loveland, Colorado, USA) after standardizing with known KCl solutions. Test tubes were then autoclaved for 20 min at 120 ^{o}C at 0.15 Mpa and conductance was measured again as an indication of maximum potential leakage from a given sample (Ibrahim and Quick, 2001). Relative injury (RI) was calculated using the equation RI = $(1-[1-(C_{55,i}/C_{55,f})] / [1-(C_{25,i}/C_{25,f})])*100)$ where C_{55} and C_{25} refer to the conductance at 55 ^{o}C and 25 ^{o}C respectively, with the subscripts i and f referring to the initial and final conductance.

Determination of Total Antioxidant Capacity

Total antioxidant capacity of the rice leaf was measured using the DPPH [2,2-diphenyl-1-picrylhydrazyl] assay from Goffman and Bergman (2004) with the modifications described below. For the DPPH assay, five leaf discs (0.0785 cm^2 each) were obtained from mid-blade while avoiding the mid-vein, and their fresh weights recorded. Leaf discs were placed in a test tube (4-ml) with 1.5-ml methyl alcohol (MeOH), then sealed. The test tubes were incubated at room temperature for 24 h in darkness to allow for complete extraction of antioxidants into the solution. Rice leaf extract of 40 µl was added to 960 µl DPPH (80 ppm) solution and the optical density determined at 515 nm after 4 h. The antiradical efficiency of the rice leaf methanolic extracts was determined after 4 h by monitoring the reduction in the absorbance (515

nm) of the methanolic solution of DPPH with the extract. The values of DPPH after adding the extract was compared with those obtained from a blank solution of DPPH (zero antiradical activity). Trolox (6-Hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid) and DPPH standard curves were developed and the values were expressed in μM trolox equivalents (TE) g^{-1} leaf (fresh weight basis) using these standard curves. The DPPH and trolox were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Total antioxidant capacity was measured at the boot stage and MD stage of the rice plant.

Final harvest was carried out between 110 and 114 DAE in experiments I, II and III. For yield determination, panicles were harvested separately from other plant components and then dried at 70 ^{o}C to constant weight. The panicles were threshed (HEGE 16 230 V, HEGE Maschinen, Domäne Hohebuch, D-74638 Waldenburg, Germany) and the paddy rice weighed.

Data Analysis

The experiments were laid out in a complete randomized design, and were repeated for a total of three times. One set of the plants was grown under ambient nighttime temperature, whereas the other set was grown under high nighttime temperature. In total, in each set, there were fifty-two plants, thirteen plants per plant growth regulator treatment. All data were analyzed using PROC GLM procedures in Statistical Analyses System (SAS) to determine the influence of nighttime temperatures, plant growth regulators, and their interactive effects on respiration rates, electrolytic leakage and total antioxidant capacities. Means were separated using Tukey's Least Significant Difference (LSD) at an alpha level of 0.05. Hotellings T-square analysis, a special case of multivariate analysis of variance (MANOVA), was also performed on the data. Differences among the experiments, nighttime temperatures, plant growth regulators and their interactive effects were also tested. If there was no significant difference among the experiments for a parameter, then the values from all the experiments for that parameter were used to obtain the mean and error. The standard errors of the mean were calculated and presented in the graphs.

Results

The ambient nighttime temperature and high nighttime temperature were maintained as desired throughout the course of the experiments and the humidity was similar between treatments (Fig. 2.1B, C). Mean daytime (06:00–19:00 h)/nighttime (19:00–6:00 h) temperatures during the cropping season (emergence to harvest) were 31.6/27.3 and 32.8/31.8 ^{o}C in ambient and ambient + 5 ^{o}C temperature treatments. The daytime/nighttime humidity during the cropping season was 14.4/15.9 and 14.3/14.4 gm m^{-3} in ambient and ambient + 5 ^{o}C temperature treatments (Fig. 2.1B, C).

There were no differences among the experiments for leaf respiration rates at boot or mid-dough (MD) stage. However, at early grain-fill (EGF) stage, in all the three experiments, plants grown under both the heat treatments (HNT and ANT) had 26% and 172% higher leaf respiration rates at EGF stage compared to boot and MD stages of the rice plants, respectively (Fig. 3.1). Leaf respiration was greater at 32^{o}C compared to 27 ^{o}C at boot stage, EGF stage and MD stage (Table 3.1; Fig. 3.1). In all the three experiments, leaf respiration rate, expressed as loss of carbon, was greater (27 %) in plants grown under HNT compared to plants grown under ANT at EGF stage.

There were differences among the plant growth regulator treatments (vitamin E, GB and SA) for leaf respiration rates at the boot and EGF stages of rice plants in both of the heat treatments. However, plants grown under HNT, as well as under ANT showed no differences among the plant growth regulator treatments for leaf respiration rates at MD stage (Table 3.1). At boot stage, plants treated with GB grown under HNT had greater loss (24%) of carbon compared to untreated plants in Exp-II and Exp-III.

However, at EGF stage, untreated plants grown under HNT showed greater loss of carbon (39%) compared to plant growth regulator-treated plants (Fig. 3.1). Plants grown under ANT responded differently to the plant growth regulators from the plants grown under HNT with respect to leaf respiration rates. At boot stage, plants grown under ANT treated with SA showed 52% and 59% decrease in respiration rate compared to untreated plants, in Exp-I and Exp-III respectively. However, in Exp-II there were no differences between untreated plants and SA-treated plants. At EGF stage, untreated plants showed 25% and 35% increase in loss of carbon compared to the treated plants in Exp-I and Exp-II, respectively. However, in Exp-III, plants grown under ANT treated with vitamin E showed a greater loss (7%) in carbon compared to untreated plants at EGF stage (Fig. 3.1). Electrolytic leakage expressed as relative injury measured at early grain-fill (EGF) stage was greater in plants grown under HNT compared to the plants grown under ANT (Fig. 3.2A). Plants grown under HNT showed a 60% increase in electrolytic leakage compared to plants grown under ANT. Application of vitamin E, GB and SA decreased electrolytic leakage by 31%, 42% and 30%, respectively, compared to untreated plants grown under ANT (Fig. 3.2A). However, under HNT, control plants showed levels of leakage similar to other treatments except SA, where plants showed a 10% decrease in electrolytic leakage compared to untreated plants (Fig. 3.2A). There were no differences among the experiments for electrolytic leakage (Table 3.1).

Table 3.1. Effects of experiment (Exp.), nighttime temperature (NT), and plant growth regulators (PGR) on respiration rates (RR), membrane thermal stability (MTS), total antioxidant capacity (TAC), and yield

	RR Boot	**RR EGF†**	**RR MDS‡**	**MTS EGF**	**TAC Boot**	**TAC MDS**	**Yield**
Exp.	NS	*	NS	NS	NS	NS	NS
NT	*	**	**	**	NS	NS	***
PGR	*	***	NS	*	NS	NS	***
Exp.* NT	NS	NS	NS	NS	NS	NS	NS
Exp.* PGR	NS	NS	NS	NS	NS	NS	NS
NT*PGR	NS	NS	NS	NS	*	*	NS
Exp*NT*PGR	NS	NS	NS	NS	NS	NS	NS

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels respectively.
† Early grain-fill (EGF) stage of rice plants.
‡ Mid-dough (MD) stage of rice plants.

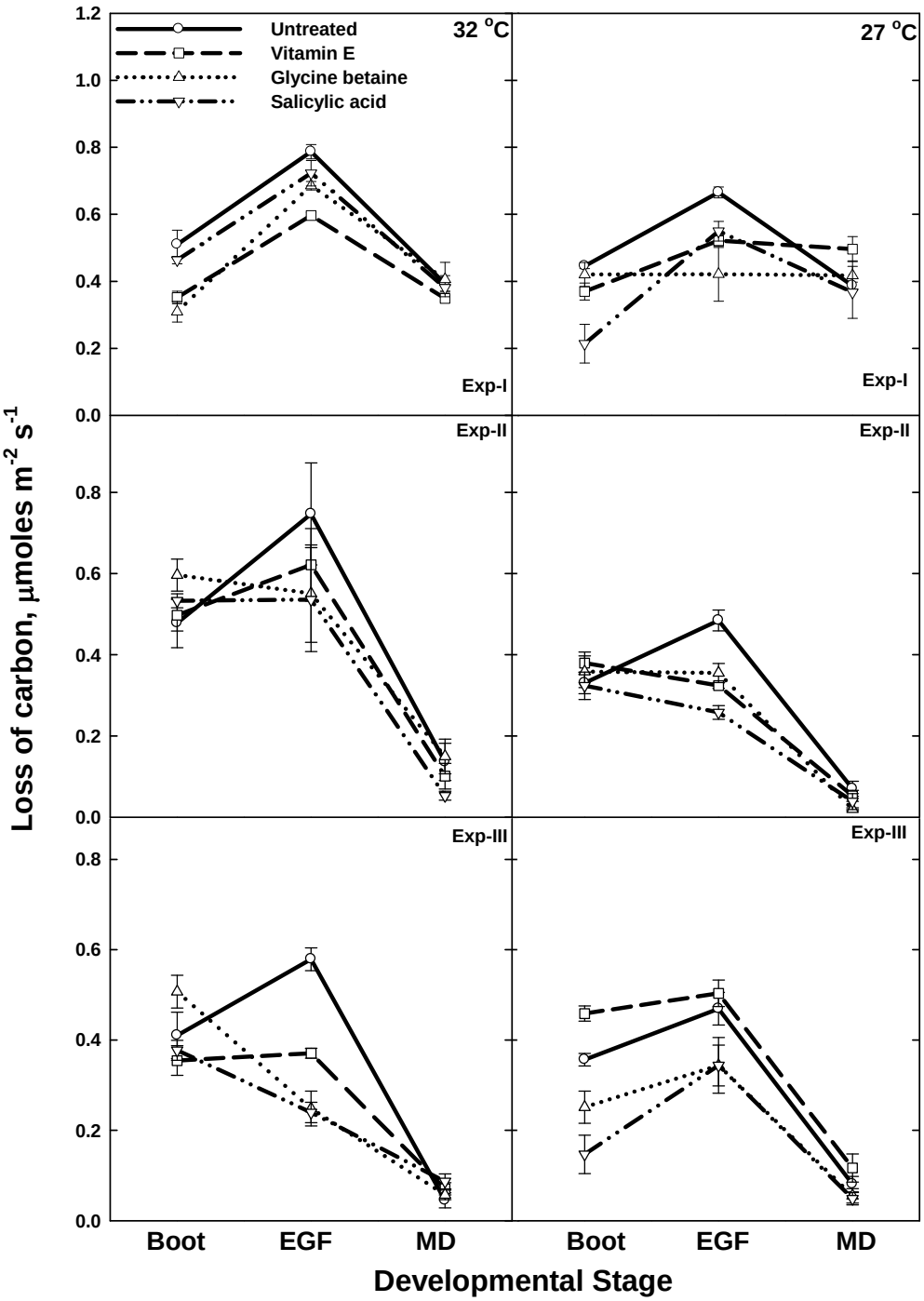

Figure 3.1. Temporal trends in leaf dark respiration of rice leaves as affected by high nighttime temperature and plant growth regulator treatments. Leaf dark respiration rates were measured at boot, early grain-fill (EGF), and mid-dough (MD) stages. The SE bars are shown if they are larger than the symbol. In Exp-I, the values are the average of three replications, whereas in Exp-II and III, the values are the average of five replications.

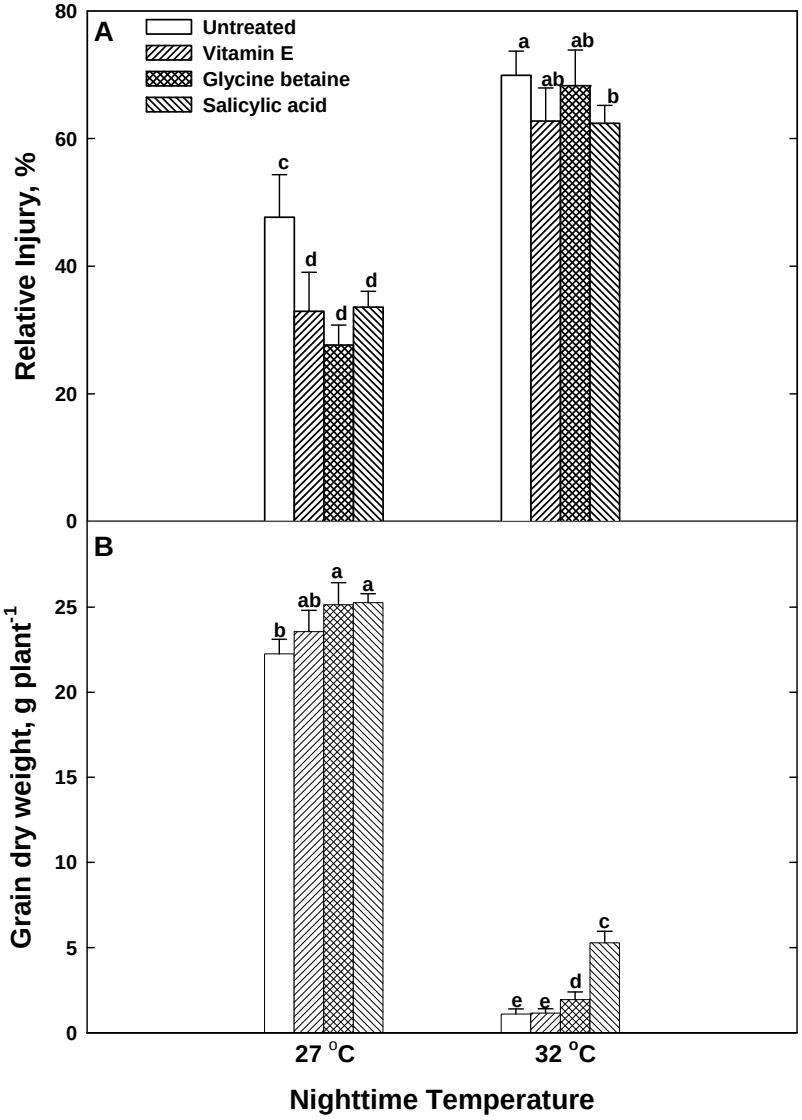

Figure 3.2. Effects of high nighttime temperature and plant growth regulators on membrane thermal stability expressed as relative injury (A) and grain dry weight (B). Membrane thermal stability was measured at early grain-fill (EGF) of the rice plant. The SE bars are shown if they are larger than the symbol. The values are averages of the results of three independent experiments. In total, there were thirteen replications for each treatment.

Total antioxidant capacities measured at boot or mid-dough (MD) stages were similar (Table 3.1). In addition, there were no differences between the heat treatments or among the plant growth regulator treatments with respect to total leaf antioxidant capacity. However, there was an interaction between the heat treatments and plant growth regulator treatments with respect to total leaf antioxidant capacity (Table 3.1). Plants grown under ANT showed no differences among the plant growth regulator treatments with respect to total leaf antioxidant capacity at boot stage and MD stage, except for plants treated with GB at boot stage (Fig. 3.3). Plants treated with GB showed a 30% increase in antioxidant capacity compared to untreated plants at boot stage. Plants grown under HNT and treated with SA showed increases of 30% and 16.7% in total antioxidant capacity at boot and MD stages, respectively (Fig. 3.3). The total antioxidant capacity declined as the plants treated with SA under HNT matured. However, there was an increase in total antioxidant capacity as the plants treated with GB under HNT matured. At MD stage, plants treated with GB grown under HNT showed a 17% increase in total antioxidant capacity (Fig. 3.3).

Plants grown under HNT showed a 90% decrease in yield compared to plants grown under ANT (Fig. 3.2B). Plants grown under ANT, treated with vitamin E, GB and SA showed a 5.8%, 12.7% and 13.5% increase in yield, respectively, compared to untreated plants. Similar results were seen in plants grown under HNT and treated with vitamin E, GB and SA. Plants treated with vitamin E, GB and SA showed 4.5%, 77% and 4-fold increase in yield, respectively, compared to untreated plants (Fig. 3.2B). The relative increases in yield as a result of GB and SA applications were different from

untreated plants in both heat treatments (Fig. 3.2B), whereas plants treated with vitamin E showed no difference from the untreated plants in either heat treatment. Rice yields were similar across experiments (Table 3.1). Our results showed strong association between leaf respiration rates, electrolytic leakage, antioxidant capacities and yield. Under HNT, increase in antioxidant capacity was associated with decrease in electrolytic leakage, which in turn was associated with decrease in respiration rates (Fig. 3.3, Fig. 3.2A and Fig. 3.1).

To evaluate patterns across the three parameters (respiration, electrolytic leakage and antioxidant capacity), the values from the untreated plant growth regulator treatment of the two heat treatments were plotted against each other (Fig. 3.4), and were analyzed using Hotellings T-square analysis, which is a special case of MANOVA for comparing two groups. The HNT and ANT differed with significant variance explained by respiration and electrolytic leakage. High nighttime temperature increased respiration and electrolytic leakage of rice plants as compared to ANT (Fig. 3.4). However, HNT had no effect on total antioxidant capacity. These parameters were strongly associated and potentially influence yield.

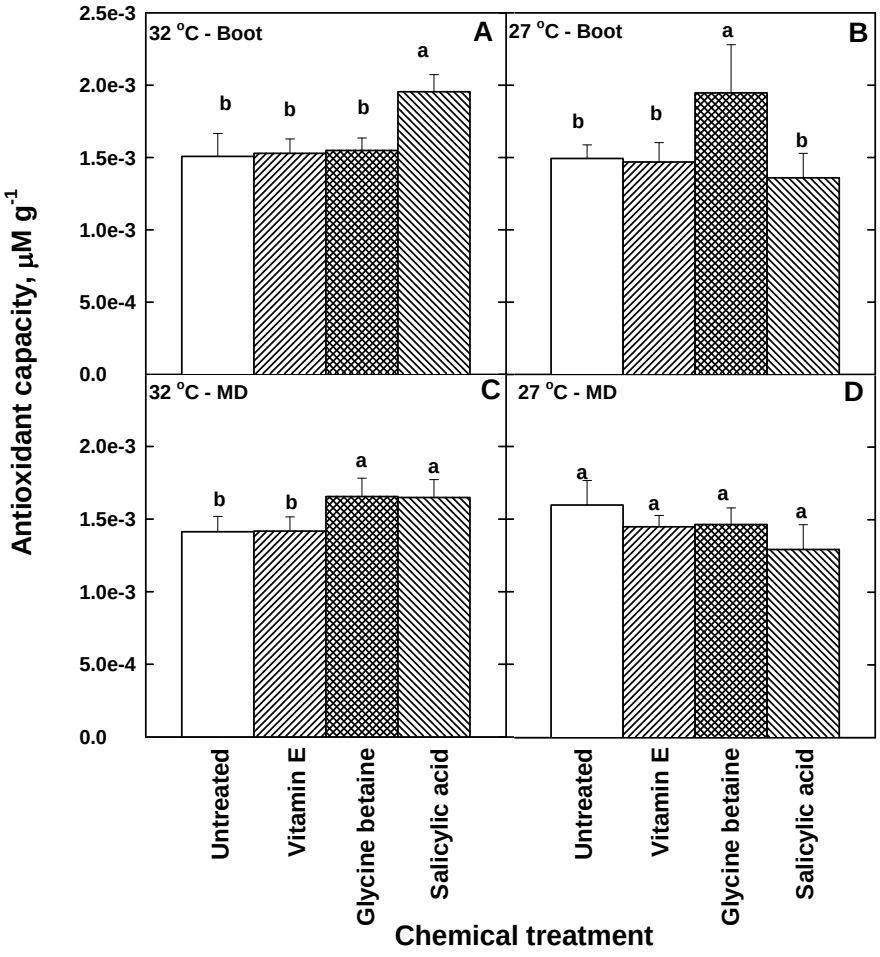

Figure 3.3. Effects of high nighttime temperature and plant growth regulators on total antioxidant capacity of rice leaves. The total antioxidant capacity was measured at boot stage and mid-dough (MD) stage of the rice plants. There are four panels (A, B, C, D) in the graph and the data shown in each panel was analyzed separately. The SE bars are shown if they are larger than the symbol. The values are averages of the results of three experiments. In total, there were thirteen replications for each treatment.

