

ORIGINAL ARTICLE

Field-based infrastructure and cyber–physical system for the study of high night air temperature stress in irrigated rice

Cherryl Quiñones¹ | Maria Arlene Adviento-Borbe² | Wenceslao Larazo¹ |
 Rodney Shea Harris¹ | Kharla Mendez¹ | Shannon S. Cunningham¹ |
 Zachary C. Campbell¹ | Karina Medina-Jimenez¹ | Nathan T. Hein³ | Dan Wagner³ |
 Brian Ottis⁴ | Harkamal Walia⁵ | Argelia Lorence^{1,6} 

¹Arkansas Biosciences Institute, Arkansas State University, Jonesboro, Arkansas, USA

²Delta Water Management Research Unit, United States Department of Agriculture–ARS, Jonesboro, Arkansas, USA

³Department of Agronomy, Kansas State University, Manhattan, Kansas, USA

⁴Global Solutions, RiceTec, Inc, Alvin, Texas, USA

⁵Department of Agronomy & Horticulture, University of Nebraska-Lincoln, Lincoln, Nebraska, USA

⁶Department of Chemistry and Physics, Arkansas State University, Jonesboro, Arkansas, USA

Correspondence

Argelia Lorence, Arkansas Biosciences Institute, Arkansas State University, P.O. Box 639, Jonesboro, AR 72467, USA.
 Email: alorence@astate.edu

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Abstract

High night air temperature (HNT) stress negatively impacts both rice (*Oryza sativa* L) yield and grain quality and has been extensively investigated because of the significant yield loss observed (10%) for every increase in air temperature (1°C). Most of the rice HNT studies have been conducted under greenhouse conditions, with limited information on field-level responses for the major rice sub-populations. This is due to a lack of a field-based phenotyping infrastructure that can accommodate a diverse set of accessions representing the wider germplasm and impose growth stage-specific stress. In this study, we built six high-tunnel greenhouses and screened 310 rice accessions from the Rice Diversity Panel 1 (RDP1) and 10 commercial hybrid cultivars in a replicated design. Each greenhouse had heating and a cyber–physical system that sensed ambient air temperature and automatically increased night air temperature to about 4°C relative to ambient temperature in the field for two cropping seasons. The system successfully imposed HNT stress of 4.0 and 3.94°C as recorded by Raspberry Pi sensors for 2 weeks in 2019 and 2020, respectively. HOBO sensors (Onset Computer Corporation) recorded a 2.9 and 2.07°C temperature differential of ambient air between control and heated greenhouses in 2019 and 2020, respectively. These greenhouses were able to withstand constant flooding, heavy rains, strong winds

Abbreviations: HNT, high night air temperature; IP, internet protocol; RDP1, Rice Diversity Panel 1; RH, relative humidity; T-FACE, temperature free-air-controlled enhancement.

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(140 mph), and thunderstorms. Selected US rice cultivars showed an average of 24% and 15% yield reduction under HNT during the 2019 and 2020 cropping seasons, respectively. Our study highlights the potential of this computer-based infrastructure for accurate implementation of HNT or other abiotic stresses under field-growing conditions.

1 | INTRODUCTION

Rice (*Oryza sativa* L.) has progressively been affected by various types of abiotic stresses, including drought, flooding, salinity, heat, and cold, which cause significant yield losses in large areas (Dar et al., 2021). While the Intergovernmental Panel on Climate Change (IPCC, 2021) report projects the world's temperature reaching or exceeding 1.5°C (2.7°F) of warming within two decades and an increase of 4.4°C by 2100, temperature remains a critical factor in rice crop growth and development. High temperatures during flowering in rice inhibit the swelling of pollen grains (Matsui et al., 2000), increase anther pore size, and reduce stigma length, pollen number, and anther-associated protein expression, thereby increasing spikelet sterility that leads to yield losses (Jagadish et al., 2010). Additionally, when the effect of high daytime air temperature stress (34/22°C, day/night) is compared to high night air temperature (HNT) stress (22/34°C, day/night), HNT causes a reduction in the final grain weight and growth rate of rice in the early and mid-stages of grain filling, along with a reduction of final grain weight and growth rate of cells (Morita et al., 2005). Peng et al. (2004) reported that rice grain yield in Asia declined by 10% for each 1°C increase in growing-season minimum night air temperature. Other agricultural crops, including wheat (*Triticum aestivum*) (Hein et al., 2020; Prasad et al., 2008), soybean (*Glycine max*) (Lin et al., 2021), maize (*Zea mays*) (Kettler et al., 2022; Wang et al., 2020), and sorghum (*Sorghum bicolor*) (Prasad & Djanaguiraman, 2011), are also affected by HNT.

Different experimental setups are used to understand crop responses to HNT, including growth chambers and greenhouses, as temperature, light, and relative humidity (RH), or a combination of these factors, are easily controlled and quantified, unlike field conditions where results are highly affected by many environmental and agronomic factors. There are very few existing field-based facilities for the study of HNT in rice fields. One existing HNT field facility is located at the International Rice Research Institute (IRRI) in the Philippines. IRRI has highly controlled walk-in chambers (glasshouse), field-based temperature-controlled tents, and temperature free-air-controlled enhancement (T-FACE) facilities (Impa et al., 2021). These facilities are either permanently installed in the field/greenhouse and/or cover < 0.1 ha of experimental

plot. For an in-depth implementation of field-based HNT stress, several studies included air temperatures from 21 to 40°C for control and HNT stress, imposed in different growth stages at 20 days after emergence (DAE) until physiological maturity, for at least 1–209 rice genotypes (Table 1). Greenhouse/chamber experiments accurately impose stress on specific growth stages with specific stress intensity and duration for phenotyping and sample collection. The highly controlled greenhouses facilitate the identification of critical temperature thresholds, timing, and sensitive growth stages that are important in the study of crop's responses to HNT (Coast et al., 2020; Mir et al., 2019; Tran & Braun, 2017). Grain yield, yield components, non-structural carbohydrates (NSC), and grain quality are among the responses of rice to HNT that were studied using greenhouses/growth chambers (Bheemanahalli et al., 2021; Coast et al., 2015; Kumar et al., 2023; Mohammed & Tarpley, 2011; Morita et al., 2005; Peraudeau et al., 2015; Sakai et al., 2022; W. Shi et al., 2022; Shi, Yin et al., 2017; Ziska & Manalo, 1996). However, in greenhouses/growth chambers, plants grown in pots become pot-bound and can impede root growth, affecting the trait-specific responses of the crop compared to field experiments (Poorter et al., 2016). Controlled experiments can also limit the sample size to a smaller number of genotypes and replications due to space limitations and the high costs involved (Mir et al., 2019; Poorter et al., 2016). Recently, there are new controlled environments that are improved and can provide high-quality and reproducible results (Mir et al., 2019). Reflecting the need for natural rice growth environment, plant breeders and agronomists prefer field experiments to correctly establish the various interactions between genetics and environment that are highly explained by quantitative agronomic traits like yield, abiotic stress tolerance, and grain quality traits for improved future food production (Bheemanahalli et al., 2021; Gupta et al., 2010).