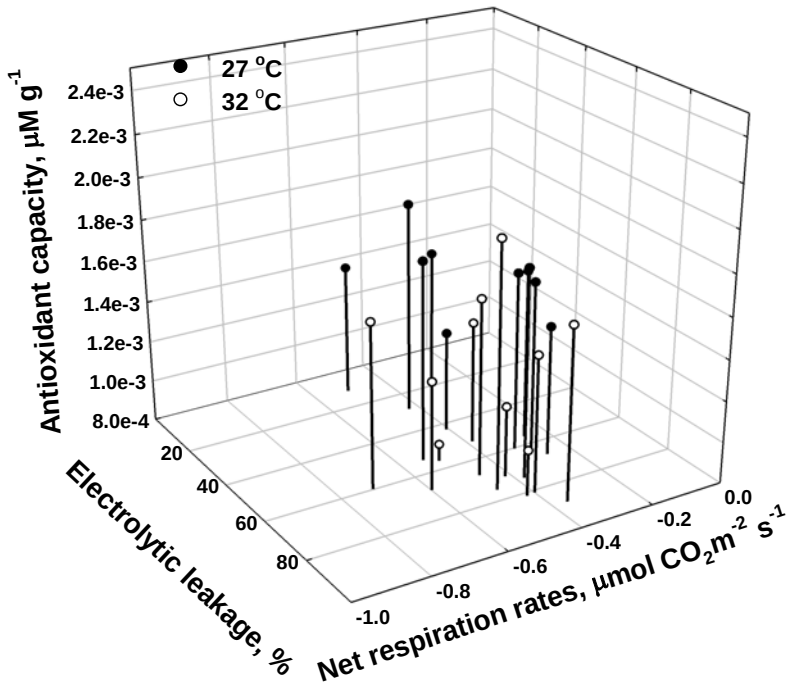

Figure 3.4. Leaf dark respiration rates, electrolytic leakage, and antioxidant capacity of leaf tissues of rice plants grown under ambient (27 ^{o}C) or high (32 ^{o}C) nighttime temperatures. Ten individual values for each parameter under each heat treatment are from untreated (no plant growth regulators) plants, which were analyzed using Hotellings T-square analysis, a special case of multivariate analysis of variance (MANOVA). The plants response to high nighttime temperature differed from the response to ambient nighttime temperature with respect to respiration rate, electrolyte leakage and antioxidant capacity in combination.

Discussion

Year-to-year variation in rice grain yield has been attributed to differences in nighttime temperatures as a result of possible global warming (Peng et al., 2004). Our results confirm conclusions from previous studies in showing an increase in respiration rates in rice plants as a result of an increase in nighttime temperature, which is associated with decrease in yield. Higher dark respiration increases the proportion of assimilates respired for maintenance and uncoupled respiration (Beevers, 1970), thereby affecting plant carbon status (Turnbull et al., 2002). Our results also indicated maximum respiration rates during grain-filling period (EGF) with decline as the plants matured (mid-dough stage). Similar trends were seen in sunflower (*Helianthus annus* L.), wheat, sorghum (*Sorghum bicolor* L. Moench.) and chick pea (*Cicer arietinum* L.), where the respiration rates peaked at grain filling stage (Albrizio and Steduto, 2003) and then decline. The decline in the rates of leaf dark respiration towards the end of the crop cycle is associated with leaf senescence (Albrizio and Steduto, 2003).

Membrane thermal stability, when measured as the conductivity of electrolytes leaking from leaf disks at high temperature, has been suggested as one of the best techniques to evaluate the performance of a plant under high temperatures (Sullivan, 1972). Our results indicated decreased membrane stability in plants grown under HNT, indicating that high temperatures of the range used in the present study lead to leaky membranes. Previous studies reported increased electrolytic leakage as a result of increased temperature in cowpea (*Vigna unguiculata* L.) (Ismail and Hall, 1999; Ibrahim and Quick, 2001). The properties of the photosynthetic system, including key enzymes

and thylakoid membrane activities depend upon the thermal stability of membranes (Björkman et al., 1980). Moreover, it is well known that a functional cell-membrane system is central to crop yield productivity and adaptation of plants to high temperature (Raison et al., 1980). Hence, leaky membranes as a result of HNT can negatively affect crop productivity. In the present study, reduction in rice yields as a result of HNT was attributed to higher respiration rates and decreased MTS, in accordance with previous studies that reported decreased rice yields as a result of HNT (Ziska et al., 1996; Baker, 2004; Peng et al., 2004; Counce et al., 2005), and many studies that have reported decreased yields due to high temperatures as a result of increased leaf respiration rates (Lambers, 1985; Albrizio and Steduto, 2003) and leaf electrolytic leakage (Reynolds et al., 1994; Ismail and Hall, 1999).

Interpretation of the relationship between temperature, respiration and yield is a difficult task as it involves many other plant processes (Johnson and Thornley, 1985; Hemming et al., 2000). Previous studies have shown association of yield with respiration rates (Lambers, 1985), sugar and starch content (Turnbull et al., 2002), membrane stability (Reynolds et al., 1994; Ismail and Hall, 1999), floral bud development (Ahmed and Hall, 1993), pollen viability (Prasad et al., 1999), flowering time and seed size (Gibson and Mullen, 1996), and developmental period or rate in many crop species (Seddigh and Jolliff, 1984; Morita et al., 2005), in response to high temperature. In the present study, rice yield showed a negative association with leaf respiration rates and a positive association with leaf membrane stability. Previous studies reported a similar negative association between yield and respiration rates (Lambers, 1985) and positive

association between yield and membrane stability (Reynolds et al., 1994; Ismail and Hall, 1999) in response to heat-stress. In addition, our results showed positive association between total antioxidant capacity and leaf membrane stability suggesting membrane leakiness is associated with oxidative stress.

In the present study, plants treated with GB or SA showed increased total antioxidant capacities, thereby decreasing the extent of damage to the membranes caused by reactive oxygen species as a result of HNT as indicated by MTS, hence minimizing yield losses under HNT. Previous studies have also shown an increase in endogenous antioxidant levels as a result of exogenous application of SA or GB (Chen et al., 1997; Diaz-Zorita et al., 2001). In the present study, both GB and SA slightly negated the detrimental effects of HNT on rice plants, by reducing the respiration rates and electrolytic leakage. Increased antioxidant levels can detoxify superoxide radicals (Bowler et al., 1992), thereby preventing oxidative damage and protecting the membranes and enzymes (Diaz-Zorita et al., 2001), hence decreasing the maintenance respiration, which is required for repair mechanisms of damaged membranes (Amthor and McCree, 1990). Based on our results, I propose that an increased antioxidant capacity as a result of application of GB or SA can reduce oxidative damage, thereby increasing MTS and reducing leaf dark respiration rates. Moreover, it has been stated that the antioxidant concentration of a plant is closely associated with its stress tolerance and survival (Smirnoff, 1995).

In conclusion, there were increases in leaf dark respiration rates and electrolytic leakage as a result of high nighttime temperatures. Unlike Prasad et al. (2006), in which there was no relationship between electrolytic leakage and yield, the results of the present study indicated that electrolytic leakage increased with temperature. We were able to identify two potential plant growth regulators (GB and SA) to ameliorate the effects of HNT. Both compounds increased total antioxidant capacity of the rice plant, thereby presumably decreasing the leaf dark respiration rates and electrolytic leakage, hence increasing the yield.

CHAPTER IV

HIGH NIGHTTIME TEMPERATURES AFFECT RICE PRODUCTIVITY THROUGH ALTERED POLLEN GERMINATION AND SPIKELET FERTILITY

Introduction

Rice (*Oryza sativa* L.), the most important food crop in terms of direct consumption, is cultivated under a wide range of environments. Global circulation models project global temperature will increase by 1.4 to 5.8°C by the end of the 21st century because of projected increases in the concentrations of greenhouse gases (Houghton et al., 2001). Much of this projected impact of global climate change is due to an increase in nighttime temperature as a result of less radiant heat loss due to increased cloudiness (Alward et al., 1999). Year-to-year variation in rice grain yield has been attributed to variation in nighttime temperature (Peng et al., 2004). If global climate change occurs as predicted, rice grain yield losses can be expected to increase in frequency and severity. Furthermore, the global increase in nighttime temperature is expected to occur at a faster rate than the increases in daytime temperature. It is well-known that high temperatures are a major constraint to crop productivity, especially when temperature extremes coincide with critical stages of plant development (McWilliam, 1980). Many analyses of crop growth and grain yield are based on daily mean air temperature, which assumes no difference in the influence of day versus night temperature (Peng et al., 2004).

Plants exposed to high nighttime temperature (HNT) in many cases operate at lower net photosynthesis rates (P_n) and/or reduced energy budgets (Deal and Raulston, 1989). The negative effects of HNT on photosynthesis are suspected to be due to a number of indirect effects on leaf chlorophyll content (Vågen et al., 2003), leaf area (Thomas and Raper, 1978), leaf nitrogen content (LNC) and various enzymes involved in photosynthesis. In addition, high temperature can reduce P_n by damaging the photosystems (Guo et al., 2006) and electron transport, particularly at the site of photosystem II (Havaux and Tardy, 1996) and by causing oxidative damage to membranes (Larkindale and Knight, 2002). High temperature can also reduce P_n by affecting intercellular CO_2 concentration (Ci) and stomatal conductance (Farquhar and Sharkey, 1982). Previous studies reported premature loss of chlorophyll (Reynolds et al., 1994), which was linked to lower P_n (Guo et al., 2006), under high temperatures. In addition, LNC showed strong association with P_n and plays an important role in thermal acclimation (Morison and Morecroft, 2006).

The reproductive stage is relatively more sensitive than the vegetative stage to heat stress in many crop species (Hall, 1992). Moreover, differential temperature sensitivity for reproductive and vegetative growth has been reported in rice (Baker et al., 1992). Huxley et al. (1976) reported a high nighttime temperature treatment of 24 ^{o}C promoted early vegetative growth compared to an average nighttime temperature treatment of 19 ^{o}C in soybean (*Glycine max* L. Merr.). However, HNT does not affect morphological characteristics such as plant height, number of auxiliary branches and number of nodes in soybean, but it does hasten physiological maturity in many crop

species, including rice, leading to decreased accumulation of photosynthates in the grain, thereby decreasing plant yield (Seddigh and Jolliff, 1984; Morita et al., 2005). In rice, nighttime temperatures above 29 ^{o}C can increase sterility with a subsequent reduction in seed set and grain yield (Satake and Yoshida, 1978). The two most susceptible stages of rice to high temperatures are flowering (anthesis and fertilization) and booting (microsporogenesis) (Satake and Yoshida, 1978). In rice, heat-induced spikelet sterility is linked to decreased anther dehiscence, decreased shedding of pollen, low germination of pollen grain on the stigma and decreased elongation of pollen tubes (Prasad et al., 2006).

Plants pre-treated with α-tocopherol (vitamin E), glycine betaine (GB), or salicylic acid (SA), the plant growth regulators used in this study, showed induced thermo-tolerance and protection against oxidative damage (Mohammed and Tarpley, 2009a). The plant growth regulators used in this study are defined as exogenously applied chemicals that have profound effects on plant growth, development or physiology at low concentrations. The α-tocopherol is one of the most effective single-oxygen quenchers and is a strong antioxidant (Munné-Bosch and Alegre, 2002), whereas GB enhances tolerance to high temperatures by protecting certain enzymes such as RUBISCO and citrate synthase against heat-induced inactivation (Mäkelä et al., 2000). Salicylic acid plays an essential role in thermo-tolerance in plants by preventing oxidative damage to the membranes by detoxifying superoxide radicals and stabilizing trimers (a trimer is a macromolecular complex formed by three, usually non-covalently bound, macromolecules like proteins and nucleic acids) of heat-shock transcription

factors (Larkindale and Knight, 2002) as well as through calcium-signaling effects (Kawano et al., 1998). Despite the importance of antioxidants in stress tolerance, little is known about the response of rice thermo-tolerance to these plant growth regulators (vitamin E, GB and SA). Our previous research indicated reduction in rice yields as a result of HNT due to higher respiration rates and decreased membrane stability (Mohammed and Tarpley, 2009a). However, the percentage decrease in the yield at HNT was much higher than the percentage increase in respiration at HNT, indicating that the relationship between temperature, respiration, and yield is a complex process and determination of yield involves many other plant processes apart from respiration. Previous studies have shown association of yield with respiration rates (Mohammed and Tarpley, 2009), sugar and starch content (Turnbull et al., 2002), membrane stability (Reynolds et al., 1994), floral bud development (Ahmed and Hall, 1993), pollen viability (Prasad et al., 2006), flowering time and seed size (Gibson and Mullen, 1996), and developmental period or rate in many crop species (Seddigh and Jolliff, 1984; Morita et al., 2005), in response to high temperature.

The goal of the present study is to understand the influence of nighttime warming on rice crop production, in particular to discern if the effects are primarily on photosynthesis, reproductive events, developmental rate, or a combination. To assist in this, several plant growth regulators were applied exogenously in an attempt to modify the rice plant response to nighttime temperatures.

Materials and Methods

Plant Culture

Three experiments were conducted in the greenhouse at the Texas A&M University System, AgriLife Research and Extension Center at Beaumont, Texas, USA. 'Cocodrie', a commonly grown tropical japonica-type U.S. rice cultivar, was used in all three experiments. Plants were grown in 3L pots that were placed in a square wooden box (0.84 m^2), 10 pots per box. The boxes were lined with black plastic (thickness = 0.15 mm; FILM-GARD, Minneapolis, Minnesota, USA) that served as a water reservoir. Pots were filled with a clay-rich soil that is common to rice farms in the area. At 20 days after emergence (DAE), the boxes were filled with water to approximately 3 cm above the top of the soil in each pot. A foam cover was placed over the water surface to prevent direct infrared heating of the water. A three-way split application of nitrogen was done as described by Mohammed et al. (2007) in each experiment. Nitrogen was applied in the form of urea and ammonium sulfate, and phosphorus in the form of P_20_5. Urea-N was applied at the rate of 112.3 kg ha^{-1} along with 45.4 kg ha^{-1} of phosphorus (P_2O_5) at planting. The remaining nitrogen fertilizations (both 44.9 kg ha^{-1} of nitrogen in the form of ammonium sulfate) were applied 20 DAE and at the panicle-differentiation stage. In experiment-I, II, and III the planting dates were March 31st (2006), March 29th (2007) and April 10th (2007) respectively. The plants were uniform in developmental stage, as indicated by tiller development, at the beginning of the heat treatments described below.

Temperature Treatments

There are three experiments presented in the study. The assignment of heat treatment to greenhouse location was random within each experiment. The average nighttime temperatures (between 2000 h to 0600 h) at Beaumont, Texas, USA (Longitude: 94^{o} 16′ 59″ W; Latitude: 30^{o} 4′ 0″ N) during the reproductive growth (panicle differentiation to harvest) of the rice plants often ranged above 25 ^{o}C (up to 28 ^{o}C) (Fig. 2.1A). Hence, the ambient nighttime temperature (ANT) was set at 27 ^{o}C. The greenhouse was maintained at 27 ^{o}C nighttime temperature and, within this, plants of the HNT treatment were subjected to elevated nighttime temperature through the use of nearly continuously controlled (sub-second response) infrared heaters (1100 W, Chromalox, Ogden, Utah, USA), as described by Mohammed and Tarpley (2009a). The infrared heaters were positioned 1.0 m above the topmost part of the plants and provided infrared temperature enrichment. In ambient nighttime temperature treatments, dummy heaters were provided to account for shading. The operating wavelengths of the infrared heaters are well above 1200 nm, and the infrared heater output is negligible below 1200 nm (Omega, 2008). Hence, there was no significant emission of photo-morphogenic wavelengths (wavelengths below 780 nm). The infrared heaters were controlled using power semi-conductor controllers (SCR Power Control, Watlow Electric Manufacturing Company, St. Louis, Missouri, USA) to enable proportioning heating action instead of on/off action, allowing maintenance of temperature within 0.5 ^{o}C. The nighttime temperature was imposed from 2000 h until 0600 h starting from 20 DAE until harvest. Daytime temperature, nighttime temperature and humidity were monitored

independently of the temperature-control system using standalone sensor/loggers (HOBO, H08-003-02, Onset Computer Corporation, Bourne, Massachusetts, USA). The HOBOs were placed in the canopy near the panicle base (few centimeters into the canopy). The devices were read every 15 minutes. Mean daytime (06:00–19:00 h)/nighttime (19:00–6:00 h) temperatures during the cropping season (emergence to harvest) were 31.6/27.3 and 32.8/31.8 ^{o}C in ambient and ambient + 5 ^{o}C temperature treatments. The daytime/nighttime humidity during the cropping season was 14.4/15.9 and 14.3/14.4 gm m^{-3} in ambient and ambient + 5 ^{o}C temperature treatments (Fig. 2.1B, C). To monitor plant physiological response to the infrared heating treatment, leaf temperatures were measured at different developmental stages of the rice plants (Boot, Early grain-fill and Mid-dough stage) using a LI-COR 6400 (when the infrared heaters were ON). The temperature depression in the leaves compared to the surrounding air was consistently less (leaf temperatures were 3 ^{o}C less than air temperatures [results not shown]) for both heat treatments, indicating the plants maintained good physiological status in response to the infrared heating.

Plant Growth Regulator Treatments

The α-tocopherol, GB, and SA were applied at the rate of 100 µl per application to the leaves using a pre-calibrated perfume-bottle sprayer. Each plant was treated three times (on a single day) to enable thorough coverage prior to imposing heat stress, for a total of 300 µl per treatment. All the plant growth regulators were dissolved in de-ionized water with 0.5% (v/v) surfactant (Latron AG-98 spreader activator, Rohm and

Haas Company, Philadelphia, Pennsylvania USA). The α-tocopherol was applied at the rate of 58 μmol per plant per spray (i.e., 580 mM); GB at 182.3 μmole per plant per spray (i.e., 1.823 M), and SA at 0.1 μmol per plant per spray (i.e., 1mM). The α-tocopherol and SA were purchased from Sigma-Aldrich (St. Louis, Missouri, USA) and GB was supplied by Capstone Food Ingredients (Marion, Massachusetts, USA).

Leaf Photosynthesis

Leaf net photosynthetic rates (P_n; photosynthesis minus dark respiration in the day per unit leaf area) were measured at the vegetative (40 DAE), boot (54 DAE), early grain-fill (EGF; 70 DAE) and mid-dough (MD; 80 DAE) stages. The P_n was measured on the penultimate leaves using a LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, Nebraska, USA) between 1000 h and 1200 h. When measuring P_n, the photosynthetic photon flux density (PPFD), provided by a 6400-02 LED light source, was set to 1500 μmol m^{-2} s^{-1}. All photosynthesis rates were expressed on a unit leaf area basis. The temperature and CO_2 concentration in the leaf cuvette (6 cm^2) were set to 25 °C and 360 ppm (ambient CO_2 concentration in the greenhouse), respectively. Humidity in the cuvette was controlled by circulation of the air through desiccant. A steady flow rate of 500 μmole sec^{-1} was maintained in the leaf chamber. Three individual leaves (penultimate position) per plant were measured for 13 plants of each temperature x plant growth regulator treatment combination. In addition, in Exp-II and Exp-III, P_n was measured on all the viable (more than 50% of green leaf area) main-stem leaves in each treatment, 75 DAE.

Chlorophyll

Chlorophyll content was estimated for the penultimate leaves at the vegetative, boot, EGF and MD stages. Three leaf discs (0.196 cm^2 each) were obtained from mid-blade, while avoiding the mid-vein, and placed in a vial (2.5-ml) with 1-ml dimethyl sulphoxide (DMSO) then incubated at room temperature for 24 h in darkness to allow for complete extraction of chlorophyll into the solution. The absorbance of the extract was measured in microtiter plates of polypropylene material using a PowerWave microplate spectrophotometer (Bio-Tek Instruments, Inc., Winooski, Vermont, USA) at 648 and 664 nm to calculate the chlorophyll a and chlorophyll b concentrations (Chappelle et al., 1992). Using the method of Chappelle et al. (1992), which utilizes a DMSO extraction, the concentrations of the pigments were calculated from the absorbance values at 648 and 664 nm using equations described by Lichtenthaler (1987). The pigment concentrations were expressed on a leaf area basis, μg cm^{-2}. Total chlorophyll was calculated by summing up the values of chlorophyll a and chlorophyll b.

Leaf Nitrogen Content (LNC)

In Exp-II and Exp-III, N was measured using a FP-528 Nitrogen/Protein analyzer (LECO Corporation, St. Joseph, Michigan, USA). For LNC analysis, the second and fourth leaves from the top were harvested at 75 DAE (between EGF stage and MD stage). Leaf nitrogen content was expressed as percentage (%; w/w).

Growth and Development

Plant height (from base of the stem to tip of the uppermost-reaching leaf), stem length (from the base of the stem to the base of the panicle) and the numbers of tillers and panicles were recorded at weekly intervals and at harvest. Daily observations were made for the appearance of panicles. The number of leaves was counted at the vegetative, EGF and MD stages, as well as at harvest. In addition, at the EGF stage, individual leaf length (from the point of contact of the leaf blade and the stem to the tip of the leaf) and leaf breadth (widest part of the leaf) were measured to calculate total leaf area (calculated leaf area). The final harvest was carried out at 100 DAE. Destructive sampling procedures were adopted: plants were dissected, numbers of leaves and non-reproductive tillers were counted, leaf areas (measured leaf area) were measured, and dry weights were determined. Leaf area was measured using a CI-251 area meter (CID Inc., Camas, Washington, USA).

Pollen Germination

In Exp-II and Exp-III, the percent pollen germination (PPG) was determined through in vitro germination on a culture medium. A preliminary study was done using different culture media. The selected culture medium for this study had the highest percent pollen germination (PPG) in the preliminary study. The culture medium was prepared in accordance with the media of Song et al. (2001), with minor modifications. The components of culture media in the present study are 15 g sucrose, 0.03 g calcium nitrate, and 0.01 g boric acid dissolved in 100 ml deionised water. Agar, 0.06 g, was

added to this solution and heated on a hot plate to dissolve the agar. When the agar was dissolved in the solution, 10 ml of this medium was poured per Petri dish (diameter 8.5 cm) and allowed to cool for 15 minutes. The pollen from different treatments was dusted on to the solidified medium as soon as the floret opened in the morning (S.O.PB. Samonte, Texas AgriLife Research and Extension Center, Beaumont, Texas, USA; personal communication). Pollen was dusted onto two sets of Petri dishes. One set was incubated at 27 ^{o}C, and the other at 32 ^{o}C, for 24 hours in the dark. Incubation temperature refers to the temperature at which the pollen was incubated and growth temperature refers to the temperature at which the plants were grown. Pollen was considered as germinated when the pollen tube length was at least equal to the pollen diameter (Luza et al., 1987) as determined using a microscope. The total numbers of pollen grain, germinated and non-germinated pollen in each Petri dish were counted, and the PGG was recorded.

Spikelet Fertility (SF)

Spikelet fertility was estimated using the procedures of Prasad et al. (2006). The ratio of filled grain to total number of florets was estimated and expressed as percentage. Each floret was pressed between the thumb and forefinger to determine if the grain was filled. The number of filled grain included both completely and partially filled grain.

Experimental Design and Data Analysis

The experiments were laid out in a complete randomized design, and were repeated for a total of three times. One set of the plants was grown under ambient nighttime temperature, whereas the other set was grown under high nighttime temperature. The temperature treatments were randomized among experiments. In the first experiment, in each set, there were 12 plants, three plants per plant growth regulator treatment, whereas in Exp-II and Exp-III, in each set, there were 20 plants, five plants per plant growth regulator treatment. All data were analyzed using PROC GLM procedures in Statistical Analysis System (SAS) to determine the influence of experiments, nighttime temperatures, plant growth regulators, and their interactive effects on growth, development and physiological parameters. Means were separated using Tukey's Least Significant Difference (LSD) at an alpha level of 0.05. If there was no significant difference among the experiments for a parameter, then the values from all the experiments for that parameter (e.g., n=13) were used to obtain the mean and standard error. The standard errors of the mean were also calculated and presented in the graphs.

Results

Unless otherwise indicated, no differences among the experiments were found for the studied variables. In addition there were no interactive effects of temperature X plant growth regulators for the studied variables.

There were no differences between leaf photosynthetic rates (P_n) comparing the nighttime temperature treatments (Fig. 4.1A). In addition, plants grown under ambient nighttime temperature (ANT) showed no differences in P_n when comparing the plant growth regulator treatments. However, P_n was 22% less in untreated plants grown under high nighttime temperature (HNT) compared to treated plants at the vegetative and early grain-filling (EGF) stages. Similar results were seen with respect to stomatal conductance (Fig. 4.1B). However, there were no differences in internal CO_2 concentration (C_i), between the nighttime temperatures and among plant growth regulator treatments, except for SA-treated plants grown under ANT. Salicylic acid-treated plants grown under ANT showed 10% and 8% decrease in C_i compared to untreated plants at EGF and mid-dough (MD) stages (Fig. 4.1C).

Plants grown under HNT showed 7% and 13% decreases in total chlorophyll content and 13% and 12% decreases in chlorophyll a content at the vegetative and boot stages, respectively, compared to plants grown under ANT (Fig. 4.2a, c). However, there were no differences between the nighttime temperatures with respect to chlorophyll b (Fig. 4.2b). In addition, there were no differences in either total chlorophyll, chlorophyll a, or chlorophyll b content at any developmental stage when comparing the plant growth regulator treatments (Fig. 4.2A, B, C).

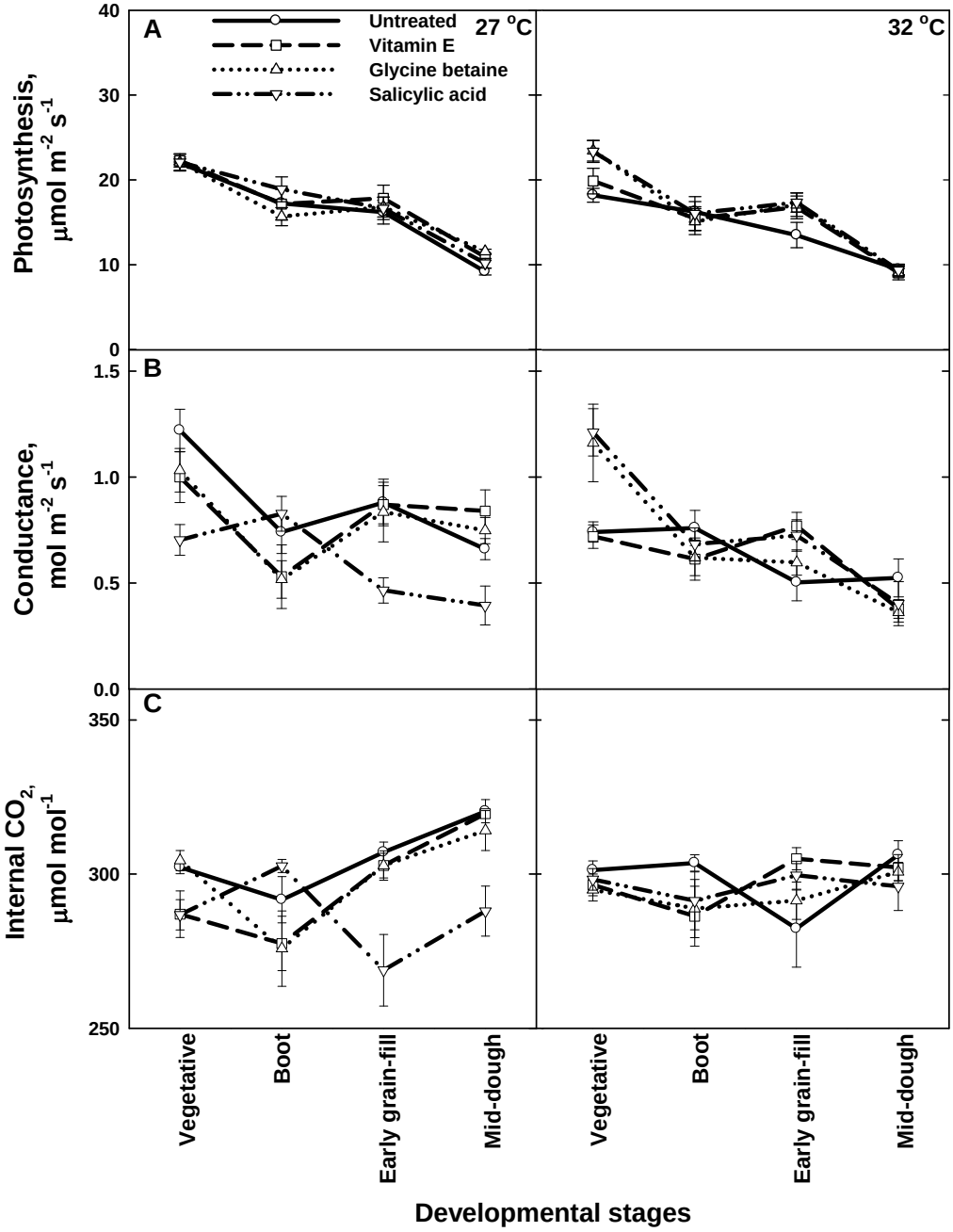

Figure 4.1. Influence of nighttime temperatures and plant growth regulators on photosynthetic parameters measured at 1500 PPFD light intensity on the penultimate leaves. All values are means ± SE; n = 13. The SE bars are shown if they are larger than the symbol.

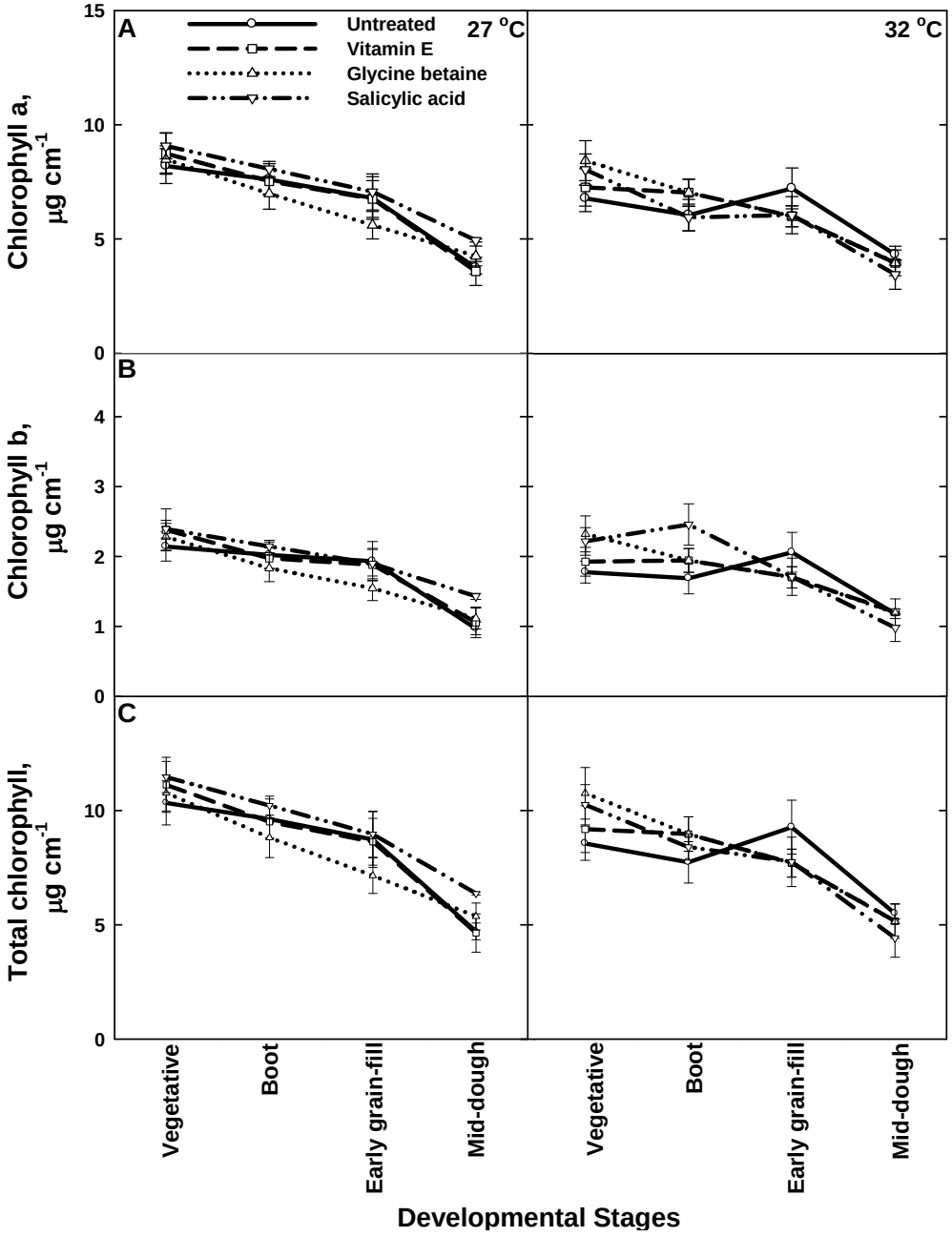

Figure 4.2. Effects of nighttime temperatures and plant growth regulators on chlorophyll a, chlorophyll b and total chlorophyll content measured on the penultimate leaves. All values are means ± SE; n = 13. The SE bars are shown if they are larger than the symbol.

Leaf photosynthetic rates measured on viable leaves (more than 50% of green leaf area) on the main-stem showed no differences between the nighttime temperatures or among the plant growth regulator treatments at any leaf position, 75 days after emergence (DAE) (Fig. 4.3A). However, P_n was lower at the lower leaf positions. Leaf nitrogen content measured on the 2nd and the 4th leaf showed differences between the nighttime temperatures, 75 DAE. Plants grown under HNT showed 29% and 18% decrease in LNC of the 2nd and the 4th leaf respectively, compared to plants grown under ANT (Fig. 4.3B). The 2nd leaf compared to the 4th leaf had 13% more LNC for plants grown under ANT. However, plants grown under HNT showed no differences between the leaf positions with respect to LNC (Fig. 4.3B). In addition, there were no differences among the plant growth regulator treatments with respect to LNC for either of the leaf positions.

On average, plants grown under HNT showed a 10% decrease in the number of viable leaves at the vegetative stage compared to plants grown under ANT (Fig. 4.4A). However, at the EGF stage, plants grown under ANT showed a 10% decrease in the number of viable leaves compared to plants grown under HNT. There were no differences among the plant growth regulator treatments with respect to number of viable leaves (Fig. 4.4A). At EGF stage, the estimated leaf area of the plants grown under HNT was twice the leaf area of the plants grown under ANT. At harvest, similar results were seen with respect to measured leaf area (Fig. 4.4B). At EGF stage, plants that were grown under ANT and treated with GB and SA showed 13% and 10% decreases in estimated leaf area, respectively. However, plants grown under HNT

showed no differences among the plant growth regulator treatments with respect to estimated leaf area (Fig. 4.4B). At harvest, plants grown under ANT and treated with vitamin E showed 50 % decrease in leaf area compared to the plants treated with other plant growth regulators (Fig. 4.4B). Glycine betaine- and SA- treated plants, grown under HNT, showed 44% and 26% decreases in leaf area compared to untreated plants.

There were no differences for plant height, stem length, tiller number and panicle number comparing the nighttime temperature treatments (Fig. 4.5A, B). In addition, there were no differences for tiller number and panicle number comparing the plant growth regulator treatments. However, GB-treated plants were shorter than the untreated plants in both the heat treatments. Plants grown under HNT showed 168%, 75% and 46% increases in non-reproductive tillers, above-ground dry weight and root dry weight respectively, compared to plants grown under ANT (Fig. 4.5B, C). However, there were no differences among the plant growth regulator treatments with respect to the above mentioned parameters in either heat treatment. Plants grown under HNT showed a 90% decrease in yield compared to plants grown under ANT (Fig. 4.5C). Plants treated with vitamin E, GB and SA showed 18%, 21% and 24%, and 5%, 77% and 5-fold increase in yield when grown under ANT and HNT respectively, compared to untreated plants (Fig. 4.5C). Our results indicated differences between the nighttime temperatures with respect to date of first panicle emergence. Plants grown under HNT showed earlier panicle emergence by 2 d, compared to plants grown under ANT (Fig. 4.6A, B). Plants grown under ANT and treated with SA showed delayed panicle emergence by 2 d compared to other plant growth regulator treatments (Fig. 4.6A). However, plants grown under HNT

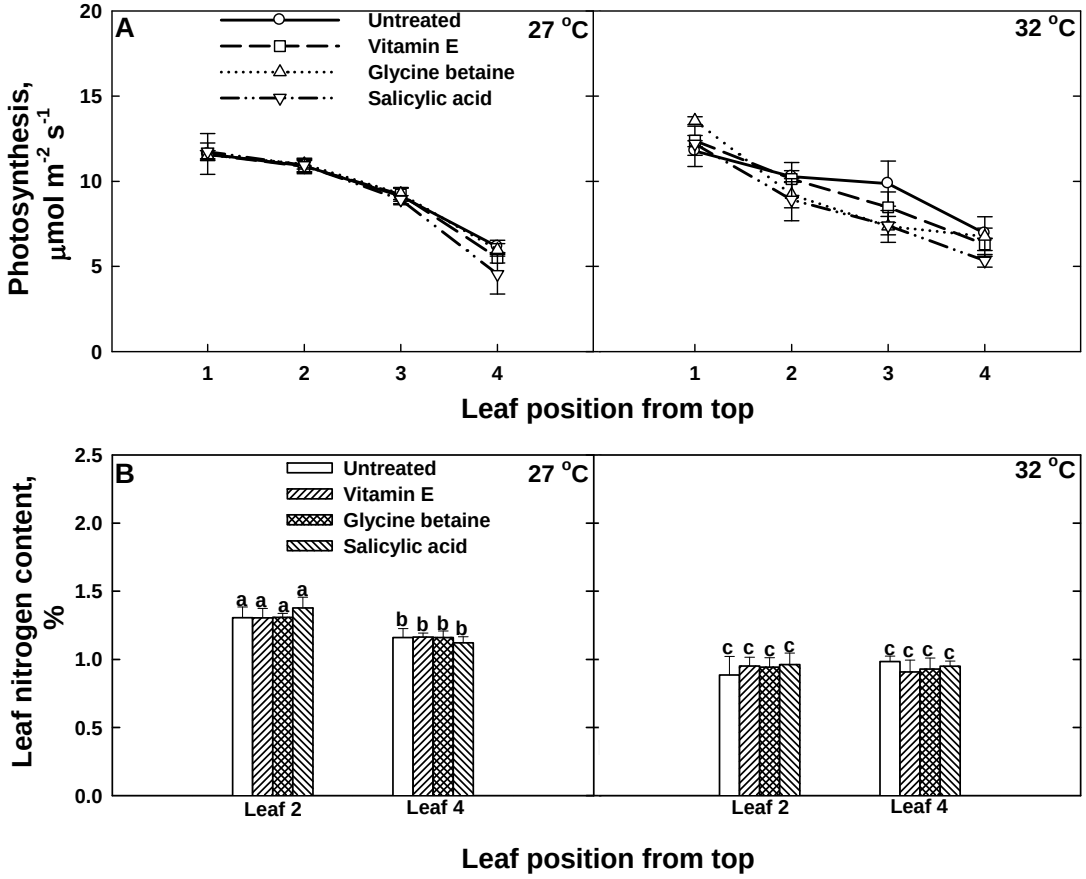

Figure 4.3. Influence of nighttime temperatures and plant growth regulators on leaf photosynthesis and leaf nitrogen content measured at 75 DAE. All values are means ± SE; n = 10. The SE bars are shown if they are larger than the symbol. Different letters indicate means are significantly different at the P<0.05 level.