Different field-based facilities have been used to further validate greenhouse/chamber studies to quantify the responses of HNT in rice (Table 1). These field-based facilities include heat tents (Bahuguna et al., 2017; Bahuguna et al., 2022; Bheemanahalli et al., 2021; Schaarschmidt et al., 2020; W. Shi et al., 2013; Shi, Xiao et al., 2017; Shi et al., 2015; Xu et al., 2021) and a field T-FACE system using an infrared heating system (Desai et al., 2021; Peraudeau, Roques et al., 2015). Additional parameters aside from yield and yield

components were collected, for example, enzymatic activities (i.e., sucrose synthase, invertase, and starch synthase) (Bahuguna et al., 2017; W. Shi et al., 2013; W. Shi, Xiao, et al., 2017), gas exchange (i.e., photosynthesis and respiration) (Bahuguna et al., 2022; Peraudeau, Roques et al., 2015), transcriptomes, and metabolites (Schaarschmidt et al., 2021), as surrogate metrics to measure rice responses to HNT stress (Impa et al., 2021). While these structures are useful, they have limitations, including size, limited planting area, and fixed setting for temperature threshold, while the outside/ambient temperature differs. These experimental setups are only suitable for a very limited number of genotypes and a fixed target air temperature and solar radiation. Consequently, these growing conditions do not reflect the variability occurring in the actual production fields. Such variabilities include diurnal fluctuations of air temperature, solar radiation, and evapotranspiration during vegetative and reproductive stages of rice plant. As technology rapidly developed in the study of plant growth, Crowder (2020) and Hein et al. (2019) addressed these growth chamber limitations and introduced a cyber-physical field-based system that allowed simultaneous phenotyping of a large number of winter wheat cultivars under HNT stress. This system was created by combining computers and physical processes; computer technology, software, and communication networks that are connected to and interact with greenhouse systems, thereby interacted with and reacted to the environment (Crowder, 2020; Hein et al., 2019). This facility for wheat study showed an average yield reduction of 3.58% per 1°C, kernel weight by 1.25% per 1°C, and grain number by 2.6% per 1°C (Hein et al., 2020). The success of Hein et al. (2019) heat stress studies in wheat cultivars made the development of new technology in field study of abiotic stress in row crops. However, field-based phenotyping infrastructure for wide rice germplasm and accurate heat stress imposition relative to ambient temperature still do not exist in rice. Therefore, our team adapted and made some changes in the Hein et al. (2019) tent technology to fit the study of HNT stress in flooded rice. The objectives for this work were to (i) build a unique field infrastructure for flooded rice that withstands typical and extreme growing field conditions in drill-seeded rice, (ii) evaluate the cyber-physical system using Raspberry Pi for HNT imposition during the reproductive stage of rice, and (iii) assess the impact of HNT on the yield of several popular US rice cultivars.

2 | MATERIALS AND METHODS

2.1 | High-tunnel greenhouses

Six high-tunnel greenhouses were built for this experiment, three for ambient conditions (control) and three for HNT stress treatment. High-tunnel greenhouses were 9.14 m (30 ft)

Core Ideas

- Six high-tunnel greenhouses equipped with Raspberry Pi computers and sensors were built in the field.
- A system was used to successfully apply high night air temperature stress (HNT) to hundreds of rice accessions for two seasons.
- The system was able to withstand flooding, heavy rains, strong winds, and thunderstorms.
- The effect of HNT stress was evaluated in a subset of rice cultivars of importance in the United States.

wide, 14.63 m (48 ft) long, and 4.39 m (14.4 ft) high and were custom-designed and fabricated by Four Season Tools. Assembly of steel pipes and building of the W-trusses, side-walls, end walls, and doors were initiated in May 2019. End walls were made simultaneously with the W-trusses and the skids, or main base. Skids were 14.63 m (48 ft) long, with ski-style ends, to facilitate the greenhouses' mobility. These main parts were connected using end-wall braces, corrosion-resistant hardware, and power tools (Figure 1). The greenhouses were enclosed with plastic and kept in place through spring wires inserted in C-channels attached to the metal pipes. The plastic used was a polyethylene film (6 mil Sun Master Pull and Cut Greenhouse Film) with 92% light transmission (Berry Global Plastics). Nylon ropes were tied and secured around the roof through eye screws installed on the sides of the roof, end walls, and sidewalls to hold the rolling up of the polyethylene plastic. The roof, sidewalls, and end walls were built with roll bars attached with motorized roll-up system (Advancing Alternatives) with 24 V DC motors to close during heat application and open during daytime for ambient conditions. Earth anchors with steel cables, "T"-rebar, and studded T-posts were installed to function as an anchoring system for greenhouses, ropes, and motorized side/end walls as per manufacturer's specifications.

Using the forged eye bolts on the pipe skid ski tips, cables were secured, strapped, or chained to each greenhouse that was used later in moving the greenhouses with the help of tractors. In pulling the greenhouses, a metal was attached between the skids to balance out the resistance of movement. Each greenhouse was arranged in the entire field in an alternating pattern to avoid shading, optimal capture of solar radiation, and ease of operation. An alleyway of 0.5 m around the perimeter of each greenhouse-covered area was made, and an alleyway outside the greenhouses was made for ease of greenhouse maintenance and access to plants.

TABLE 1 Systems that have been used to study high night air temperature (HNT) stress responses in rice.

Temperature (°C)						
Control	HNT	Facility/system	Stress imposition	Number of genotypes	Area (m²)/ dimensions (m)	Reference
Greenhouse (GH) and growth chambers (GC)						
25	33	Outdoor chambers	Entire crop duration	1	3.4 m²	Ziska & Manalo, 1996
22	34	Glasshouse	Grain-filling	1	NA	Morita et al., 2005
27	32	Glasshouse (infrared heaters)	20 DAE to maturity	1	NA	Mohammed & Tarpley, 2011
25	28	Growth chambers	Post anthesis	2	4.5 m (l) × 1.5 m (w) × 1.7 m (h)	Dong et al., 2014
21	26–29	Greenhouses and growth chambers	Panicle initiation to anthesis	2	6.2 m × 6.2 m (GH) & 3 m × 3 m (GC)	Peraudeau et al., 2015
24	35	Walk-in growth chambers	Anthesis	10	3.3 m × 3.2 m × 2.7 m; 10.6 m²	Coast et al., 2015
23	30	Walk-in growth chambers	Panicle initiation to maturity	5	3.3 m × 3.2 m × 2.7 m; 10.6 m²	Shi, Yin et al., 2017
26	29	Walk-in growth chambers	Panicle initiation to maturity	209 <i>Indica</i> Rice Diversity Panel (Phenomics of Rice Adaptation and Yield [PRAY])	3.3 m × 3.2 m × 2.7 m; 10.6 m²	Bheemanahalli et al., 2021
22	28	Greenhouse	Booting to maturity	185 RILs (MY2 population) + two parents	NA	Kumar et al., 2023
22	32	Growth chamber	Anthesis	3	4 m (l) × 2 m (w) × 2 m (h)	Sakai et al., 2022
Field-based facilities						
22	28	Field heat tents	Panicle initiation to maturity	2	6 m (l) × 3 m (w) × 2 m (h)	W. Shi et al., 2013
23	29		Panicle initiation to maturity	8	6 m (l) × 3 m (w) × 2 m (h)	Shi et al., 2015
22	28		Panicle initiation to maturity	3	6 m (l) × 3 m (w) × 2 m (h)	W. Shi, Xiao et al., 2016
23	29		Panicle initiation to maturity	3	6 m (l) × 3 m (w) × 2 m (h)	Bahuguna et al., 2017

(Continues)

TABLE 1 (Continued)