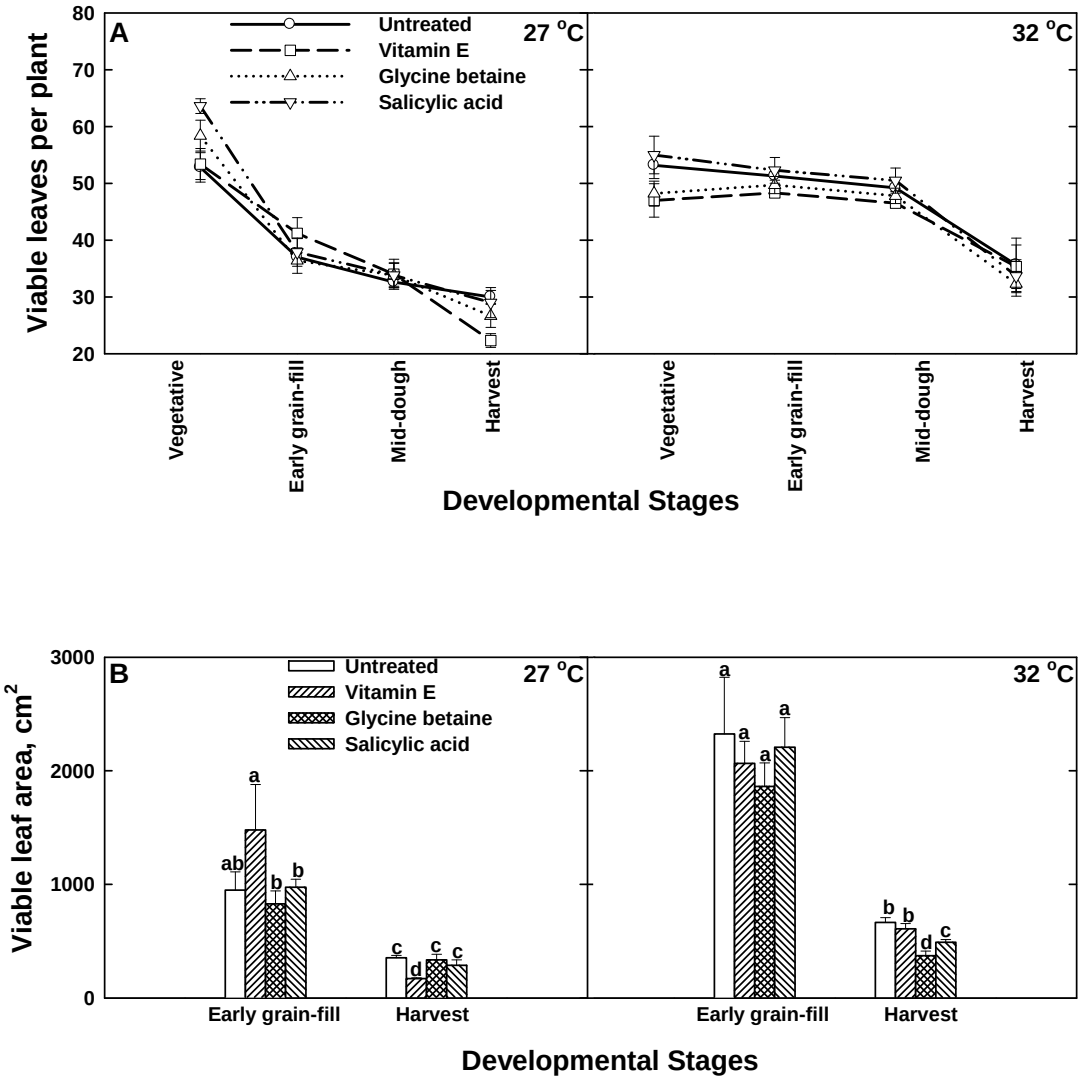

Figure 4.4. Effects of nighttime temperatures and plant growth regulators on patterns of panicle emergence. All values are means $\pm$ SE; n = 13. The SE bars are shown if they are larger than the symbol.

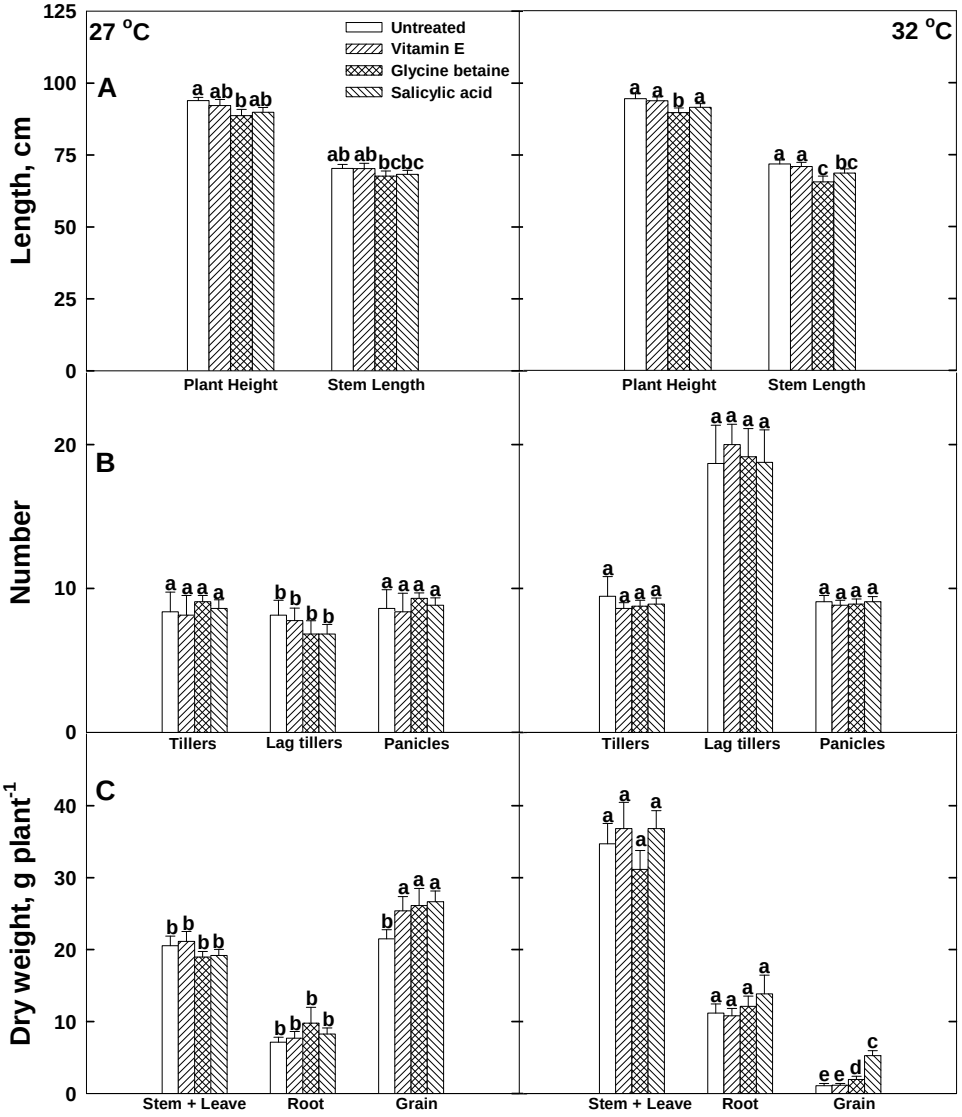

Figure 4.5. Effects of nighttime temperatures and plant growth regulators on pollen germination and spikelet fertility. For pollen germination, all values are means $\pm$ SE; n = 10; and for spikelet fertility, all values are means $\pm$ SE; n = 13. Different letters indicate means are significantly different at the $P<0.05$ level.

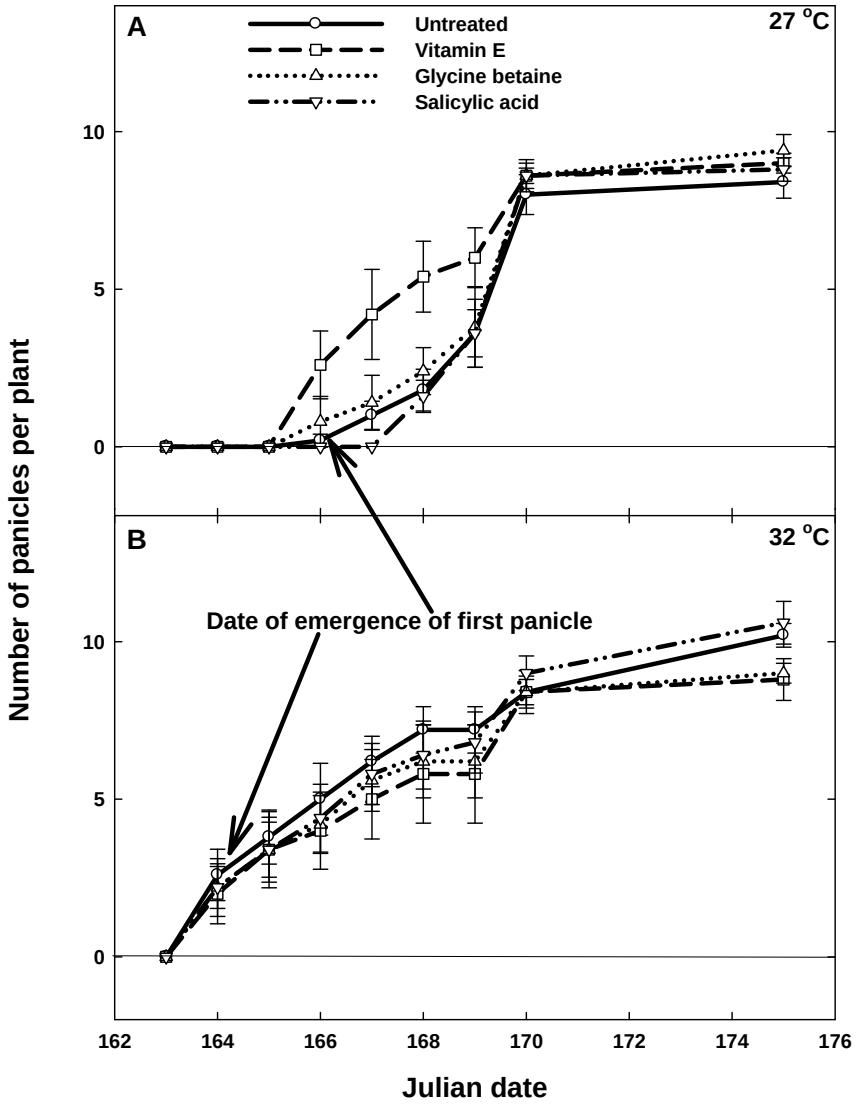

Figure 4.6. Effects of nighttime temperatures and plant growth regulators on leaf number and leaf area per plant. All values are means $\pm$ SE; n = 13. The SE bars are shown if they are larger than the symbol. Different letters indicate means are significantly different at the $P<0.05$ level at a particular nighttime temperature.

showed no differences among the plant growth regulators with respect to the first panicle emergence date (Fig. 4.6B).

There were effects of incubation as well as growth temperature on PPG (Fig. 4.7A). On average, pollen incubated at a higher temperature (32 ^{o}C) showed 20% decrease in germination, compared to pollen incubated at lower temperature (27 ^{o}C). On average, untreated plants and plants treated with vitamin E, GB, and SA showed 34%, 18%, 22% and 6% decrease in PPG respectively, when pollen was incubated at 32 ^{o}C compared to 27 ^{o}C (Fig. 4.7A). The untreated plants and plants treated with vitamin E, grown under ANT, showed 38% and 20% decrease in PPG respectively, when pollen was incubated at 32 ^{o}C compared to 27 ^{o}C. However, plants treated with GB or SA, grown under ANT, showed no difference for PPG between incubation temperatures. Plants grown under HNT showed no differences among the plant growth regulator treatments with respect to PPG when the pollen was incubated at 32 ^{o}C (Fig. 4.7A).

There were differences between the nighttime temperatures and among the plant growth regulator treatments, with respect to SF (Fig. 4.7B). On average, plants grown under HNT showed 72% decrease in SF, compared to ANT. Plants treated with one of the plant growth regulators generally had greater SF than untreated plants in ANT and HNT (Fig. 4.7B). Plants treated with vitamin E, GB and SA showed 9%, 15% and 18%, and 47%, 120% and 195% increase in SF when grown under ANT and HNT respectively, compared to untreated plants (Fig. 4.7B).

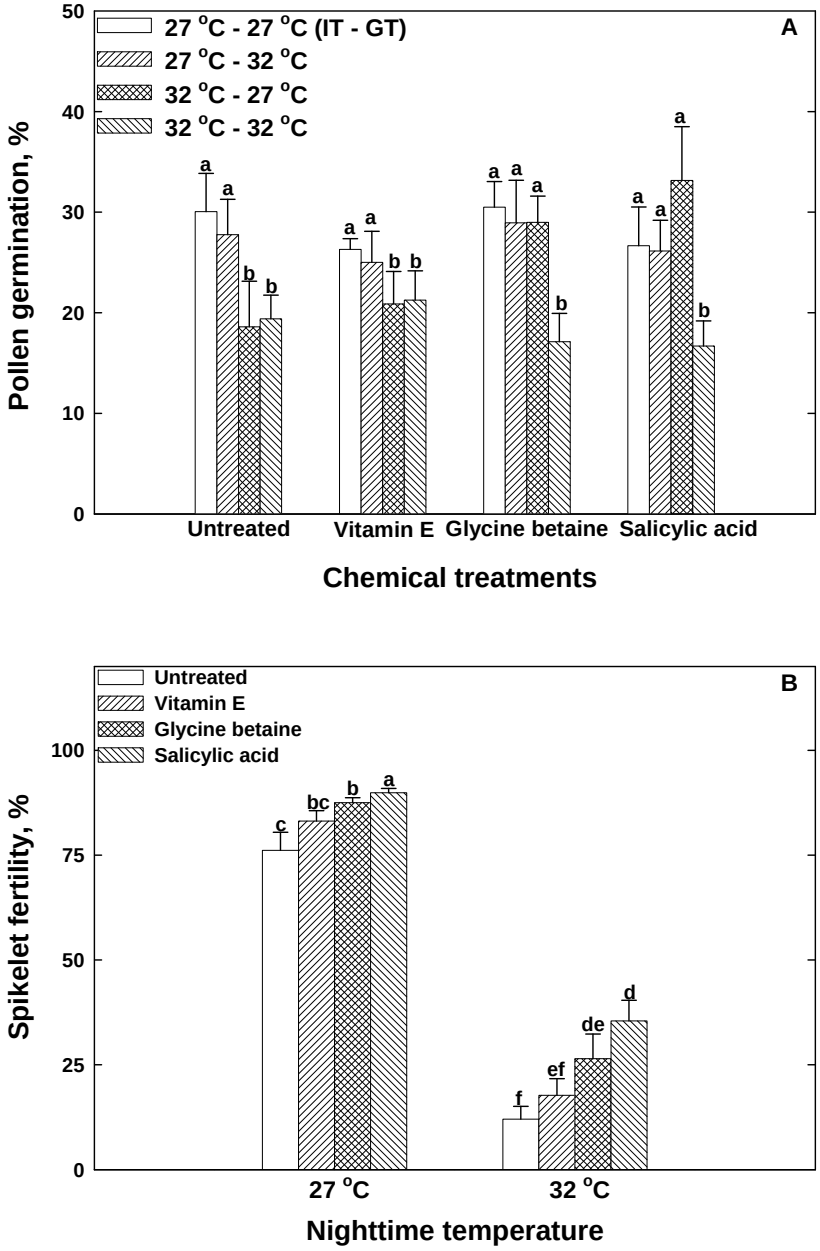

Figure 4.7. Effects of nighttime temperatures and plant growth regulators on morphological characteristics of rice plants. All values are means ± SE; n = 13. The SE bars are shown if they are larger than the symbol. Different letters indicate means are significantly different at the $P<0.05$ level for that particular parameter under ambient and high nighttime temperature.

Discussion

Our findings are consistent with those of earlier studies in showing decreased rice yields as a result of high nighttime temperature (HNT) (Morita et al. 2005). However, in the present work, HNT did not affect the following daytime's P_n for any of the tested developmental stages or leaf positions. This differs from the findings of Turnbull et al. (2002), which stated that HNT can increase the following daytime's P_n by reducing carbohydrate-induced inhibition of photosynthesis. Our findings also indicated no relationship of P_n with stomatal conductance or internal CO_2 concentration (C_i), suggesting that apart from stomatal conductance and C_i, a number of factors including the activity of Rubisco enzyme and photo-systems (Pastenes and Horton, 1996a, b) govern photosynthesis. Photosynthetic capacity is also often closely associated with chlorophyll content and leaf nitrogen content (LNC) (Mae, 1997) and LNC in particular has been used to estimate photosynthetic capacities (Llorens et al., 2003). Previous research indicated a decline in chlorophyll content (Guo et. al., 2006) as a result of premature loss of chlorophyll (Reynolds et al., 1994) and a decline in LNC at high temperatures (Wollenweber et al., 2003; Xu and Zhou, 2006). In the present work, similar results of decrease in chlorophyll content and LNC were noticed. However, neither the chlorophyll content nor the LNC were associated with P_n. Xu and Zhou (2006) reported a similar lack of association between LNC and P_n in a perennial grass. Sicher and Bunce (1997) stated that high temperature leads to the suppression of P_n by mainly decreasing the proportion of soluble protein to total leaf N, thereby adversely

affecting Rubisco protein and activity. The ratio of soluble protein to total leaf N was not estimated in the present study.

High nighttime temperature had no effects on plant height, stem length, tiller number, and panicle number per plant. Similar results were reported for soybean for plant height and stem length (Seddigh and Jolliff, 1984). High nighttime temperature decreased the number of leaves per plant at the vegetative stage in contrast to the findings of Thomas and Raper (1978), which stated that HNT had no effect on the number of leaves per plant. In the present study, HNT increased the number of non-reproductive tillers, leaf area, above-ground weight, and root dry weight per plant; however, the crop growth duration was decreased by HNT as indicated by earlier panicle emergence. Similar results of decreased crop growth duration were reported as a result of high daytime temperatures for rice (Prasad et al., 2006). Cantarero et al. (1999) indicated decreased corn (*Zea mays* L.) yields under HNT were due to reduced accumulation of photosynthates as a result of decreased crop growth duration. In the present study, in addition to decreased crop growth duration under HNT, decreases in percent pollen germination (PPG) and spikelet fertility (SF) also contributed to decreased yields. PPG decreased in many crops with an increase in temperature (Hall, 1992; Matsui et al., 2001). The decrease in PPG at high temperatures results from poor anther dehiscence and pollen reception (Prasad et al., 2006) and reduced pollen swelling and decreased anther pore size (Matsui and Kagata, 2003). In the present work, decreased SF was partially due to decreased PPG. However, at higher temperatures, SF is also governed by hormonal balance in the floret (Micheal and Beringer, 1980) or

availability and transport of photosynthates to the kernel (Afuakwa et al., 1984) or inability of floral buds to mobilize carbohydrates under heat stress (Dinar and Rudich, 1985) or altered activities of starch and sugar biosynthesis enzymes (Keeling et al., 1994; Singletary et al., 1994) In the present study, decreased PPG reduced the number of reproductive sinks apparently leading to an increase in dry matter partitioning to vegetative structures such as stem, leaves and roots, thereby increasing the number of non-reproductive tillers, leaves, leaf area, and above-ground weight and root dry weight.

The treatments of plants with GB and SA were effective in reducing the negative effects of HNT. Exogenous application of GB and SA did not affect plant morphological parameters; however, each of these treatments had a significant effect on PPG and SF, and hence on yield. Exogenous application of GB and SA increased endogenous antioxidant levels in rice plants, thereby preventing oxidative damage to the membranes (Mohammed and Tarpley, 2009a). Membrane stability can play an important role in water, ion, and organic-solute movement across membranes (Christiansen, 1978). In addition, increased antioxidant levels can protect the enzymes against heat-induced reactive oxygen species (ROS)-mediated degradation (Sen Gupta et al., 1993). Movement of water and photosynthates across membranes and the activities of enzymes (Singletary et al., 1994) play an important role in the grain-filling process.

In conclusion, the decrease in 'Cocodrie' rice yields as a result of HNT was not correlated with net photosynthesis. The photosynthesis-governing parameters, such as stomatal conductance, internal CO_2 concentration, chlorophyll content and leaf nitrogen, were also not associated with photosynthesis rates per unit leaf area. There was no effect

of HNT on photosynthesis per unit leaf area and morphological parameters. However, there was a decrease in yield at HNT, which was attributed to decreased crop growth duration, percent pollen germination and spikelet fertility. Exogenous application of GB and SA increased yields under HNT, possibly acting through previously observed increased antioxidant levels, which might have protected the membranes and enzymes against heat-induced ROS-mediated degradation.

CHAPTER V

NIGHTTIME TEMPERATURE EFFECTS ON PRODUCTIVE TILLERS, SPIKELET FERTILITY, GRAIN CHARACTERISTICS AND YIELD OF RICE (*ORYZA SATIVA* L.) PLANTS

Introduction

In the last decade, nighttime temperatures on the Earth's surface increased three times more than the corresponding daytime temperature (Karl et al., 1991). Moreover, differential increases in daytime and nighttime temperatures during a recent 25-year period (1979 to 2003) have putatively been associated with global warming (Peng et al., 2004). Rice grain yield and biomass production showed strong negative linear relationships with nighttime temperatures (Peng et al., 2004). Although the physiological effects of high daytime temperatures are well understood (Ziska et al., 1996), the effects of increased nighttime temperature on rice production are poorly understood (Peng et al., 2004).

Productive tillers (per unit ground area), spikelet fertility and grain weight are important components of yield (Sheehy et al., 2001) that are affected by the cultivation system and by environmental factors, among which temperature is considered to be important (Singla et al., 1997). Tiller production is sensitive to temperature (Mitchell, 1953) and in rice it is an important agronomical trait (Li et al., 2003). Furthermore, tiller number in small grain crops is highly correlated with panicle dry weight (Paulsen, 1987; Samonte et al., 1998). Spikelet fertility in rice is also sensitive to temperature, where the degree of sensitivity depends upon the developmental stage of the spikelet (Zakaria et

al., 2002). Moreover, high nighttime temperature (HNT) (>29 ^{o}C) decreases spikelet fertility of rice with a subsequent reduction in seed-set and grain yield (Satake and Yoshida, 1978; Ziska et al., 1996). There is a strong negative linear relationship between spikelets per m^2 and nighttime temperature (Peng et al., 2004).

The grain weights for a cultivar are almost constant in a stress-free environment (Yoshida, 1981). However, under stress conditions, the kernels at the tip of the panicle are the largest and heaviest because they are normally filled first, while large numbers of blanks occur at the base of the panicle (Stansel, 1975). Hence, under stress conditions, there is increased competition among the grain located at different positions within the panicle. The decrease in individual grain weight under high nighttime temperature is not associated with a deficit of carbohydrates in the leaves (Morita et al., 2005), but is associated with decrease in grain width (Counce et al., 2005). However, previous studies reported an increase in respiration rates as a result of high temperatures, which might decrease assimilate supply to the spikelet (Hirai et al., 2003). Decrease in carbon accumulation by the kernel as a result of stress usually increases grain nitrogen concentration (Triboi and Triboi-Blondel, 2002). In rice, it has been reported that a change in temperature away from the optimum (27/22 ^{o}C) reduces carbon accumulation but increases grain nitrogen content (Tashiro and Wardlaw, 1991).

The severity of heat-induced reactive oxygen species (ROS)-damage is dependent on the antioxidant status of the plant and, in some circumstances, the antioxidant status of the plant is closely associated with stress tolerance (Smirnoff, 1995). Angiosperms possess several enzymatic and non-enzymatic scavenging systems

to minimize deleterious effects of heat-induced ROS-damage, which include lipid-soluble antioxidants (e.g. α-tocopherol and β-carotene), water-soluble reactants (e.g. ascorbic acid and glutathione), and enzymatic antioxidants (e.g. superoxide dismutase, catalase, and enzymes of the ascorbate and glutathione cycle) (Zhang and Kirkham, 1996). In addition, application of some plant growth regulators (e.g. salicylic acid (SA), glycine betaine (GB), cytokinin) can also induce stress tolerance in plants. The α-tocopherol, GB and SA – the plant growth regulators used in this study – are all important in plant growth and development and are also associated with thermo-tolerance in plants. The plant growth regulators used in this study are defined as exogenously applied chemicals that have profound effects on plant growth, development or physiology at low concentrations. Plants treated with α-tocopherol showed induced thermo-tolerance and protection against oxidative damage (Fryer, 1992), GB (Diaz-Zorita et al., 2001), or SA (Larkindale and Knight, 2002). The α-tocopherol is one of the most effective single-oxygen quenchers and is a strong antioxidant, whereas GB enhances tolerance to high temperatures by protecting RUBISCO and citrate synthase against heat-induced inactivation (Caldas et al., 1999; Mäkelä et al., 2000). Salicylic acid plays an essential role in preventing oxidative damage in plants by detoxifying superoxide radicals (Bowler et al., 1992) and stabilizing trimers (a trimer is a macromolecular complex formed by three, usually non-covalently bound, macromolecules like proteins and nucleic acids) of heat shock transcription factors (Larkindale and Knight, 2002). Salicylic acid is also involved in calcium signaling (Kawano et al., 1998) and induces thermo-tolerance (Larkindale and Knight, 2002).

Despite the importance of antioxidants in stress tolerance, little is known about the response of rice thermo-tolerance to these plant growth regulators (vitamin E, GB and SA).

Based on the reports of diverse effects of HNT on plant physiology, development and yield, and of the selected plant growth regulators providing thermo-tolerance by protecting against oxidative stress, this study tested the hypothesis that an increase in nighttime temperature can negatively affect yield by affecting multiple yield-determining parameters, including productive tillers, spikelet fertility, and grain length and width, and that the exogenous application of plant growth regulators known to provide thermo-tolerance can provide protection against high nighttime temperature by increasing antioxidant capacity of the rice plant. The objectives were to determine 1) the effects of HNT on productive tillers, spikelet fertility and grain characteristics, and 2) if application of plant growth regulators (vitamin E, GB and SA) can render protection to the rice plants against HNT by increasing the plant's antioxidant capacity.

Materials and Methods

Experimental Conditions and Plant Culture

Three experiments were conducted in the greenhouse at the Texas A&M University System, AgriLife Research and Extension Center at Beaumont, Texas, USA. 'Cocodrie', a commonly grown U.S. tropical japonica rice cultivar, was used in all three experiments. As described by Mohammed and Tarpley (2009), plants were grown in 3L pots that were placed in a square wooden box (0.84 m^2), 10 pots per box. The boxes were lined with black plastic (thickness = 0.15 mm; FILM-GARD, Minneapolis, Minnesota, USA) that served as a water reservoir. Pots were filled with a clay soil that is common to rice farms in the area. At 20 days after emergence (DAE), the boxes were filled with water to approximately 3 cm above the top of the soil in each pot. A foam cover was placed over the water surface to prevent direct infrared heating of water. A three-way split application of nitrogen was provided as described by Mohammed et al. (2007). At planting, urea-N was applied at the rate of 112.3 kg ha^{-1} along with 45.4 kg ha^{-1} phosphorus (P_20_5). The second and third nitrogen fertilizations (both 44.9 kg ha^{-1} nitrogen in the form of ammonium sulfate) were applied 20 DAE and at the panicle-differentiation stage. The plants were uniform in terms of developmental stage, as indicated by tiller development, at the beginning of the heat treatments described below.

Temperature Treatments

The greenhouse ambient nighttime temperature was maintained at 27 ^oC, which is similar to the ambient nighttime temperature during the reproductive growth of rice at

the Texas A&M University System, AgriLife Research and Extension Center at Beaumont in 2005 (Fig. 2.1A). Plants of the HNT treatment were subjected to elevated nighttime temperature through the use of nearly continuously controlled (sub-second response) infrared heaters (Chromalox, Ogden, Utah, USA) as described by Mohammed and Tarpley (2009a). The infrared heaters were positioned 1.0 m above the topmost part of the plants and provided free-air enrichment of the temperature. Air temperatures surrounding the plants were controlled at predetermined set points (27 ^{o}C and 32 ^{o}C). When the temperature was below the set point as determined by the readings from the thermocouples positioned within a few centimeters of the uppermost or reproductive parts of the plant, semi-conductor controllers (SCR Power Control, Waltlow Electrical Manufacturing Company, St. Louis, Missouri, USA) sent a signal to the infrared heaters to provide a short slightly elevated heating event, as needed, to raise the temperature to the desired set point. The nighttime temperature was imposed from 2000 h until 0600 h starting from 20 DAE until harvest. Daytime temperature, nighttime temperature and absolute humidity were also monitored using standalone sensor/loggers (HOBOs, Onset Computer Corporation, Bourne, Massachusetts, USA). Nighttime air temperatures were maintained within the set points $\pm$ 0.5 ^{o}C (Fig. 2.1B, C).

Plant Growth Regulator Treatments

Plants were treated three times (on the same day) to ensure thorough coverage, prior to imposing heat stress. The α-tocopherol, GB, and SA were applied at the rate of 100 µL per application to the leaves using a pre-calibrated perfume-bottle sprayer. The

α-tocopherol was applied at the rate of 58 μmol per plant per spray (i.e., 580 mM); GB was applied at the rate of 182.3 μmole per plant per spray (i.e., 1.823 M), and SA at the rate 0.1 μmol per plant per spray (i.e., 1mM). The α-tocopherol and SA were purchased from Sigma-Aldrich (St. Louis, Missouri, USA) and the GB was supplied by Capstone Food Ingredients (Marion, Massachusetts, USA).

Productive Tillers and Yield

The number of reproductive tillers, defined as tillers that produced panicles, was counted at weekly intervals and at harvest. The mainstem panicles were tagged at panicle emergence and harvested separately from the tiller panicles and dried at 70 ^{o}C. Mainstem panicle length and number of primary branches were recorded. Mainstem and tiller panicles were harvested separated, dried at 70 ^{o}C to constant weight and separately threshed using a thresher (HEGE 16 230 V, HEGE Maschinen, Domäne Hohebuch, D-74638 Waldenburg, Germany), and manually hulled, then the grain was weighed. Final harvest was between 104 and 114 DAE in experiment-I (Exp-I), II and III.

Spikelet Fertility

Spikelet fertility (SF) was determined using the procedures from Prasad et al. (2006), defined as the ratio of filled grain to total number of reproductive spikelets, and expressed as a percentage. Each spikelet was pressed between the thumb and forefinger to determine if the grain was filled. Number of filled grain included both completely and partially filled grain. In experiment-I (Exp-I) and –II (Exp-II), the effects of HNT and

plant growth regulators on spikelet fertility were examined. In Exp-III, main-stem panicle was divided into three equal parts (lower middle and upper) and SF determined for each part.

Grain Dimensions

In Exp-I and II, the grain length and width of brown (dehulled) rice were determined using a Winseedle (Regent Instruments, Inc. Quebec, Canada), which analyzes scanned color images of grain to calculate these parameters. In Exp-III, the impact of HNT and plant growth regulator treatments on grain located at different positions within the main-stem panicle, including grain length and width of dehulled rice from the different parts (lower, middle and upper) of the panicle as determined using a Winseedle. In Exp-I, II and III, the weight of 100 grain was determined.

Grain Nitrogen Concentration

For each experiment, grain nitrogen concentration (GNC) was measured using a FP-528 Nitrogen/Protein analyzer (LECO Corporation, St. Joseph, Michigan, USA). Mainstem panicles were harvested and dried and the grain were hulled manually**.** The de-hulled (brown) rice was packed in a capsule (QUIK CAPS, LECO Corporation, St. Joseph, Michigan, USA) for nitrogen analysis. Grain nitrogen concentration was expressed as mg g^{-1}. For Exp-III, GNC was determined for grain located at different positions (lower, middle and upper) within the mainstem panicle.

Determination of Total Antioxidant Capacity

Total antioxidant capacity of rice leaves was measured during the grain-filling period using the DPPH [2,2-diphenyl-1-picrylhydrazyl] assay procedure from Mohammed and Tarpley (2009a). Five leaf discs (0.0785 cm^2 each) from penultimate leaves were obtained from mid-blade while avoiding the mid-vein. After weighing, leaf discs were placed in a test tube (4-ml) with 1.5-ml methyl alcohol (MeOH), which was then sealed. For complete extraction of antioxidants into the solution, the test tubes were incubated at room temperature for 24 h in darkness. Rice leaf extract of 40 µl was added to 960 µl DPPH (0.2 mM) solution and the optical density determined at 515 nm after 4 h. The antiradical efficiency of the rice leaf methanolic extracts was determined after 4 h by the reduction in absorbance (515 nm) of the methanolic solution of DPPH with the extract. The values of DPPH after adding the extract was compared with those obtained from a blank solution of DPPH (zero antiradical activity). Trolox (6-Hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid) and DPPH standard curves were developed and the values were expressed in µM trolox equivalents (TE) g^{-1} leaf (fresh weight basis) using these standard curves. The DPPH and trolox were purchased from Sigma-Aldrich (St. Louis, MO).

Statistical Analysis

Three experiments were conducted using a complete randomized design. One set of the plants was grown under ambient nighttime temperature, whereas the other set was grown under high nighttime temperature. In Exp I and II, there were 20 plants, five

plants per plant growth regulator treatment, under each nighttime temperature regime. In Exp III, there were 12 plants, three plants per plant growth regulator treatment, under each nighttime temperature regime. The first two experiments studied the effects of HNT and plant growth regulators on reproductive tillers, SF and grain characteristics, such as grain length, width, weight and nitrogen content. In the third experiment, the effects of HNT and plant growth regulators on SF and the above-mentioned grain characteristics were examined at different positions within the mainstem panicle. All data were analyzed using PROC GLM procedures in Statistical Analysis System (SAS) to determine the influence of experiments, nighttime temperatures, plant growth regulators, and their interactive effects on the above-mentioned physiological and grain parameters. Means were separated using Tukey's Least Significant Difference (LSD) at an alpha level of 0.05. If there was no significant difference among the experiments for a parameter, so the values from all the experiments for that parameter were used to estimate the mean and standard error.

Results

None of the treatments significantly affected the number of reproductive tillers per plant (Fig. 5.1A). However, an increase in nighttime temperature from 27 ^{o}C to 32 ^{o}C decreased SF by 73% (Fig. 5.1B). Plants grown under ambient nighttime temperature (ANT) and treated with vitamin E, GB or SA showed a 9%, 15% and 18% increase in SF, respectively, compared to untreated plants (Fig. 5.1B). Plants grown under HNT showed an increase in SF by 47%, 120% and 195% when treated with vitamin E, GB or SA, respectively, compared to untreated plants (Fig. 5.1B).

Nighttime temperature had no effects on mainstem panicle length and number of primary branches per panicle (Table 5.1). In addition, the application of plant growth regulators showed no effect on the number of primary branches per panicle in the ANT or HNT regime. However, plants grown under ANT treated with GB had a 5% shorter panicle length compared to untreated plants and plants treated with vitamin E or SA (Table 5.1).

Nighttime temperature and plant growth regulators affected grain length and width. Grain length and width both decreased by 2% in the high nighttime temperature treatment (Table 5.2). Plants grown under ANT and treated with plant growth regulators showed decreased grain length compared to untreated plants. However, plants grown under HNT and treated with plant growth regulators showed an increased grain length compared to plants under HNT but without any plant growth regulator treatment (Table 5.2).

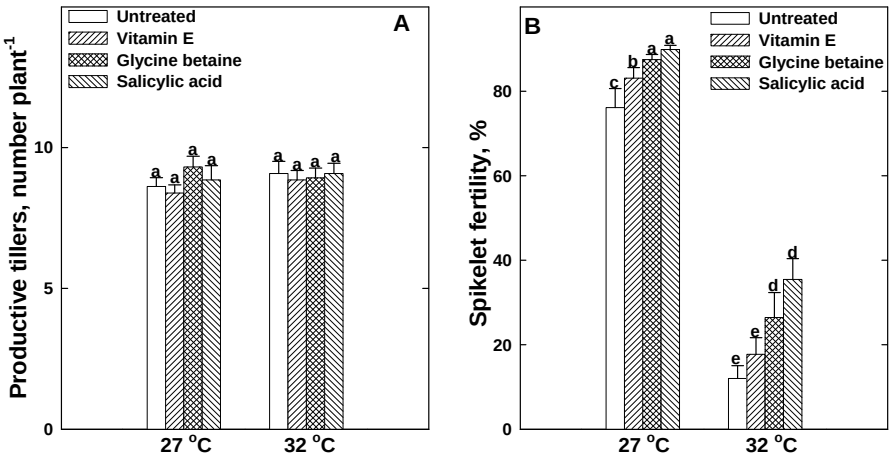

Figure 5.1. Effects of nighttime temperature and plant growth regulators on rice productive tillers (A) and spikelet fertility (B). The SE bars are shown if they are larger than the symbol.