Temperature (°C)						
Control	HNT	Facility/system	Stress imposition	Number of genotypes	Area (m ²)/ dimensions (m)	Reference
22	28		Panicle initiation to maturity	8	6 m (l) × 3 m (w) × 2 m (h)	Schaarschmidt et al., 2020
24	29		Panicle initiation to maturity	209 (PRAY diversity panel)	6 m (l) × 3 m (w) × 2 m (h)	Bheemanahalli et al., 2021
24	27		Panicle initiation to maturity	432 genotypes (MAGIC ^{heat} population) + three checks	6 m (l) × 3 m (w) × 2 m (h)	Xu et al., 2021
22	27	Field heat tents (heat radiators)	Panicle initiation to maturity	5	8 m (l) × 4 m(w) × 3 m (h)	Bahuguna et al., 2022
25	27	Field T-FACE system (infrared heating system)	20 DAPI to mid grain-filling stage	3	4.5 m ²	Peraudeau et al., 2015
24	30	Field T-FACE system (infrared heating system)	Panicle initiation to maturity	1	4.5 m ²	Desai et al., 2021
Ambient temperature	+4°C ambient temperature	Cyber-physical system	Anthesis	320 (Rice Diversity Panel 1)	9.14 m (l) × 14.6 m (w) × 4.4 m (h)	This study

Abbreviations: DAE, days after emergence; DAPI, days after panicle initiation; NA, not available; RIL, recombinant inbred line; T-FACE, temperature free-air-controlled enhancement.

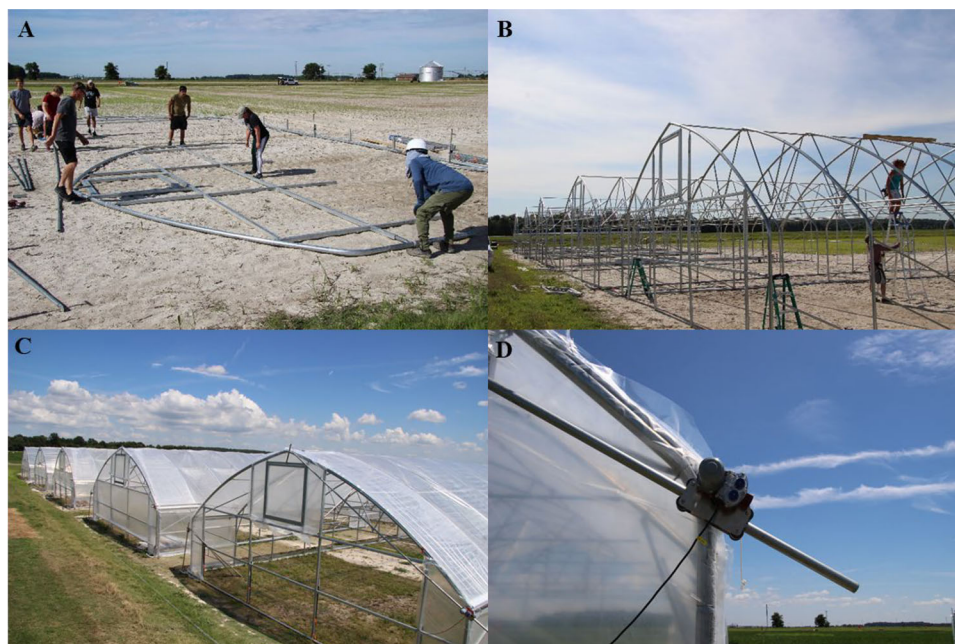


FIGURE 1 Greenhouse construction. (A) Assembly of metal pipes for W-trusses. (B) End wall assembly, skids, hoops braced together. (C) Plastic installed enclosing the greenhouses. (D) Motor installed on roofs, sidewalls and end walls for alternating the plastic position.

2.2 | High-tunnel greenhouses service and maintenance

High-tunnel greenhouses were checked before heat stress imposition. When small tears in the plastic were detected, repairs were made using poly patch tape, applied inside and outside of the material. Prior to the 2020 season, greenhouses were repaired due to damage caused by high winds in a 2019 tornado occurrence and heavy rains. In 2020, 1 month prior to seeding, we used 1,114 m² (12,000 ft²) of plastic films to replace several parts of the greenhouse, such as the convection tubing, the roofs, end walls, and side walls. Roll bars for the end wall were also replaced. Spring wires were removed before installing the new or reused plastic in its respective C-channels. The reused plastics were cleaned using a soap solution and water. Plastic that was damaged and unable to be reused was recycled by Delta Plastics.

2.3 | Heating system

A 20-kW diesel-fueled generator (Sunbelt Rentals Equipment Co.) was used to supply the electricity needed for both the 2019 and 2020 cropping seasons. The generator was placed in front of the greenhouses for ease of operation. Two 50 A portable power distribution centers were wired to the generator to allow the distribution of electricity to all greenhouses using various lengths and gauges of electrical cables attached to wooden stakes with plastic pipes to prevent wires from touching the floodwater (Figure S1). A

total of 82.48 L (21.79 gallons) of diesel per night were consumed to run the system. A liquefied petroleum gas (LPG) propane heater was installed in each heated greenhouse (liquefied propane supplied by Craft Propane Inc.). An average of 38.2 kg (84.21 lb) and 36.7 kg (81 lb) of propane per night were consumed in 2019 and 2020, respectively. The propane heater (HDB100 Modine) had 100,000 BTU, where BTU is British thermal unit, (105,505.59 kJ) capacity with an airflow range of 1326.93 CMH, where CMH is cubic meters per hour, (781 FPM, where FPM is feet per minute). The heater was augmented with a duct transition to allow the attachment of convection tubing. The tubing itself was 45.7 cm in diameter and 13.7 m, punctured every 1.2 m with round openings with a diameter of 5.7 cm at 3 o'clock and 9 o'clock to force the heated air to escape parallel to the field. Two 30.5-cm horizontal airflow fans (J&D Manufacturing) with an airflow rate of 1733 CMH (1020 CFM, where CFM is cubic feet per minute) were hung from the bottom chord of the trusses in opposite corners to ensure even distribution of air within the greenhouse during the night. The larger heating system with convection tubing and dual circulation fans allowed a single heater to distribute hot air completely and equally inside each HNT greenhouse.

The greenhouses under control temperature had a similar setup without the implementation of heat. To maintain air movement over the plants, a 45.72-cm (18-in.) tube fan (Coolair) was installed, and convection tubing ran with the same hole set up as the heated greenhouses. The same horizontal airflow fans were also installed to circulate the air throughout each of the three control greenhouses.

2.4 | Raspberry Pi thermostat controllers

The temperature was controlled by a thermostat system in each greenhouse, as designed by Kansas State University. This system was used to monitor and record the temperature within each greenhouse and transmit this data wirelessly from the control greenhouse to the corresponding HNT greenhouse. The thermostat controller system consisted of a Raspberry Pi (Raspberry Pi Foundation) and six MCP9808 temperature sensors (Adafruit) randomly distributed in each greenhouse. The MCP9808 digital temperature sensors converted temperatures between -20 and $+100^{\circ}\text{C}$ to a digital word with $\pm 0.25/\pm 0.5^{\circ}\text{C}$ (typical/maximum) accuracy. Each heated greenhouse contained a four-channel solid-state relay (Keyes KY-019 Relay Module, Songle Relay) for controlling the heater. The target temperature difference between the ambient greenhouses and the HNT greenhouse at night was 4°C (7.2°F) (Hein et al., 2020). The 4°C temperature increase was imposed in the HNT treatment to ensure a strong response of rice plants to heat stress and create a future HNT scenario as predicted by weather models (IPCC, 2019). Elevated air temperature in the HNT treatments was achieved by heating the greenhouses when temperatures in HNT greenhouses were below or equal to the temperature of control greenhouses. The increase in air temperature inside the HNT greenhouses was relative to the average ambient air temperature measured by temperature sensors installed in the control greenhouses. Actual air temperatures in both control and HNT greenhouses were continuously measured and monitored to maintain temperature differentials during the rice growth stage. The overall system is illustrated in Figure 2.