Table 5.1. Effects of nighttime temperatures and plant growth regulators on mainstem panicle length and number of primary branches per panicle

Treatments	Panicle length (cm)	Primary branches per panicle
27°C-Untreated	22.53 ± 0.49 a[1]	16.08 ± 1.33 a
27°C-Vitamin E	21.95 ± 0.44 ab	16.85 ± 1.27 a
27°C-Glycine betaine	21.27 ± 0.35 b	15.54 ± 1.62 a
27°C-Salicylic acid	21.81 ± 0.42 ab	18.54 ± 2.01 a
Mean	**21.89 ± 0.22**	**16.75 ± 0.79**
32°C-Untreated	21.67 ± 0.69 ab	16.85 ± 1.24 a
32°C-Vitamin E	21.58 ± 0.60 ab	16.92 ± 1.13 a
32°C-Glycine betaine	22.43 ± 0.44 a	16.77 ± 1.40 a
32°C-Salicylic acid	21.93 ± 0.33 a	17.85 ± 1.77 a
Mean	**21.92 ± 0.26**	**17.10 ± 0.69**
Difference between group means	NS[2]	NS

Each value is the mean with standard error (+S.E.). [1]Means within a column followed by a different letter differed significantly ($P < 0.05$). [2]Group means significant at $P < 0.05$; otherwise, not significant (NS).

There was no difference among the plant growth regulators with respect to grain width for plants grown under ANT. However, plants grown under HNT and treated with vitamin E or GB showed 2% and 4% decreases in grain width, respectively, compared to untreated plants (Table 5.2).

On average, plants grown under HNT showed a 44% increase in GNC compared to plants grown under ANT. Plants grown under ANT and treated with vitamin E showed a 4.5% decrease in GNC, and plants grown under HNT and treated with SA showed an 8.75% decrease in GNC compared to untreated plants of the respective temperature regime (Table 5.2).

Plants grown under HNT showed a 20% decrease in 100-grain weight compared to plants grown under ANT (Table 5.2). There were no differences among the plant growth regulator treatments with respect to 100-grain weight under ANT or HNT (Table 5.2). Plant yield also decreased as a result of HNT. On average, plants grown under HNT across the plant growth regulator treatments showed a 90% decrease in yield compared to the corresponding treatments under ANT (Table 5.2). Plants grown under ANT and treated with vitamin E, GB or SA showed a 6%, 13% and 13.5% increase in yield, respectively, compared to untreated plants. Compared to untreated plants grown under HNT, plants treated with vitamin E, GB or SA showed 4.5% (N.S.), 77% ($P<0.05$) and 380% ($P<0.05$) increase in yield, respectively, when grown under HNT (Table 5.2).

Table 5.2. Effects of nighttime temperatures and plant growth regulators on grain length, grain width, grain nitrogen concentration, and grain yield

Treatments	Grain length (mm)	Grain Width (mm)	100-grain weight (g)	Nitrogen (mg g^{-1})	Grain dry weight (g $plant^{-1}$)
27°C-Untreated	7.51 ± 0.04 a[1]	2.18 ± 0.01 a	1.80 ± 0.25 a	11.00 ± 0.20 c	22.25 ± 0.86 b
27°C-Vitamin E	7.36 ± 0.03 bc	2.20 ± 0.01 a	1.89 ± 0.21 a	10.50 ± 0.20 d	23.56 ± 1.24 ab
27°C-Glycine betaine	7.29 ± 0.07 c	2.20 ± 0.01 a	1.87 ± 0.19 a	10.80 ± 0.20 cd	25.14 ± 1.30 a
27°C-Salicylic acid	7.34 ± 0.03 c	2.21 ± 0.01 a	1.94 ± 0.21 a	11.00 ± 0.20 c	25.26 ± 0.53 a
Mean	**7.37 ± 0.01**	**2.20 ± 0.01**	**1.88 ± 0.10**	**10.90 ± 0.10**	**24.05 ± 0.53**
32°C-Untreated	6.86 ± 0.07 d	2.19 ± 0.01 a	1.47 ± 0.26 a	16.00 ± 0.40 a	1.09 ± 0.30 e
32°C-Vitamin E	7.26 ± 0.05 c	2.15 ± 0.01 b	1.43 ± 0.19 a	16.30 ± 0.30 a	1.15 ± 0.25 e
32°C-Glycine betaine	7.30 ± 0.05 c	2.10 ± 0.01 c	1.49 ± 0.15 a	16.00 ± 0.60 a	1.94 ± 0.45 d
32°C-Salicylic acid	7.42 ± 0.04 b	2.18 ± 0.01 a	1.66 ± 0.18 a	14.60 ± 0.40 b	5.28 ± 0.68 c
Mean	**7.25 ± 0.03**	**2.15 ± 0.01**	**1.51 ± 0.09**	**15.70 ± 0.20**	**2.37 ± 0.35**
Difference between group means	*	*	*	*	*

Each value is the mean with standard error (+S.E.). [1]Means within a column followed by a different letter differed significantly (P > 0.05).

Exp-III studied the effects of nighttime temperatures and plant growth regulators on SF and grain characteristics at different positions within the panicle. In general, HNT decreased SF, and grain length and width across plant growth regulator treatments, irrespective of position within the panicle (Fig. 5.2A, B, C, D, E, F). For untreated plants grown under ANT, SF was lowest at the base of the panicle compared to the middle and upper parts of the panicle (Fig. 5.2A). However, application of GB or SA increased SF in the lower part of the panicle. Application of plant growth regulators increased SF in the lower, middle and upper parts of the panicle, compared to untreated plants, grown under HNT (Fig. 5.2B).

For untreated plants grown under ANT and HNT, kernels located at the base of the panicle were smaller in length and width, compared to kernels located in the middle and upper part of the panicle (Fig. 5.2C, D, E, F). However, plants grown under ANT and treated with GB showed increased grain length at the base of the panicle. Similarly, application of vitamin E or SA increased grain length of the grain located at the base of the panicle, for plants grown under HNT. In addition, application of vitamin E, GB or SA increased grain width of the grain located at the base of the panicle, when grown under ANT (Fig. 5.2E). However, under HNT, SA application increased grain width of the grain at the base of the panicle.

In general, across plant growth regulator treatments, HNT increased GNC irrespective of grain position within the panicle. In untreated plants, nitrogen content was greater in grain located at the base of the panicle compared to the middle and upper parts of the panicle, in both the heat treatments (Fig. 5.3A, B). Plants grown under ANT

and treated with vitamin E or GB showed increased nitrogen content in the grain from the upper part of the panicle compared to the grain from the middle and lower parts of the panicle. The nitrogen content of the grain located at the different positions within the panicle was inversely related to grain weight.

Irrespective of positions within the panicle, HNT decreased grain weight across all of the plant growth regulator treatments (Fig. 5.3C, D). In general, in both the heat treatments, application of plant growth regulators increased grain weight in each part (lower, middle and upper) of the panicle compared to untreated plants (Fig. 5.3C, D). Plants grown under ANT and treated with vitamin E or GB, and plants grown under HNT and treated with vitamin E or SA showed greater increases in weight of grain located at the base of the panicle compared to the middle and upper parts of the panicle (Fig. 5.3D).

There was no difference between any treatments with respect to total leaf antioxidant capacity. However, there was a significant interaction between the heat treatments and plant growth regulator treatments with respect to total leaf antioxidant capacity (Fig. 5.4A, B). Plants grown under ANT showed no difference among the plant growth regulators with respect to their effect on total leaf antioxidant capacity (Fig. 5.4A). However, GB-treated or SA-treated plants grown under HNT showed a 17% increase in total antioxidant capacity during the grain-filling period compared to untreated plants grown under HNT (Fig. 5.4B).

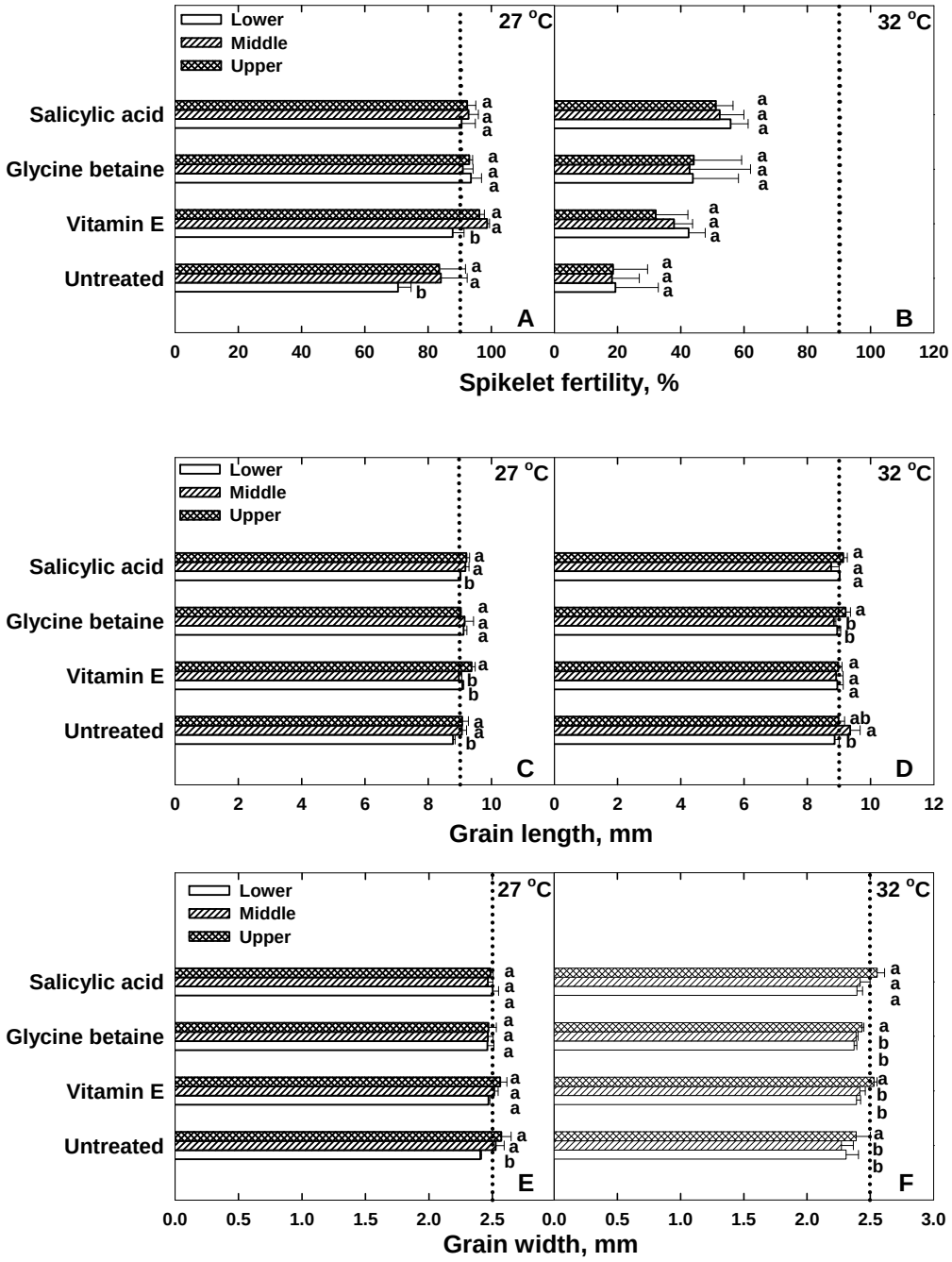

Figure 5.2. Effects of nighttime temperature and plant growth regulators on rice spikelet fertility (A, B), grain length (C, D) and grain width (E, F) examined at different positions within the main-stem panicle. The SE bars are shown if they are larger than the symbol.

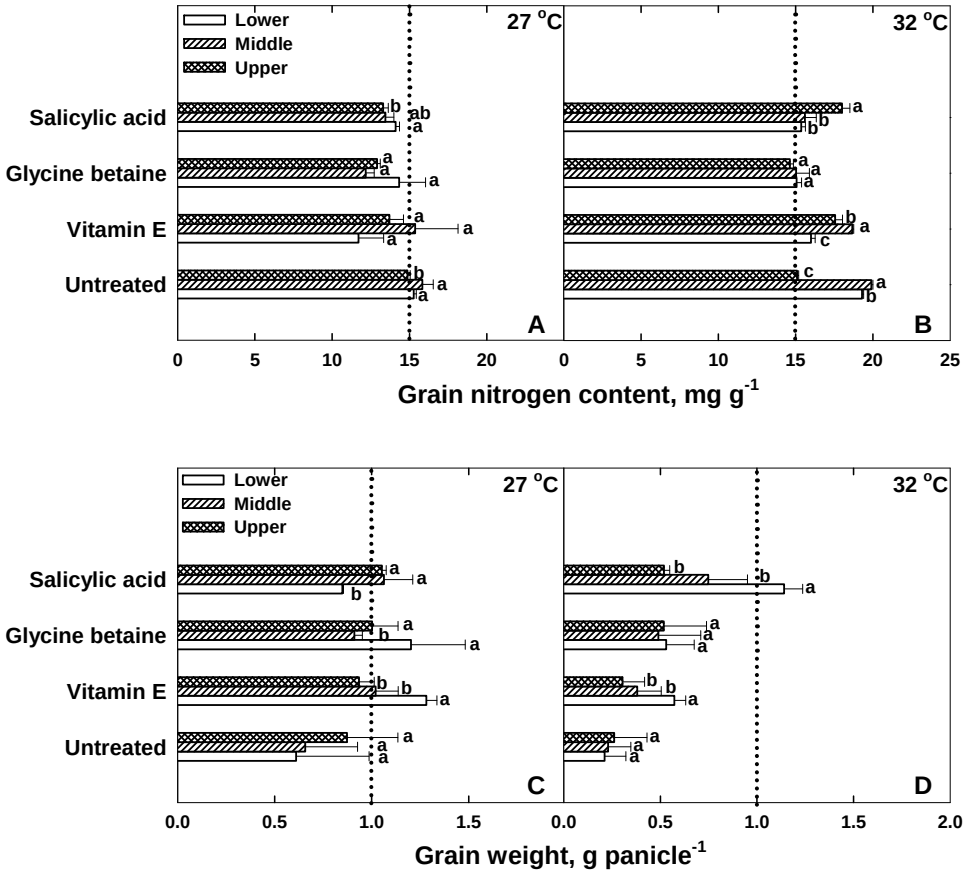

Figure 5.3. Effects of nighttime temperature and plant growth regulators on rice grain nitrogen content (A, B) and grain weight (C, D). The SE error bars are shown if they are larger than the symbol.

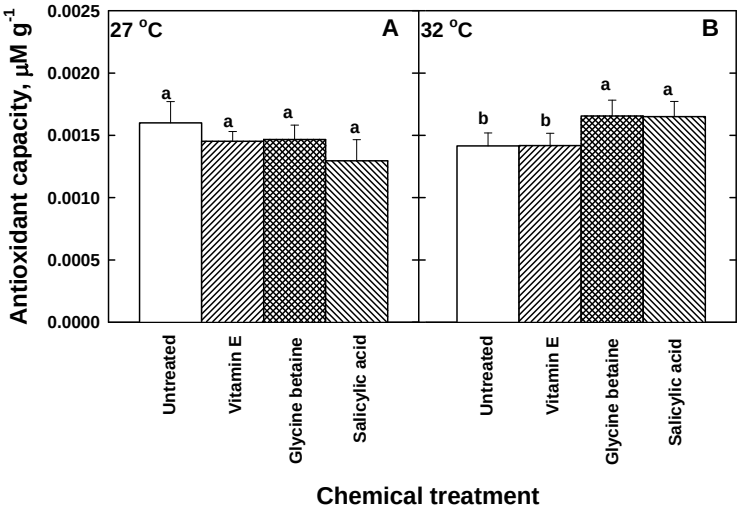

Figure 5.4. Effects of high nighttime temperature and plant growth regulators on total antioxidant capacity of rice leaves. The total antioxidant capacity was measured during the grain-filling period of the rice plants. There are two panels (A, B) in the graph and the data shown in each panel was analyzed separately. The SE bars are shown if they are larger than the symbol.

Discussion

Year-to-year variation in rice grain yield has been attributed to changes in nighttime temperature (Peng et al., 2004) due to global warming, which appears to be increasing at a faster rate than the corresponding daytime temperature (Karl et al., 1991). Our results indicate decreased plant grain yield under HNT was due to decreases in spikelet fertility (SF), grain length, and grain width. Similar results of decreased rice yield due to decreased SF as a result of high daytime (Prasad et al., 2006) or increased nighttime temperature (Ziska et al., 1996) have been reported in rice. Moreover, nighttime temperatures greater than 29 ^{o}C can decrease SF in rice with a subsequent reduction in seed-set and grain yield (Satake and Yoshida, 1978; Ziska et al., 1996). Based on previous research, the decrease in SF under HNT is related to pollen germination (Mohammed and Tarpley, 2009b). Fertility in rice is associated with number of pollen or germinated pollen on the stigma (Matsui et al., 2000; Farrell et al., 2006; Prasad et al., 2006) and anther dehiscence (Matsui et al., 2001), both of which are acutely sensitive to temperature. In the present study, nighttime temperatures did not affect production of reproductive tillers, panicle length or number of primary branches per panicle. Similar research with soybean (*Glycine max* L.) failed to show an impact of HNT on plant height, number of auxiliary branches and number of nodes (Seddigh and Jolliff, 1984).

The increase in nighttime temperature from 27 ^{o}C to 32 ^{o}C decreased grain length and width, thereby decreasing plant grain yield. Previous studies reported a decrease in grain yield due to decreased grain widths as a result of HNT (Counce et al., 2005; Morita

et al., 2005). The decreases in rice grain length and width might be associated with a reduction in average endosperm cell area observed under HNT (Morita et al., 2005). Moreover, it is known that the capacity of endosperm to accumulate dry matter is determined by endosperm cell number (Bingham, 1969), which is affected by high temperature (Commuri and Jones, 1999). In addition, cereals generally respond to high temperatures through an increase in the rate of kernel growth, which can lead to a decrease in the duration of dry matter accumulation (Zakaria et al., 2002). Decreased dry matter accumulation can lead to production of smaller grain or imperfect grain (Yoshida, 1981; Tashiro and Wardlaw, 1991). The apparent decrease in grain density due to HNT suggests that HNT led to production of kernels that were smaller. In the present study, grain weight was inversely related to GNC. Similar relationships between carbon and nitrogen accumulation have been reported in rice (Tashiro and Wardlow, 1991) and wheat (*Triticum esculentum* L.) (Triboi and Triboi-Blondel, 2002) cultivated under high daytime temperature. High temperatures can affect carbon and nitrogen flow to the grain. However, flow of carbon to the grain is more sensitive to temperature (Triboi and Triboi-Blondel, 2002).

The lower, middle and upper parts of the panicle exhibited similar patterns of decrease in SF, grain length, width, weight and increase in GNC as that of the whole mainstem panicle as a result of increase in nighttime temperature. However, for both the heat treatments, the lower part of the panicle displayed the lowest SF and smallest grain dimensions. When rice plants are under stress or if there are more florets than can be filled with carbohydrates, similar results have been noted (Stansel, 1975). A larger

number of blanks typically occur at the base of the panicle because kernels at the tip of the panicle are normally filled first (Stansel, 1975). Hence, there is greater competition for photosynthates by grain located lower on the panicle.

Previous studies have shown an increase in endogenous antioxidant levels as a result of exogenous application of antioxidants (Chen et al., 1997; Diaz-Zorita et al., 2001). Similar results were seen in our study in that exogenous application of GB and SA to the plants increased total antioxidant capacities, under HNT. Bohnert and Jensen (1996) and Rao et al. (1997) stated that GB and SA can alter antioxidant levels in plants, thereby preventing oxidative damage and protecting the membranes and enzymes (Harinasut et al., 1996; Rajasekaran et al., 1997; Diaz-Zorita et al., 2001) by detoxifying superoxide radicals (Bowler et al., 1992). In my study, an increase in total antioxidant capacity as a result of GB or SA application affected spikelet fertility, thereby significantly increasing yield. Based on our results, we propose that increased antioxidant capacity as a result from GB or SA application can limit the heat-induced ROS-damage to the membrane, thus protecting cellular integrity, including the enzymes involved in translocation of photosynthates, thereby increasing spikelet fertility, hence plant grain yield.

CHAPTER VI

SOLUBLE SUGARS AND STARCH BIOSYNTHESIS AND INVERTASE ACTIVITY OF RICE PLANTS SUBJECTED TO HIGH NIGHTTIME TEMPERATURES DURING EARLY-GRAIN FILLING STAGE

Introduction

The reproductive phase of many crops is relatively more sensitive than the vegetative phase to heat stress (Hall, 1992). High temperatures have also been reported to reduce kernel dry weight and induce kernel abortion in maize (Cheikh and Jones, 1995). In wheat (*Triticum esculentum* L.), kernel weight decreased with an increase in nighttime temperature (25°C) without an impact on total above ground biomass (Banowetz et al., 1999). The ovary appears to be an important sink for carbohydrates during growth and development of the kernel (Mäkelä et al., 2005). Disruption of carbon flow into the ovary during key stages induces kernel abortion. For example, starch degradation in the ovary is the primary factor for kernel abortion in maize (*Zea mays* L.) (Zinselmeier et al., 1999). Reduction in photoassimilate supply as a result of stress conditions can decrease the flow of sucrose into the ovary, thereby decreasing starch production (Zinselmeier et al., 1999) and affecting grain size and number of seeds retained.

In rice (*Oryza sativa* L.), 90% of the final dry weight of an unpolished grain is starch (Yoshida and Hara 1977). The actual process of starch accumulation is the grain

filling (Yang et. al., 2003), and the first step in starch synthesis is cleavage of sucrose to its constituent monosaccharides, which are either used in biosynthetic processes or maintenance processes (Ranwala and Miller 1998). Sucrose is the primary sugar transported in rice plants (Nelson and Cox, 2003), and invertase (Enzyme Commission 3.2.1.26) activity in the hydrolysis of sucrose is suspected to have a large influence during early ovary development for kernel retention, starch concentration and maintenance of sink strength. The invertase activity likely influences the survivability of the zygote during stress conditions (Andersen et al., 2002) by playing an important role in the hydrolysis of sucrose for starch production by producing substrates upstream for starch synthesis in ovaries (Zinselmeier et al., 1999). Sucrose catabolism is a major determinant of sink strength in nearly all actively growing plants and affects sucrose partitioning to growing sinks, thereby affecting sink size and carbohydrate concentration of the sink. Invertase and sucrose synthase (Enzyme Commission 2.4.1.13) activities in the sink tissue can be taken as a reflection of the mobilization strength of the sink (Claussen et al., 1986).

High temperatures can reduce crop yields by influencing sucrose availability (Afuakwa et al., 1984) and by affecting enzymes involved in grain filling (Singletary et al., 1994). When exposed to HNT, mature leaves of roses (*Rosa hybrida* cv Golden Times) showed an increase in invertase activity, whereas the young shoots showed a decline in the activity (Khayat and Zieslin, 1987).

Many studies have been conducted looking at the effects of daytime temperatures and water stress on these enzymes. However, effects of short term exposure of high nighttime temperature (HNT) on carbohydrate composition and invertase activity have not been well characterized. The objective of this study was to investigate the effects of HNT and plant growth regulators on carbohydrate profiles and invertase activity of rice leaves and grain. The plant growth regulators used in this study are defined as exogenously applied chemicals that have profound effects on plant growth, development or physiology at low concentrations.

Materials and Methods

Plant Culture

Two experiments were conducted in the greenhouse at the Texas A&M Universisty System, AgriLife Research and Extension Center at Beaumont, Texas, USA. 'Cocodrie', a common southern U.S. rice cultivar of tropical japonica background, was used. In each experiment, a set of twenty plants, five plants per plant growth regulator treatment, was grown under ANT, and another set was grown under HNT. Plants were grown in 3L pots placed in a square wooden box (0.84 m^2), 10 pots per box. The boxes were lined with black plastic (thickness = 0.15 mm; FILM-GARD, Minneapolis) that served as a water reservoir. Pots were filled with a clay soil (fine montmorillonite and thermic Entic Pelludert (Chen et al., 1989) that is common to rice farms in the area. At 20 days after emergence (DAE), the boxes were filled with water to approximately 3 cm above the top of the soil in each pot. A reflective foam cover was placed over the water surface to prevent direct infrared heating of water. A three-way split application of nitrogen was used as described by Mohammed et al. (2007). Nitrogen was applied in the form of urea and ammonium sulfate, and phosphorus in the form of P_20_5. At planting, urea-N was applied at the rate of 112.3 kg ha^{-1} along with 45.4 kg ha^{-1} phosphorus (P_20_5). The second and third nitrogen fertilizations (both 44.9 kg ha^{-1} nitrogen in the form of ammonium sulfate) were applied 20 DAE and at panicle-differentiation. The plants were uniform in terms of developmental stage, as indicated by grain development, at the beginning of the heat treatments described below.

Temperature Treatments

The assignment of heat treatment to greenhouse location was random within each experiment. For 2005, the average nighttime temperatures (between 2000h to 0600h) at Beaumont, Texas, USA (Longitude: 94^{o} 16′ 59″ W; Latitude: 30^{o} 4′ 0″ N) during the reproductive growth (panicle differentiation to harvest) of the rice plants ranged between 26 ^{o}C and 28 ^{o}C (Mohammed and Tarpley, 2009a). Hence, the ambient nighttime temperature (ANT) was set at 27 ^{o}C. The greenhouse was maintained at 27 ^{o}C nighttime temperature and, within this, plants of the HNT treatment were subjected to elevated nighttime temperature through the use of nearly continuously controlled (sub-second response) infrared heaters (Chromalox, Ogden, Utah, USA), which were positioned 1.0 m above the topmost part of the plants. In the ambient nighttime temperature treatments, dummy heaters were provided to account for shading. The single fixed element radiant process heater was mounted in an aluminum housing (frame). The length and breadth of the frame is 77.8 cm and 9.4 cm, respectively. The length of the heating element is 57.8 cm and the diameter is 1cm. The working voltage of the heating element is 120 volts. The operating wavelengths of the infrared heaters are well above 1200 nm, and the infrared heater output is negligible below 1200 nm (Omega, 2008). Hence, there was no significant emission of photo-morphogenic (wavelength below 780 nm) wavelengths. The infrared heaters were controlled using semi-conductor controllers (SCR Power Controller, Watlow Electric Manufacturing Company, St. Louis, Missouri, USA) to enable proportioning heating action instead of on/off action. Air temperatures surrounding the plants were controlled at predetermined set points (27 ^{o}C and 32 ^{o}C).

When the temperature was below the set point as determined by the air-temperature thermocouples positioned within a few centimeters of the uppermost parts of the plant, the controller sent a signal to the infrared heaters, which provided short slightly elevated heating events, as needed, to raise the temperature to the desired set point. Air temperatures were monitored and maintained within the set points $\pm$ 0.5 ^{o}C (Fig. 6.1A, B). The nighttime temperature was imposed from 2000 h until 0600 h during the grain-filling period for 5 nights. Daytime temperature, nighttime temperature and humidity were independently monitored using standalone sensor/loggers (HOBOs, Onset Computer Corporation, Bourne, Massachusetts, USA) (Fig 6.1A, B).

Plant Growth Regulator Treatments

The α-tocopherol (vitamin E), glycine betaine (GB), and salicylic acid (SA) were applied at the rate of 100 μL per application to the leaves using a pre-calibrated perfume-bottle sprayer. Each plant was treated three times (on a single day) to enable thorough coverage, prior to imposing heat stress. The α-tocopherol was applied at the rate of 58 μmol per plant per spray (i.e., 580 mM); GB was applied at 182.3 μmole per plant per spray (i.e., 1.823 M), and SA at 0.1 μmol per plant per spray (i.e., 1mM). Vitamin E and SA were purchased from Sigma-Aldrich (St. Louis, Missouri, USA), and GB was supplied by Capstone Food Ingredients (Marion, Massachusetts, USA).

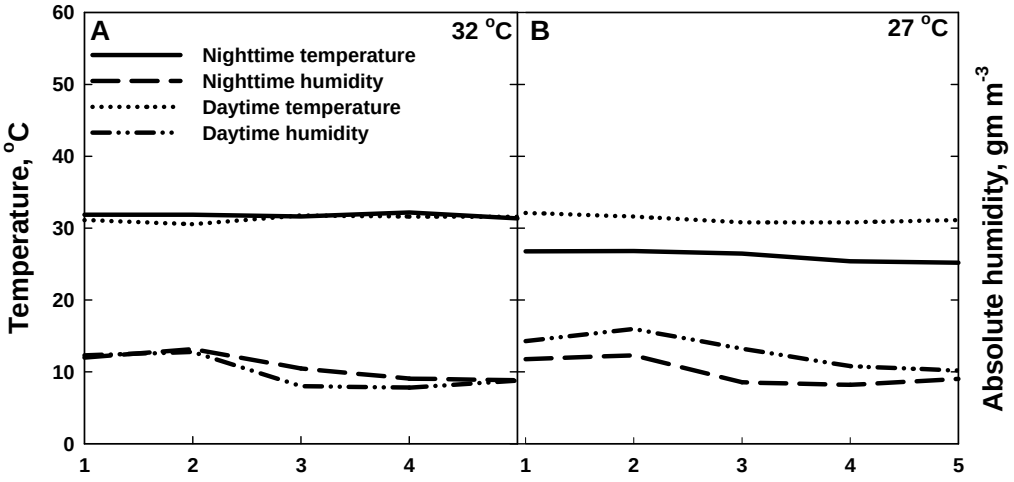

Figure 6.1. Average nighttime temperatures imposed during early grain-fill stage for five nights using infrared heaters along with average daytime temperature during the same period. Average daytime and nighttime humidity are also shown under the two nighttime temperature regimes. Panel A represents the high nighttime temperature regime, and panel B the ambient nighttime temperature regime.

Plant Sampling

The penultimate (second leaf from the top) leaves and the panicles from the mainstem and tillers were harvested after imposing the heat treatment (five nights). The samples were collected in liquid nitrogen and stored in an ultra-cold freezer (Environmental Equipment, Cincinnati, Ohio, USA) at -75 ^{o}C for later use. Before pulverizing, the grain was separated from the panicle under liquid nitrogen, then pulverized in a custom steel trough-shaped mortar with a chisel. The ground samples were aliquoted into five subsamples of 50 mg each and four subsamples were stored in the ultra-cold freezer until use. One subsample was placed into a drying oven (Blue Island, Illinois, USA) at 70°C for four days to remove all moisture and used to determine dry weight (DW).

Carbohydrate Extraction and Assay

The frozen pulverized plant material was placed into 13.7 *M* (80%) ethanol within a vial sitting in liquid N_2. The vial was then transferred to hot bath conditions (70 ^{o}C) for extraction of soluble sugars. The pooled extract obtained from repeated extraction (3 times at approximately 8 h each) was treated with neutralized decolorizing-type activated charcoal (Sigma Plant growth regulator Co., St. Louis, Missouri, USA.) to adsorb chlorophylls and phenols that might interfere with the enzymes used to assay the sugars (Tarpley et al. 1993). The extract was stored at -20 ^{o}C for sugar analysis. Aliquots of aqueous sugar-containing solution were assayed for glucose, fructose and sucrose using enzymatic assays (Tarpley and Sassenrath, 2006). This procedure involves the

addition of phospho-glucose isomerase (for fructose assay) or invertase (sucrose assay) to a glucose assay mix to enable quantification of fructose, sucrose, and glucose. The sum of glucose, fructose and sucrose was expressed as total soluble sugars and the sum of total soluble sugars and starch as total nonstructural carbohydrates (TNC). The sugars were quantified through stiochiometric production of β-nicotinamide adenine dinucleotide (reduced form) (NADH) at 340 nm using a Power $Wave_X$ microplate spectrophotometer (Bio-Tek Instruments, Inc., Winooski, Vermont, USA). Glucose-assay (hexokinase-based) kits from Sigma-Aldrich (St. Louis, Missouri, USA.) were used at ½ x recommended reagent strength. Standards were prepared and their absorbances were used to construct a standard curve for each microplate from which the sugar concentrations of samples were determined. All microplate standard curves were linear with an $r^2 > 0.98$. The sugars were assayed separately rather than sequentially. The amount of glucose, fructose and sucrose were expressed as μg gDW^{-1}.

Enzyme Extraction

Plant materials stored at -75 ^oC were homogenized according to the procedures of Tarpley et al. (1994), as modified by Rounds and Tarpley (personal communication). Pulverized plant samples (50 mg) were mixed with 1.0 mL homogenization medium (90 mM HEPES (N-[2-hydroxyethyl] piperazine-N-[2-ethanesulfonic acid]), 5 mM $MgCl_2$, 10 mM L-Cysteine, 10% glycerol and 1 mM EDTA (ethylene-diamine-tetraacetic acid, with pH 7.2, 0.2 mL acid-washed polyvinylpolypyrrolidone and 10 μL of 50 X protease inhibitor cocktail) and homogenized with a Tissue Tearor™ (Biospec Products, Inc.,

Racine, Wisconsin, USA) while kept cold by immersion in an ice bath for 1 minute, until a uniform slurry was formed. This slurry was passed through desalting columns containing Sephedex G-25 pre-soaked and infiltrated with desalting buffer (homogenization medium excluding polyvinylpolypyrrolidone and protease inhibitor cocktail). The desalting columns were spun (Marathon 3200R Centrifuge, Fisher Scientific, Pittsburgh, Pennsylvania, USA) at 3200 X *g*-force (4000 rpm) at 4˚C for 5 minutes. The eluate was collected and separated into 0.5-mL aliquots and placed into 2 mL polypropylene vials and stored in the ultra-cold freezer (-75˚C) until used for enzyme assay.

pH and Temperature Optimization for Invertase Activity

A pH curve was established from pH 4.0 to pH 8.0 in increments of pH 1.0, using selected buffers. The buffer stock solutions were prepared at 250 mM concentration. Citric acid was used for pH 4.0 and pH 5.0, MES (morpholinoethanesulfonic acid) for pH 6.0, PIPES (Piperazine-1,4-bis[2-ethanesulfonic acid]) for pH 7.0 and Glycyl-Glycine for pH 8.

The temperatures tested for invertase activity optimization were between 22°C and 47°C at 5°C increments. The enzyme mix and the samples were exposed to different temperatures using a water bath (Water Bath, Magni Whirl, Blue M, New Columbia, Pennsylvania, USA). The reaction for invertase activity in the pH and temperature response curve was performed with 25 µL 500 mM sucrose, 50 µL selected buffer, 25 µL enzyme preparation, and brought to a final volume of 150 µL with de-ionized water

(diH_2O). The activity of invertase was measured across pH without concern for the buffer used. The amount of NADH present, as determined by absorbance at 340 nm, is stoichiometric to the amount of glucose and fructose present in the samples.

The pH and temperature curves showed similar patterns for leaf and grain samples (Fig. 6.2). The peak in absorbance was seen at pH 6 for both leaf and grain (Fig. 6.2A). The peak in absorbance was seen at 37 ^{o}C for both leaf and grain (Fig. 6.2B). Hence, invertase assay was carried out at pH 6 and 37 ^{o}C.

Enzyme Assay

The assay was carried out at pH 6 at 37 ^{o}C for 15 minutes. The reaction was stopped by heating the mixture at 95 ˚C for 4 min in a dry bath incubator (Fisher Scientific, Pittsburgh, Pennsylvania, USA) with the wells of the heating block partially filled with water to provide good heat transfer. After the reaction was stopped, the amount of product resulting from invertase action was measured spectrophotometrically using enzymatic assay. For each plate, 24 wells of the 96-well plate were loaded with 100 µL of ½ X fructose assay reagent and 5 µL of standards. The remaining 72 wells were loaded with 100 µL of ½ X fructose assay reagent and 20 µL of sample. The loaded plates were incubated at room temperature for 45 min before the plates were read using a $PowerWave_X$ Spectrophotometer at 340 nm. The amount of NADH present, as determined by absorbance at 340 nm, is stoichiometric to the amount of glucose and fructose present in the samples. The activity of invertase is expressed as µmole sucrose cleaved s^{-1} kg DW^{-1}. The plant growth regulators involved in this procedure were

purchased from Sigma-Aldrich (St. Louis, Missouri, USA), except for the protease inhibitor cocktail and EDTA, which were purchased from Calbiochem (San Diego, California, USA) and Acros Organics (Morris Plains, New Jersey, USA), respectively.

Data Analysis

Both experiments used a complete randomized design. One set of plants was grown under ambient nighttime temperature, whereas the other set was grown under high nighttime temperature. In each set, there were 40 plants, 10 per plant growth regulator treatment. All data were analyzed using PROC GLM procedures in Statistical Analysis System (SAS) to determine the influence of experiments, nighttime temperatures, plant growth regulators, and their interactive effects on sugars, starch and invertase activity. Means were separated using Tukey's Least Significant Difference (LSD) at an alpha level of 0.05. If there was no significant difference among the experiments for a parameter, the values from both experiments for that parameter were used to obtain the mean and error.