2.5 | Hardware set up, connections, and software

A cyber–physical system was used to monitor the temperature in the control greenhouse and to regulate the temperature of the HNT greenhouse (Hein et al., 2020). The Raspberry Pi within each controller was connected to the temperature sensors and clock module. Each temperature sensor and clock module shared the same four wire lines and were soldered directly through the pins of the electronics to guarantee continuity. Each sensor was connected in parallel to others to ensure the system could accommodate a different number of sensors. Both control and HNT greenhouses had the same wiring, but heated greenhouses had additional wiring to manage the interface with the relays that controlled the heater. These relays used five pins on the Pi, the power supply pins, while the other three pins were connected to relay ports. The output of the relays was connected to the heaters within each greenhouse. The normally open (NO) port of each relay was connected back to the 24VAC connection on

the heater's control board. Other wires to the heater (i.e., fan heater, first stage heater, and second stage heater) were connected to the common (COM) ports (i.e., fan heater port, first stage heater port, and second stage heater port) to ensure that the heater was turned off by default. The MCP9808 temperature sensors required the mentioned ports and had an I2C address. Pins were powered to 5 V to make the sensor readable. MCP9808 sensors recorded and averaged the temperature readings. However, if the temperature differential was below the set temperature threshold (4°C above actual ambient temperature), the relays were engaged. When errors existed in reading the sensors, the greenhouses were stopped and rebooted.

All the controllers used Python 2.7 to run the software. The Python MCP9808 library from Adafruit was required for successful communication between temperature sensors (Hein et al., 2020). All control greenhouses had their own access points, which allowed the corresponding HNT greenhouses to connect and retrieve the temperature. Each control greenhouse was given an internet protocol (IP) address with a corresponding HNT greenhouse IP address. The systems communicated through their IP addresses when downloading the temperature data.

In downloading the temperature data, a host system (i.e., a laptop) was connected to each control greenhouse wireless network to access the logs for each pair of associated control and HNT greenhouses. The WinSCP program was used to copy the files from each set of controllers to the local machine. Upon data retrieval, two main log files were recorded throughout the system's operation. The *control.log* file consisted of the system's health information and general program information/debugging. The *sensors.csv* file was a comma-delimited file containing each individual sensor reading. The transfer of files was done through click and drag from the remote machine to the local machine. The temperatures were logged with a 1-min interval. The average of temperatures was taken to compare the temperatures between the control greenhouse and its corresponding paired heated greenhouse.

Continuous monitoring of temperature during the stress duration ensured proper heat stress imposition. The same method was followed for the 2020 planting season. Temperature data were downloaded every day after nighttime heat imposition. Average temperatures were taken to monitor the heat stress condition and health of the system.

2.6 | HOBO temperature data

Raspberry Pi (MCP9808 sensors) temperature data were validated using HOBO temperature sensors and data loggers (HOBO MX2303, Onset Computer Corp.) installed inside and outside of the greenhouses. Temperatures were logged with a

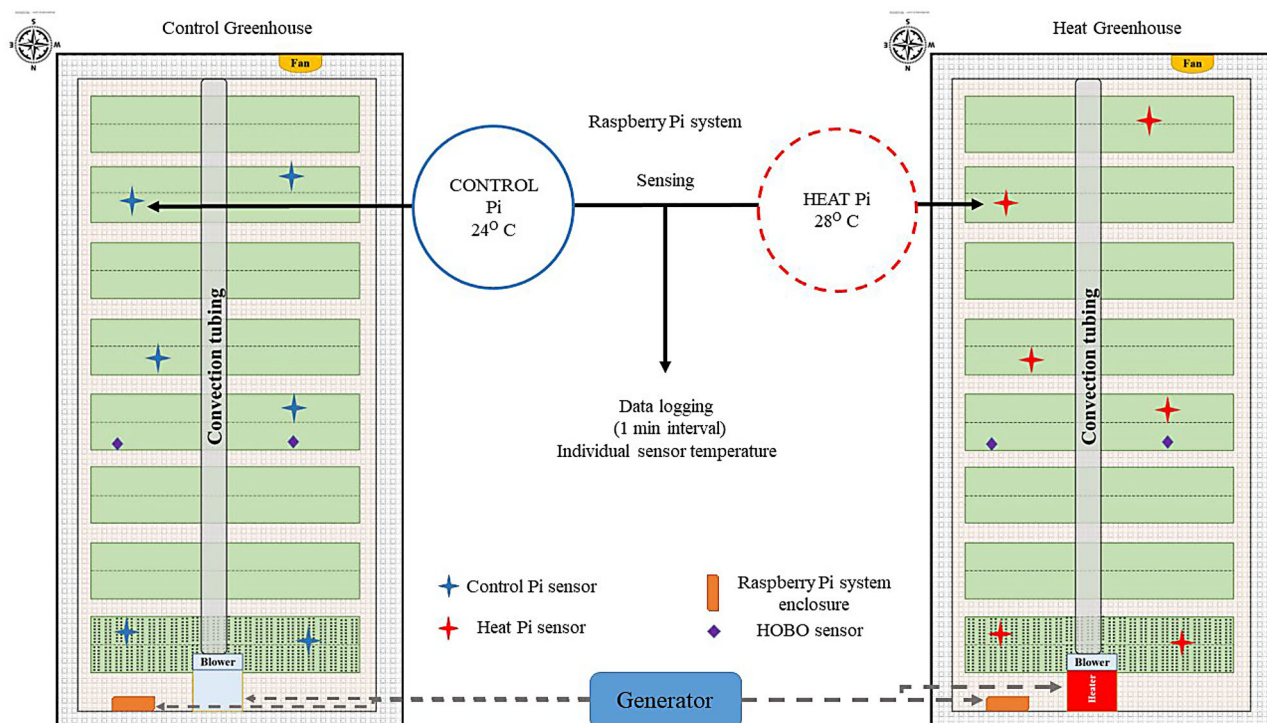


FIGURE 2 Illustration of a paired control and heated greenhouse using the Raspberry Pi system. The cyber-physical system ensures an approximately 4°C increase of temperature in the high night air temperature (HNT) greenhouse relative to control temperature throughout the stress imposition of 14 nights.

15-min interval throughout the experiment. Two HOBOS sensors were distributed at two different locations (west and east) inside each greenhouse. Two HOBOS sensors were also used to record the ambient temperature outside the greenhouse. The same method was repeated during the 2020 planting season with two HOBOS additional sensors installed (west and east) in an additional ambient plot (unhoused).

Temperatures were downloaded as comma-delimited (.csv) files. Averages of temperatures of installed HOBOS sensors were taken to compare the temperatures inside and outside of the greenhouses and the temperatures of control greenhouses with their corresponding pair of heated greenhouses.

2.7 | Crop cultivation

The field experiments were conducted for two cropping years (2019 and 2020) at the RiceTec Experimental Station in Harrisburg, AR (2019: 35.66675 N, -90.70938 W, and 2020: 35.6675 N, -90.71236 W). The field experiment was conducted on a 1.6-ha rice field. Inside the rice experimental field, the plot area covered by one greenhouse had a dimension of 9.14 m (30 ft) × 10.97 m (36 ft). Standard crop management practices common to the region were followed except for planting and harvesting, which were completed manually. Each greenhouse-covered plot had eight varietal sub-blocks consisting of 40 rice accessions in each sub-block.