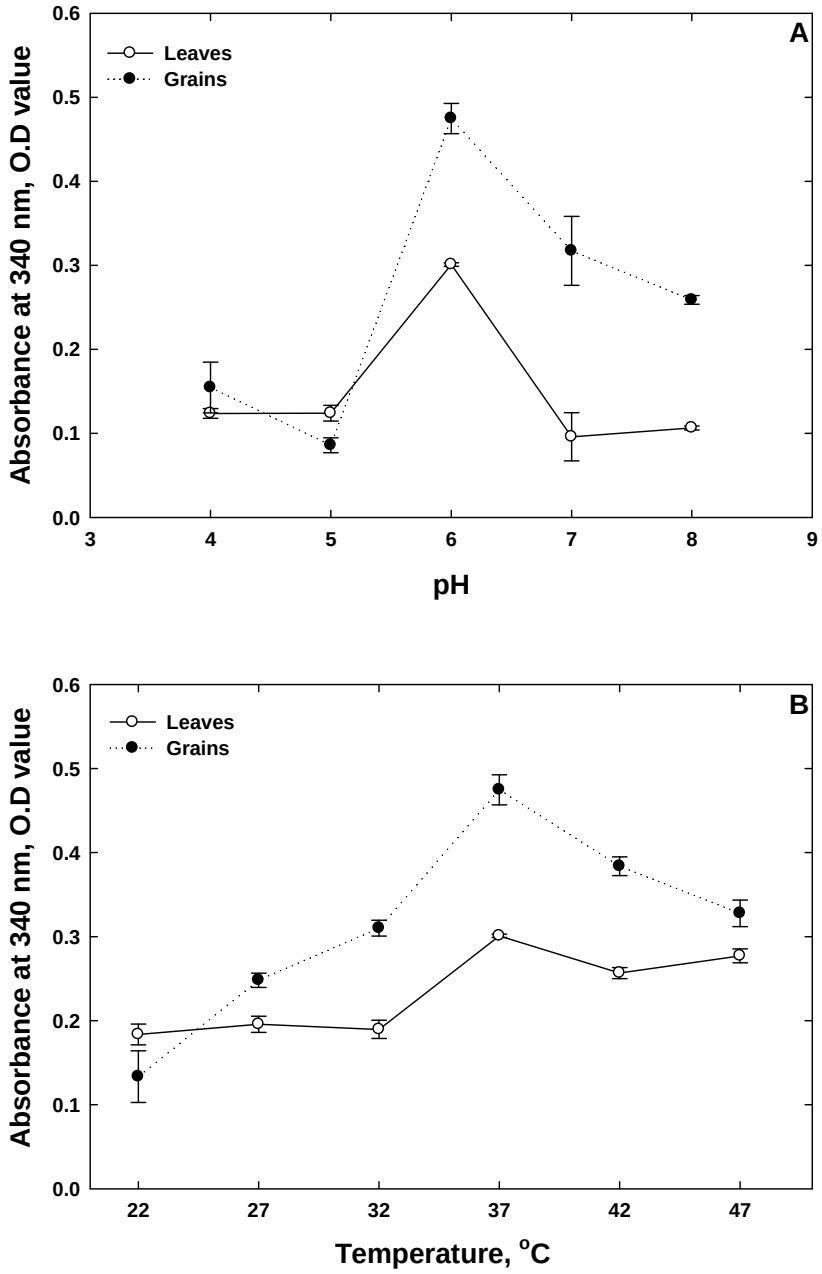

Figure 6.2. The pH (A) and temperature (B) optimization curves for the extractable invertase reaction.

Results

At early grain-fill (EGF), averaged across the nighttime temperatures and plant growth regulator treatments, rice leaves had greater glucose (93%), fructose (32%) and sucrose (98%) concentrations compared to grain (Fig. 6.3). However, the grain had a greater (87-fold) starch concentration than leaves at EGF. Across the plant growth regulator treatments, HNT did not affect glucose and sucrose concentrations in the leaves or fructose, sucrose and starch concentrations in the grain. However, HNT increased fructose concentration by 46% and decreased starch concentration by 14% in leaves, compared to the ambient nighttime temperature (ANT) treatments (Fig. 6.3). In the grain, across the plant growth regulator treatments, HNT decreased glucose concentration by 14%, compared to ANT. Across the nighttime temperature treatments, vitamin E- and salicylic acid (SA)-treated plants showed 16% and 47%, and 25% and 41%, increases in glucose concentration in the leaves and grain, respectively, compared to untreated plants. Fructose concentration in the leaf and grain decreased by 23% and 21%, respectively, in GB-treated plants, compared to untreated plants. However, SA-treated plants showed a 40% increase in leaf fructose concentration, compared to untreated plants (Fig. 6.3). Glycine betaine-treated plants showed a 12% decrease in leaf sucrose concentration, whereas vitamin E-treated plants showed a 20% increase in grain fructose concentration, compared to untreated plants. Vitamin E-treated plants showed a 27% increase in leaf starch concentration, whereas SA-treated plants showed a 10% decrease in grain starch concentration, compared to untreated plants (Fig. 6.3).

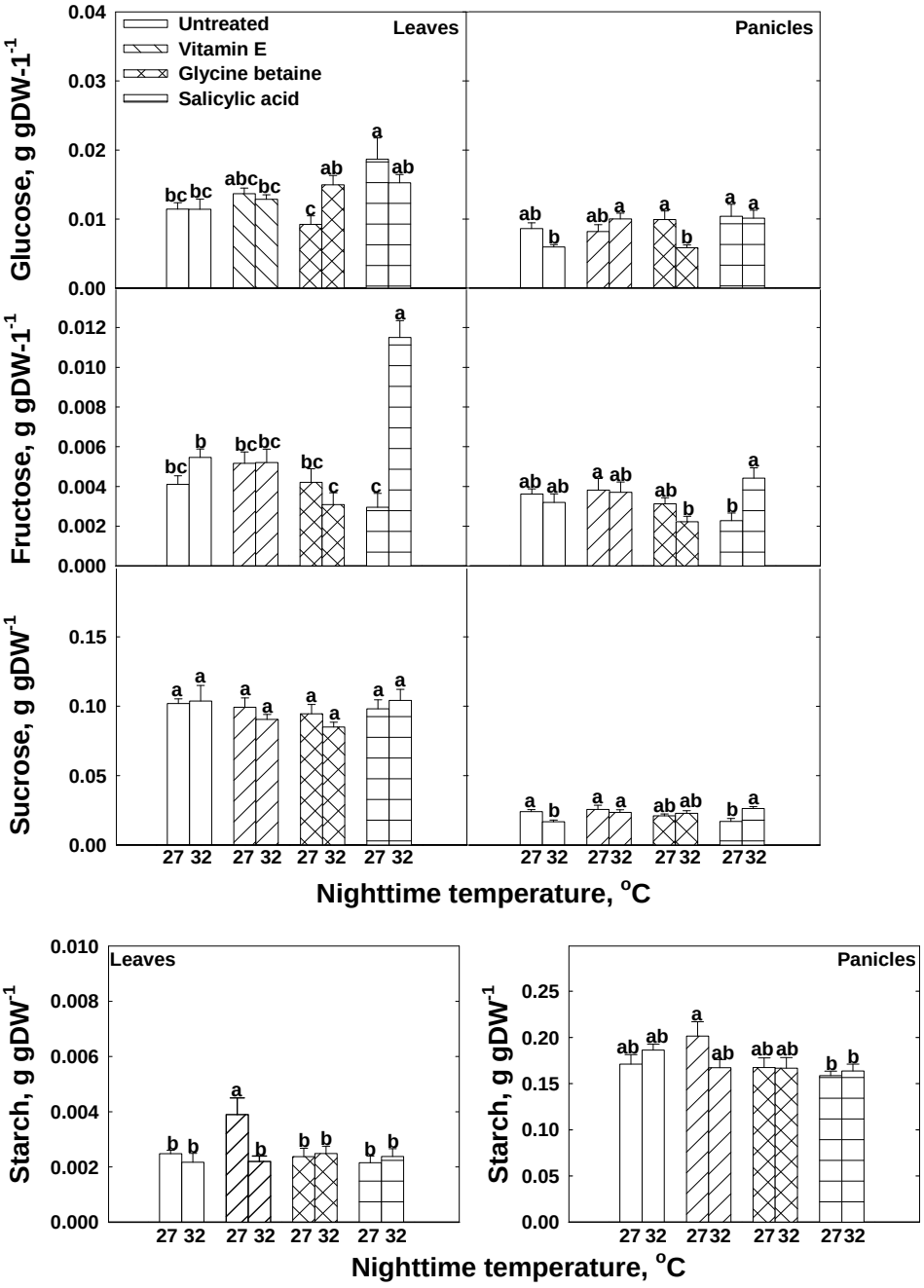

Figure 6.3. Effects of nighttime temperature and plant growth regulators on glucose, fructose, sucrose and starch concentrations. Glucose, fructose, sucrose and starch concentrations were measured in the penultimate leaf and the grain at the early grain-fill stage of the rice plant. The SE bars are shown if they are larger than the symbol. The values are averages of the results of two independent experiments (N=10).

Across the nighttime temperature and plant growth regulator treatments at EGF, rice leaves had a greater soluble-sugar concentrations (71%) and a lower total nonstructural carbohydrate (TNC) (81%) concentration compared to grain (Fig. 6.4). Across the plant growth regulator treatments, there were no differences between the nighttime temperature treatments for soluble sugar and TNC in the leaves. However, HNT decreased TNC concentration by 9% in the grain. Across the nighttime temperature treatments, SA-treated plants showed a 13% increase in leaf soluble-sugar concentration, whereas vitamin E- and GB-treated plants showed 23% and 16% increases in grain soluble-sugar concentration, compared to untreated plants. There were no differences among the plant growth regulator treatments for leaf TNC concentration; however, SA-treated plants had an 8% lower grain TNC concentration, compared to untreated plants (Fig. 6.4).

Across the nighttime temperature and plant growth regulator treatments at EGF, rice leaves had greater (30%) invertase activity compared to grain (Fig. 6.5). Across the plant growth regulator treatments, HNT decreased invertase activity in the grain by 19% (Fig. 6.5). However, there was no difference between the nighttime temperatures for leaf invertase activity. Across the nighttime temperatures, GB and SA-treated plants showed 20% and 23% increases, respectively, in invertase activity in the leaf, compared to untreated plants (Fig. 6.5). However, there was no difference among the plant growth regulator treatments for grain invertase activity.

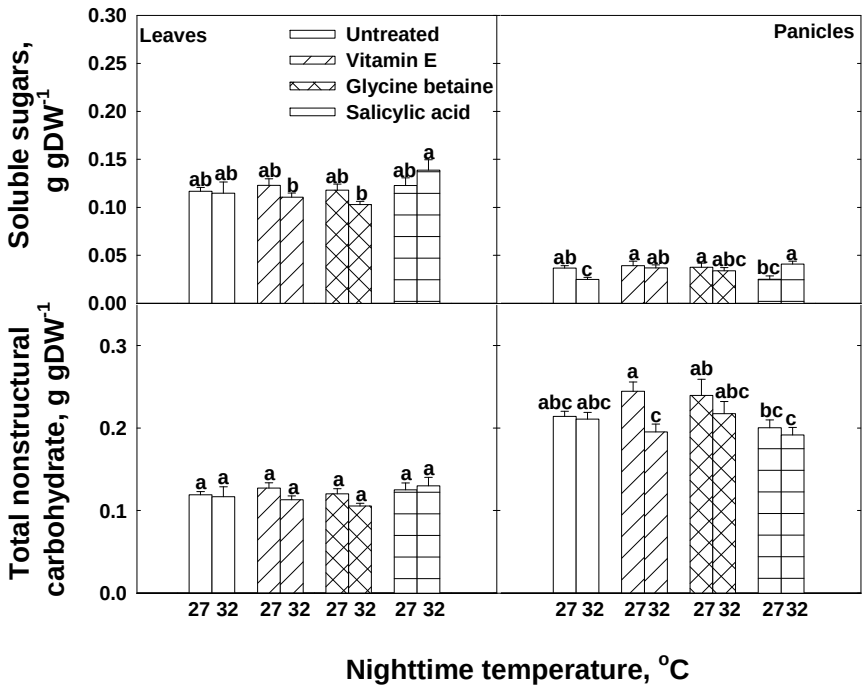

Figure 6.4. Effects of nighttime temperature and plant growth regulator treatments on soluble sugars and total nonstructural carbohydrate concentrations. Soluble-sugar and TNC concentrations were measured in the penultimate leaf and the grain at the early grain-fill stage of the rice plant. The SE bars are shown if they are larger than the symbol. The values are averages of the results of two independent experiments (N=10).

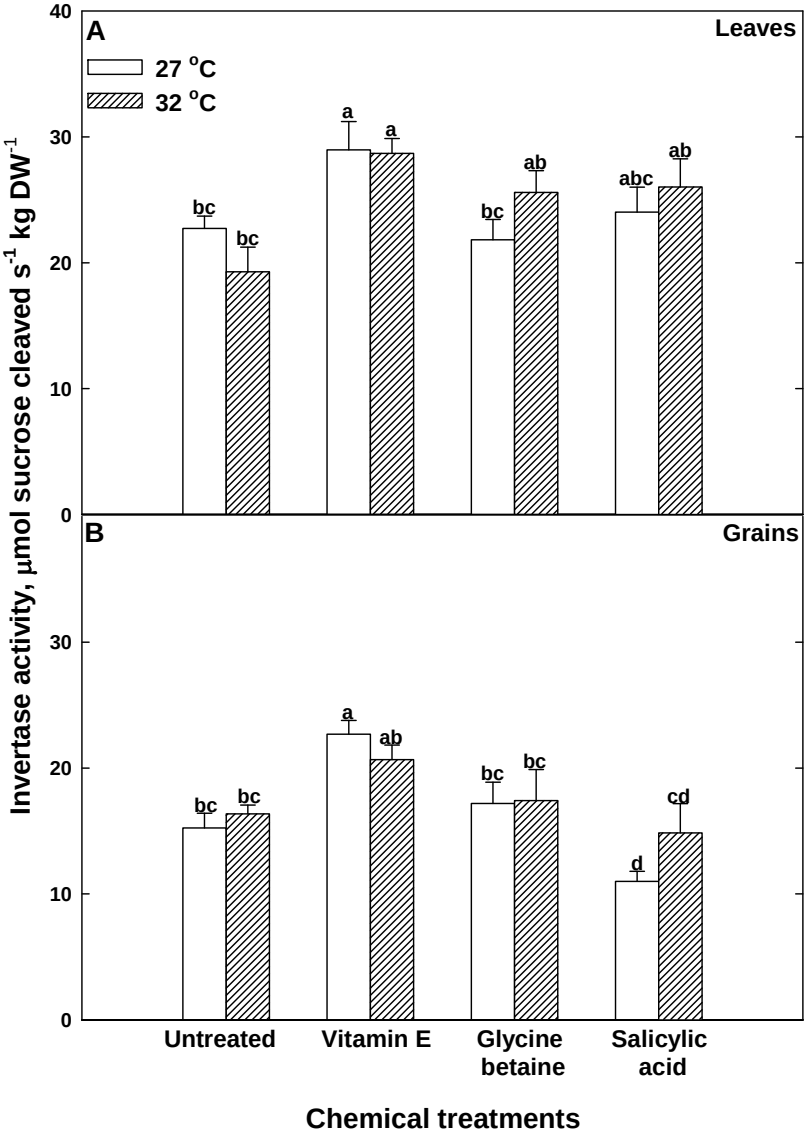

Figure 6.5. Effects of nighttime temperature and plant growth regulator treatments on invertase activity on a dry weight basis. The invertase activity was measured in the penultimate leaf and the grain at the early grain-fill stage of the rice plant. The SE bars are shown if they are larger than the symbol. The values are averages of the results of two independent experiments (N=10).

Discussion

In the present study, HNT decreased grain glucose concentration, total nonstructural carbohydrate (TNC) concentration and invertase activity. Sucrose and starch concentrations were inversely related and not associated with invertase activity; however glucose and TNC concentrations were associated with invertase activity, indicating that degradation of sucrose by invertase may not be rate limiting and may not be required for transfer of carbohydrates into the filling grain, suggesting degradation of sucrose through the sucrose synthase pathway might be important for grain filling. Moreover, it has been reported that invertase activity was highest in the pre-storage phase rather than in the storage phase of rice grain development (Ishimaru et al. 2005), further supporting the notion that sucrose degradation leading to starch biosynthesis might include substantial involvement of the sucrose synthase pathway in rice grain. In the present study, invertase activity was less in the grain compared to the leaf which is in contrast to the findings of Nakamura et al. (1989), which reported higher invertase activity in rice grain compared to leaves. Exposure to HNT for five nights decreased invertase activity in rice grain without affecting leaf invertase activity, indicating extractable invertase activity on a dry-matter basis in the grain is more sensitive to HNT, as imposed in this study, than invertase activity in leaves. Previous study by Aloni et al. (1992) showed invertase activity in pepper (*Capsicum annuum* L.) leaves decreased after seven days of heat exposure.

Application of vitamin E and SA prior to imposing high nighttime temperatures increased leaf starch and leaf soluble sugar concentrations, respectively. Vitamin E- and

GB- treated plants showed increase in grain soluble sugar concentrations, whereas SA-treated plants showed decreased grain starch concentration. Glycine betaine- and SA-treated plants showed increased leaf invertase activity. Glycine betaine enhances tolerance to high temperatures by protecting some enzymes, potentially including invertase, against heat-induced inactivation (Paleg et al., 1981), whereas vitamin E and SA render protection against oxidative damage (Fryer, 1992, Larkindale and Huang, 2004) as a result of high temperatures. Moreover, application of GB or SA increases total antioxidant capacities thereby rendering membrane stability under HNT (Mohammed and Tarpley 2009a) and protecting the enzymes (Rajasekaran et al., 1997; Rao et al., 1997; Diaz-Zorita et al., 2001), possibly including invertase. Although invertase activity has been implicated as being important to the survival of the zygote during stress conditions (Anderson et al., 2002) by playing an important role in the hydrolysis of sucrose to provide substrates upstream for starch synthesis in ovaries (Zinselmeier et al., 1999), it is unclear whether the decline in invertase activity due to HNT in the present study was sufficient to severely affect grain filling. These results make it difficult to clearly assign an importance for invertase during rice grain filling under high nighttime temperatures.

In conclusion, HNT decreased leaf starch concentration, grain TNC (soluble sugars plus starch) concentration, and grain invertase activity. Vitamin E- and GB-treated plants showed increased grain soluble sugars, whereas SA-treated plants showed increased leaf soluble sugar and decreased grain TNC concentrations. Glycine betaine- and SA-treated plants showed increased leaf invertase activity. The plant growth

regulator treatment effects on soluble-sugars, starch and invertase are probably indirect through their different mechanisms when acting as preventatives of abiotic stress. Our results indicate invertase is not rate-limiting and is not required for sucrose degradation in support of starch synthesis in rice grain under short-term high nighttime temperatures exposures during grain filling.

CHAPTER VI

SUMMARY

A heating system was developed that uses overhead infrared heaters and provides relatively inexpensive, accurate, and precise rapid control of temperature. The system uses remote computer-based data acquisition and control via the internet, which provides the ability to use complex temperature regimes and real-time monitoring. Due to its easy mobility, the heating system can be allotted in the open field or in the greenhouse within an experimental setup. The infrared heating system maintained constant set-point temperature $\pm$ 0.5 ^{o}C and was used to evaluate rice plant physiological response to high nighttime temperatures (HNT).

I hypothesized that HNT affects production (decrease), consumption (increase) and transfer (decrease) of photosynthates into grain, and further impacts morphology, developmental rates and reproduction in rice plants. I also hypothesized application of plant growth regulators renders protection against HNT, by increasing the antioxidant status in the plant. In the present study, I investigated the effects of HNT on rice leaf respiration, leaf membrane stability, total leaf antioxidant capacity, leaf photosynthetic rates, morphology, developmental rates, pollen germination, reproductive tillers, spikelet fertility, and grain length, width, and weight. In addition, I determined if application of plant growth regulators (α-tocopherol [vitamin E], glycine betaine [GB] or salicylic acid [SA]) can negate the negative effects of HNT in rice plants.

My results indicate no effect of HNT on production of photosynthates (on a per unit leaf area basis) or rice morphology; however, profound effects on leaf production,

consumption of photosynthates (increased), developmental rates (increased) and reproduction (decreased) were noticed. Exogenous application of GB and SA increased yields under HNT, possibly acting through increased antioxidant levels, which might have protected the membranes and enzymes against heat-induced ROS-mediated degradation.

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