Three hundred and ten rice accessions from the Rice Diversity Panel 1 (RDP1, Zhang et al., 2011) and 10 hybrids from RiceTec were sown in a marked furrow for each entry in a 60-cm long × 20-cm wide between rows in each of the six plots. The distance between rows of rice accessions was 20 cm, and the distance between plants was 7 cm. For the 2020 cropping season, 302 rice accessions from the RDP1 and 10 rice hybrids from RiceTec were planted.

The field trials were arranged in a randomized complete block design with three replications. The six greenhouses were placed in the middle of the field and 15 m away (in all directions) from other greenhouses to promote normal air circulation and avoid shading and bias during the morning increase of solar radiation. Rice accessions were grouped according to their height, from short rice plants facing east to avoid shadowing taller rice plants to shorter rice plants, and then randomly ordered within the height groups. The Diamond rice cultivar (filler rice) was seeded in all areas outside of the six greenhouses at a rate of 62 kg/ha. Urea fertilizer was applied 2–3 days prior to permanent flooding at 120 kg N/ha (260 lb/ac).

2.8 | Water management and border rice

Two weeks after seeding, rice plots were flushed once, and a permanent flood was applied when rice plants reached the

three- to four-leaf stage. The area outside of greenhouses was planted with filler rice to mimic a production rice farm and to avoid excessive evapotranspiration and heat reflectance during the growing period. The multiple-inlet rice irrigation (MIRI) method (Vories et al., 2005) was implemented in all greenhouses and filler rice in both cropping seasons. Poly-pipe tubing was used to uniformly distribute the water across the whole field. Floodwater was maintained at 3.6–16.6 cm during the whole growing season, including periods of stress implementation. The field was drained 4 weeks before harvest.

2.9 | Greenhouse operation and heat stress imposition

Initially, the greenhouses were stationed 46 m away from the actual rice plots. Greenhouses were moved using tractors to their respective rice plots when 50% of all the rice plants were at the flowering stage. HNT stress was imposed from August 15 to 28 for the 2019 cropping season and from August 22 to September 4 for the 2020 cropping season. The stress period for the experiment continued for 2 weeks, from the flowering stage to the grain filling stage. The greenhouses were closed at 18:00 h, and the heat treatment started at 19:00 h. The stress duration lasted until 05:00 h, and the greenhouses were opened by 05:30 h. With the greenhouses fully opened, the plants were exposed to the natural environment during the day. After 2 weeks of HNT implementation, the six greenhouses remained open throughout the growing season. The overall greenhouse view and layout are shown in Figure 3.

2.10 | Estimation of grain yield

At physiological maturity, sixteen rice plants from the different rice accessions of the RDP1 panel were manually harvested for both 2019 and 2020. Eleven most commonly planted or commonly grown rice accessions in the United States, specifically Lemont (*O. sativa* ssp. japonica), Cybonnet (*O. sativa* ssp. japonica), Lacrosse (*O. sativa* ssp. japonica), Cocodrie (*O. sativa* ssp. japonica), Rosemont (*O. sativa* ssp. japonica), Carolina Gold (*O. sativa* ssp. japonica), Kaybonnet (*O. sativa* ssp. japonica), LaGrue (*O. sativa* ssp. japonica), M-202 (*O. sativa* ssp. japonica), and L-202 (*O. sativa* ssp. japonica) were selected to assess the impact of HNT stress on grain yield. Grain threshing was done manually, and grains were cleaned by separating the filled grains from the empty grains using a seed blower (Seedburo South Dakota Seed Blower, 4-in. cap). Grains were air-dried until they reached 14% moisture content and weighed.

2.11 | Statistical analysis

Average air temperature and standard errors were calculated for each replicate treatment plot using basic statistics (MS Excel). Differences in grain yield in each cultivar and air temperature among greenhouses due to main effects such as heat treatment were analyzed at p value < 0.05 using R v.3.4.4. Means and standard errors and analysis of variance (ANOVA) of air temperature between outside and inside greenhouses were also performed with package “lsmeans” (R core team, 2019).

3 | RESULTS AND DISCUSSION

3.1 | The large field-based infrastructure used in phenotyping wheat and rice for HNT stress

There are major similarities between the infrastructure used for wheat (Hein et al., 2020) and rice (Table 2) for HNT phenotyping. The high-tunnel structure, heating system, and cyber-physical system used in wheat and rice are the same. The size of the high-tunnel greenhouses accommodated at least 320 wheat and rice accessions and can house tall crops like maize, sorghum, and small row crops. The roof, sidewalls, and end walls were mechanically rolled up for proper ventilation during the day. This field infrastructure is built on skids, which helped in moving the greenhouses from one area of the field to another. These features are some of the differences between the other field heat tents, wherein roofs, side, and end walls are opened or closed manually and greenhouses are fixed in field plots (Bahuguna et al., 2022; W. Shi, Xiao et al., 2017). The heating system of the large field-based infrastructure used in wheat and rice was propane, and heat was distributed efficiently and uniformly using convection tubing and additional blowers inside the greenhouses. The heating system relies on the Raspberry Pi system. Within each of the paired greenhouses, air temperature was measured by six MCP9808 sensors installed inside the control greenhouse and inside the HNT greenhouse. During the heat treatment period, average air temperature data from the control greenhouse were transmitted wirelessly using a Wi-Fi hotspot at 1-min time intervals to the HNT greenhouse with the Raspberry Pi module. Once the air temperature was received by the module from the control greenhouse, the heater raised the temperature inside the heated greenhouse by about 4°C. Temperature for HNT stress was based on the control greenhouse temperature, which isolated unaccounted external variables. The system includes the capacity to download temperature data through wireless communication between the sensors and the laptop. This cyber-physical system used in this study is the major

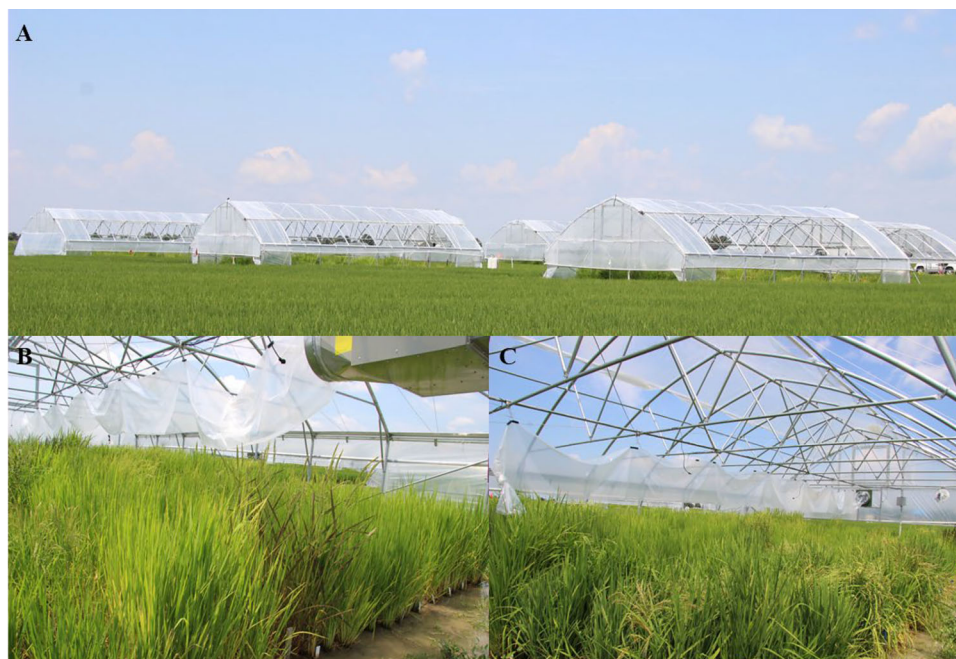


FIGURE 3 Overall view of high-tunnel greenhouses and plot layout. (A) Six high-tunnel greenhouses arranged in a 1.6-ha experimental field. (B) Interior view of a high night air temperature (HNT) greenhouse. The heater was attached to a convection tube for equal distribution of heat. (C) Interior view of the control greenhouse. An 18-in. fan was attached to a convection tubing for equal air distribution. Roofs, sidewalls, and end walls were rolled up during the daytime to expose plants to natural light conditions.

difference from the mentioned field heat tents and facilities presented in Table 1, which uses a fixed target temperature.

The main difference between the large field-based infrastructure used in rice and wheat is the growing condition/location and the environment of each experiment. Wheat accessions were grown in well-drained soil, while rice accessions in this study were grown in a fully-flooded condition, which posed several challenges, including tissue sampling for analyses and data gathering. Another challenge was to avoid submerging wires in the water. As a solution, wires were tied to a stick, placing it above the rice canopy. This infrastructure in the study withstood severe weather conditions like heavy rains, strong winds (140 mph), and high temperatures for two consecutive cropping seasons. This facility offers an opportunity to improve the ability to translate results and findings on HNT responses in rice under field conditions to improve its resilience.

3.2 | Distribution of heat and temperature differential by Raspberry Pi system

A uniform and consistent distribution of heat was achieved for both 2019 and 2020 cropping seasons in the HNT greenhouses. The cyber-physical system using Raspberry Pi sensors array was able to record an average temperature difference of 4.0 and 3.94°C between control greenhouses and HNT greenhouses during the 14-day heat implementation in 2019 and 2020, respectively (Figure 4). An average of

0.19°C difference was observed among six MCP9808 sensors in control greenhouses, while an average difference of 0.38°C was observed among six MCP9808 sensors in HNT greenhouses during heat stress imposition in 2019 cropping season (Table S1). During 2020, MCP9808 sensors and their system recorded an average difference of 0.18 and 0.53°C among six MCP9808 sensors for control and HNT greenhouses, respectively (Table S2). With this difference, the cyber-physical system was able to maintain a difference on average of 4°C between control greenhouses and heated greenhouses for two cropping seasons (Figure 4; Figure S2).

Using HOBO sensors and loggers, the air temperature outside the greenhouses was measured to assess if there was any temperature variation between the control greenhouse temperature measured by MCP9808 sensors and outside ambient growing conditions during the heat treatment period. Air temperatures from MCP9808 sensors inside the three control greenhouses and ambient (outside) using HOBO sensors did not differ significantly ($p = 0.13$) during 2019. Similarly, the average air temperature of 22.7°C recorded by HOBO sensors did not differ significantly from the average air temperature inside the control greenhouses ($p = 0.97$) recorded by MCP9808 in 2020.

Air temperatures fluctuated from 19.4 to 26.1°C for control greenhouses and from 26.9 to 31°C for HNT greenhouses in 2019. In 2020, air temperatures fluctuated from 18.4 to 24.1°C for control greenhouses and from 22.2 to 28.4°C for HNT greenhouses. The fluctuations of air temperature within

TABLE 2 Features of the field-based high-tunnel greenhouses and cyber-physical system used in this study compared to the ones published by Hein et al. (2020).

Component	Feature	High-tunnel greenhouses (Hein et al., 2020)	High-tunnel greenhouses (This study)
High tunnel structure	Dimensions	9.1 m × 14.6 m × 4.4 m	9.14 m × 10.97 m × 4.4 m
	Number of genotypes	320	320
	Crops/field condition	Wheat, sorghum/dry	Rice/fully flooded
	Ventilation	Roof, sidewalls, and end-walls mechanical roll-up	Roof, sidewalls, and end-walls mechanical roll-up
	Mobility	Built on skids—towed using tractors	Built on skids—towed using tractors
Heating system	Number of replicates	Three heat and three control greenhouses	Three heat and three control greenhouses
	Heater	Propane heater	Propane heater
	Heat distribution	Blower fan on the heater with convection tubing allowing uniform heat distribution	Blower fan on the heater with convection tubing allowing uniform heat distribution
	Ventilation	Direct ventilation of combustion exhaust to the exterior of the greenhouse	Direct ventilation of combustion exhaust to the exterior of the greenhouse
Cyber-physical system	Basic function	Multiple relays to function as thermostat	Multiple relays to function as thermostat
	Sensors	Six temperature sensors	Six temperature sensors
	Communication	Wireless communication between control and heat stress at 1-min intervals	Wireless communication between control and heat stress at 1-min interval
	Control environment	Ambient conditions but within a greenhouse to separate unaccounted external factors	Ambient conditions but within a greenhouse to separate unaccounted external factors
Electrical system	Source	Diesel-generator	Diesel-generator. Wires fixed above rice canopy to avoid submergence in water

replicated greenhouses during 2019 were not significantly different from the temperature fluctuations in 2020 ($p = 0.88$) across all three paired greenhouses. To illustrate, the average temperature fluctuation inside the control greenhouses was 0.26°C during the 2019 cropping season, while an average air temperature fluctuation of 0.23°C in the 2020 cropping season (Figure 5). These results provide evidence that the Raspberry Pi and temperature sensor system were able to mirror the diurnal fluctuations of ambient air temperature at an elevated air temperature of about 4°C throughout the HNT stress period for two consecutive years.

3.3 | Nighttime air temperature validation during HNT stress imposition by HOBO sensors

Comparing the two cropping years during heat treatment, average daily ambient nighttime air temperature outside of the six greenhouses was 24.1 and 22.7°C during the 2019 and 2020 cropping seasons, respectively. Nighttime air temperatures were warmer in 2019 compared to 2020 cropping. There were six more days of air temperatures above 26°C in 2019 than in 2020.

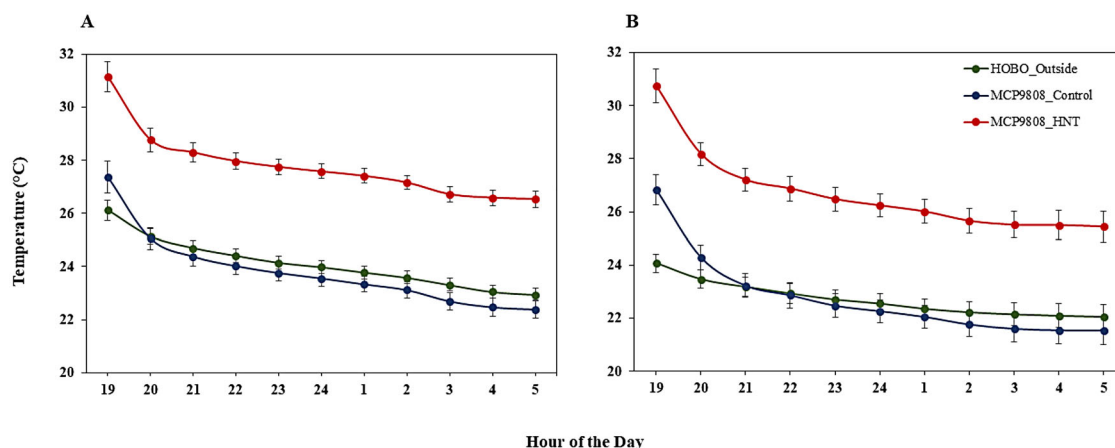


FIGURE 4 Comparison of average air temperatures between MCP9808 sensors inside the control, HNT greenhouses, and ambient (outside greenhouses) over the 11-h heat stress imposition for 14 nights during the 2019 (A) and 2020 (B) cropping seasons. Blue and red solid lines are the average air temperatures by MCP9808 sensors inside control and HNT greenhouses, respectively, and the green solid line is the average air temperature by HOB0 sensors in ambient conditions (outside greenhouses) with standard errors of the mean.

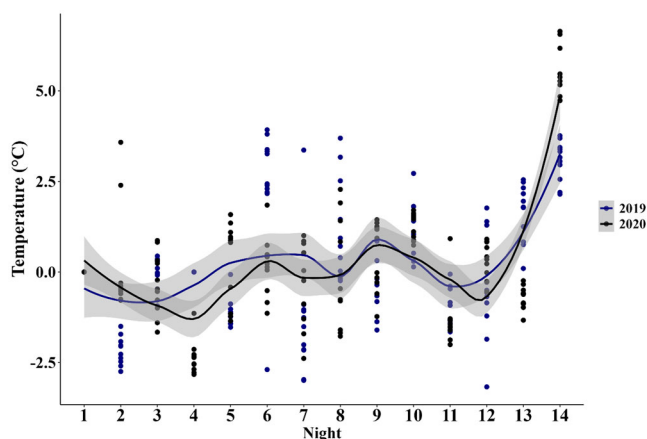


FIGURE 5 Comparison of air temperature fluctuations in control conditions recorded by MCP9808 sensors over the heat stress imposition during 2019 and 2020 cropping seasons. Blue and black solid lines denote nightly air temperature fluctuations during 2019 and 2020 cropping seasons, respectively, while the shaded area denotes 95% confidence intervals.

In 2019, the average daily nighttime air temperature ranged from 21.4 to 30.5°C inside the control greenhouses and 21.3–28.8°C for ambient field conditions (outside of greenhouses). As a result, average nighttime air temperatures inside the control greenhouses differed from ambient field conditions (outside) by approximately 1.2°C in 2019 cropping season ($p < 0.0001$). Likewise, in 2020, the average nighttime air temperature ranged from 18.9 to 26.7°C for inside control greenhouses and 17.9 to 25.6°C for ambient field conditions. Relative to ambient nighttime air temperature, control greenhouse differed on average by 0.8°C. Our study shows that

while the average air temperature in the HNT greenhouses was about 4°C, the nighttime air temperature inside the control greenhouses was approximately 1°C higher than the actual ambient field condition. Although the elevated temperature was implemented in HNT greenhouses, the air temperatures recorded by HOB0 sensors inside the control greenhouses were 1°C higher than the field condition. The slight deviation of nighttime air temperatures of enclosed greenhouses can be attributed to changes in RH. RH increased following the enclosure of the greenhouse due to heat loss from floodwater, inner wall and roof of the greenhouse, evaporation, and condensation (Alberto et al., 2014; K. Garzoli, 1985; Seginer & Kantz, 1989; Tong et al., 2009). The heat loss in the inner wall and roof is made up of energy transfer that takes place at the inner and outer surfaces of the plastic and by the transfer of heat directly through the material itself. The energy transfer processes are influenced by convection from the air inside, latent heat condensation on the inside surface, and thermal radiation from the interior of the greenhouse (K. V. Garzoli & Blackwell, 1981). At nighttime, heat energy is being transferred from the surface of floodwater through evaporation from and/or condenses onto the surface. Generally, flooded rice fields have higher latent heat flux than aerobic fields. Also, water has a specific heat capacity, which makes flooded fields warm and much slower giving up heat (Alberto et al., 2014). Given the influence of differential ambient nighttime air temperature on rice physiology, we performed a comparison of rice responses to HNT stress under enclosed greenhouses. Here, the heat stress responses of rice plants were measured with the accompanying control greenhouses to account for any changes caused by enclosures and diurnal variation during the rice growth.

3.4 | Ambient daytime temperature validation between the inside and outside of greenhouses

The growth of rice is strongly affected by environmental conditions. Hence, a change in weather temperature and soil conditions can contribute to the annual variability in plant growth and reproduction in the field. Our study measured ambient daytime temperatures over the heat stress duration using HOBO sensors. Ambient field daytime air temperature ranged from 20.7 to 33.7°C in 2019 and 17.6 to 30.7°C in 2020. During the 2019 cropping season, from 16:00 to 18:00 h, average air temperatures outside ambient field conditions and inside control and HNT greenhouses did not differ significantly ($p = 0.95$) with an overall average temperature of 28.3°C. Air temperatures in HNT greenhouses started to diverge from control greenhouses starting at 18:00 h since the closure of greenhouses was initiated. The increase in temperature continued due to HNT imposition and was maintained until the following morning. Air temperature decreased to ambient conditions by 06:00 h as the heating was turned off and greenhouses were re-opened. Average daytime air temperatures inside the greenhouses (control and HNT) were like the average ambient field daytime air temperature within 2 h of opening the greenhouses, with an average air temperature difference of 0.7°C between control and HNT greenhouses ($p = 0.08$) (Figure 6). During the 2020 cropping season, average daytime air temperatures between 16:00 and 18:00 h in ambient field, control, and HNT greenhouse conditions differed significantly ($p = 0.00$), with average air temperatures of 26, 27.1, and 28.3°C, respectively. The air temperature inside HNT greenhouses started to increase at 18:00 h as heat stress was implemented and decreased to ambient conditions by 06:00 h as heating was turned off. Average daytime air temperatures inside greenhouses were 1°C warmer than air temperatures outside ambient conditions ($p = 0.00$). There was a slight deviation of microclimate of greenhouses from the actual ambient field temperature for both years. As discussed above, the heat built up inside the greenhouses was caused by several processes from heat-releasing surfaces such as plastic covers, floodwater, and rice plants. While greenhouses showed slight warming of ambient air temperature after the heat stress treatment duration, our results show that the temperature conditions generated inside the greenhouses were not far from the real open-field conditions. The rice plants' responses overall were more similar to those of rice plants outside the greenhouses because rice plants inside the greenhouses showed no signs of nutrient deficiency, disease symptoms, or chemical burn. No traces of abiotic or biotic stress except heat stress were observed across all rice selections because the infrastructures were placed over the plants 93 days after optimal vegetative growth had been

achieved. The rice responses to HNT stress were compared systematically to plant responses inside the control treatment.

3.5 | Grain yield: Response to HNT stress

The selected rice cultivars Lemont, Cybonnet, Lacrosse, Cocodrie, Rosemont, Carolina Gold 12033, Carolina Gold 12034, Kaybonnet, LaGrue, M-202, and L-202 had varying grain yield responses to HNT stress. Across treatments and years, grain yield of the selected cultivars ranged from 424.3 to 1994.8 g/m². During 2019, significant yield reductions caused by HNT stress occurred in Lemont and Carolina Gold 12033 ($p = 0.03$). However, L-202, M-202, Rosemont, Kaybonnet, Lacrosse, LaGrue, Carolina Gold 12034, Cybonnet, and Cocodrie yield losses under HNT treatment were not significant because of large replication errors; however, there was an observed overall yield loss of 24% (Table 3). During the 2020 cropping season, the effect of HNT stress on grain yield of the selected cultivars was not apparent during the 14-day heat treatment period. Although heat treatment did not significantly affect the yield ($p = 0.6$), here the magnitude of grain yields was reduced by an average of 15.2% under HNT compared to control conditions. In both 2019 and 2020 cropping seasons, grain yields of Cocodrie, Kaybonnet, L-202, LaGrue, and Lemont were consistently reduced (by 2%–45.2%) with the greatest yield penalty in L-202 (Table 3). The decrease in grain yield in Lemont and Carolina Gold 12033 might be due to a decrease in the aboveground biomass (sum of dry weights of rachis, straw, filled grains, and unfilled grains, g/m²) and an increase in unfilled grains weight under HNT conditions shown by Carolina Gold 12033 (Table S3). Similarly, W. Shi et al. (2013) observed grain yield reduction because of significant reduction in biomass and grain weight when rice was exposed to HNT stress from panicle initiation to maturity. Mohammed and Tarpley (2011) reported that HNT stress mainly increased spikelet sterility and negatively affected seed set and grain yield, but not the number of productive tillers, panicle length, or number of primary branches in the panicle. When HNT (25°C) stress was applied during the reproductive period, reduction of yield occurred due to increased plant's dark respiration rate and spikelet degeneration (which consequently reduced sink size), leading to a decrease in biomass production (Laza et al., 2015; Peraudeau et al., 2015). In the case of Cocodrie cultivar, grain yield decreased due to decreased pollen germination, increased leaf dark respiration rates, electrolytic leakage, spikelet fertility, and decreased dry partitioning in grains when exposed to 30–32°C nighttime air temperature in the greenhouse (Mohammed & Tarpley, 2009a, 2009b, 2014). Counce et al. (2005) reported that LaGrue decreased head rice yield and grain widths when exposed to 24°C under chamber

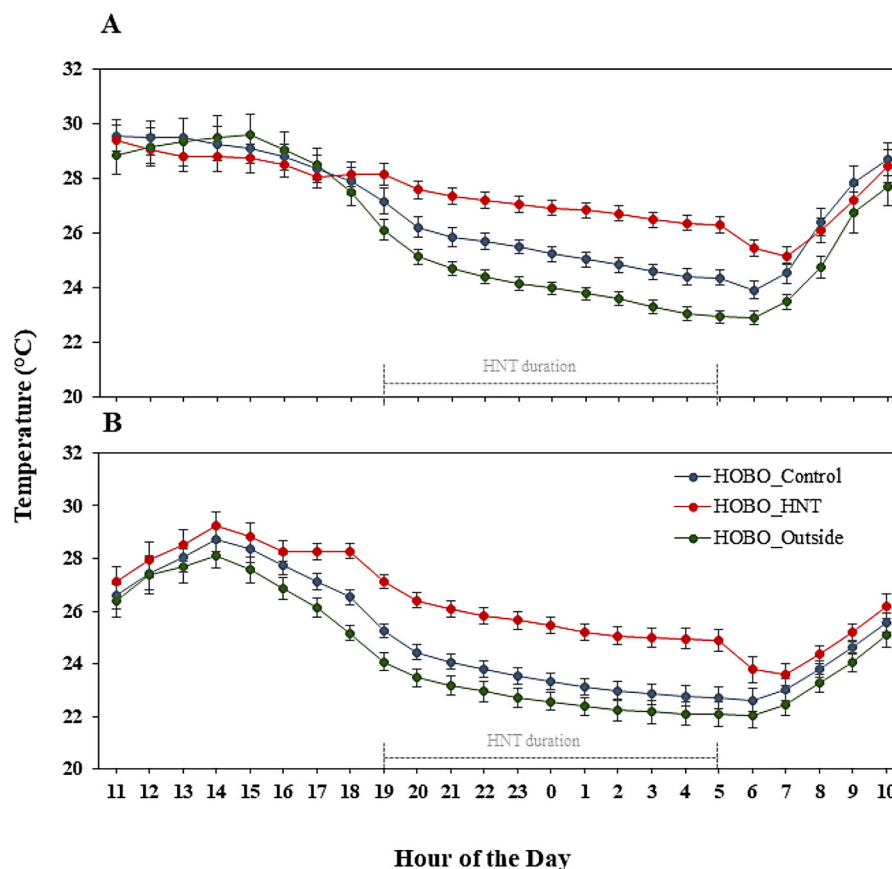


FIGURE 6 Comparison of average air temperatures between HOBO sensors within control, heated greenhouses, and ambient (outside) conditions during 24-h time over the heat stress period (14 nights) during 2019 (A) and 2020 (B) cropping seasons. Blue and red solid lines denote the average air temperature inside the control and HNT greenhouses, respectively, while green solid lines denote the average air temperature ambient conditions (outside the greenhouses) with standard errors of the mean

TABLE 3 Grain yield response (g/m^2) of selected rice cultivars to control and high night air temperature (HNT) treatments during 2019 and 2020 cropping season.

Rice cultivar	2019			2020		
	Control	HNT	% change	Control	HNT	% change
Carolina Gold 12033	1095.2 $\pm$ 265.7a	864.1 $\pm$ 236.2b	−21.1	1324.9 $\pm$ 219.6a	1994.8 $\pm$ 103.8a	50.6
Carolina Gold 12034	1046.6 $\pm$ 162.3a	1080.1 $\pm$ 503.3a	3.2	1516.0 $\pm$ 353.5a	1485.9 $\pm$ 213.8a	−2.0
Cocodrie	821.1 $\pm$ 111.8a	804.5 $\pm$ 124.1a	−2.0	620.5 $\pm$ 243.1a	597.2 $\pm$ 162.4a	−3.7
Cybonnet	741.0 $\pm$ 274.9a	741.4 $\pm$ 244.7a	0.1	453.3 $\pm$ 41.1a	498.7 $\pm$ 128.9a	10.0
Kaybonnet	1555.9 $\pm$ 492.9a	1307.5 $\pm$ 288.1a	−16.0	1186.0 $\pm$ 29.3a	953.5 $\pm$ 334.4a	−19.6
L-202	1139.7 $\pm$ 79.4a	701.7 $\pm$ 239.4a	−38.4	1001.3 $\pm$ 230.0a	747.9 $\pm$ 150.0a	−25.3
Lacrosse	1444.2 $\pm$ 341.9a	1210.8 $\pm$ 34.6a	−16.2	907.5 $\pm$ 151.1a	1186.8 $\pm$ 163.3a	30.8
LaGrue	947.6 $\pm$ 43.1a	819.9 $\pm$ 228.1a	−13.5	1494.4 $\pm$ 606.5a	1086.2 $\pm$ 239.1a	−27.3
Lemont	1275.5 $\pm$ 105.5a	698.6 $\pm$ 51.0b	−45.2	763.1 $\pm$ 230.2a	231.4 $\pm$ 231.4a	−13.3
M-202	1232.4 $\pm$ 311.7a	930.1 $\pm$ 126.6a	−24.5	567.6 $\pm$ 138.8a	1065.9 $\pm$ 42.0a	87.8
Rosemont	1497.9 $\pm$ 408.3a	876.1 $\pm$ 342.2a	−41.5	424.3 $\pm$ 92.5a	681.1 $\pm$ 239.4a	60.5
Overall average	1163.4 $\pm$ 236.1a	912.2 $\pm$ 219.8b	−19.6	932.6 $\pm$ 212.3a	996.3 $\pm$ 182.6a	13.5

Note: Means within each cultivar and year followed by the same letter were not significant at $p < 0.05$. Least significant difference (LSD) test at $p = 0.05$ was used to compare means in a randomized complete block design analysis of variance (Table S4). Standard errors were computed from three replications.

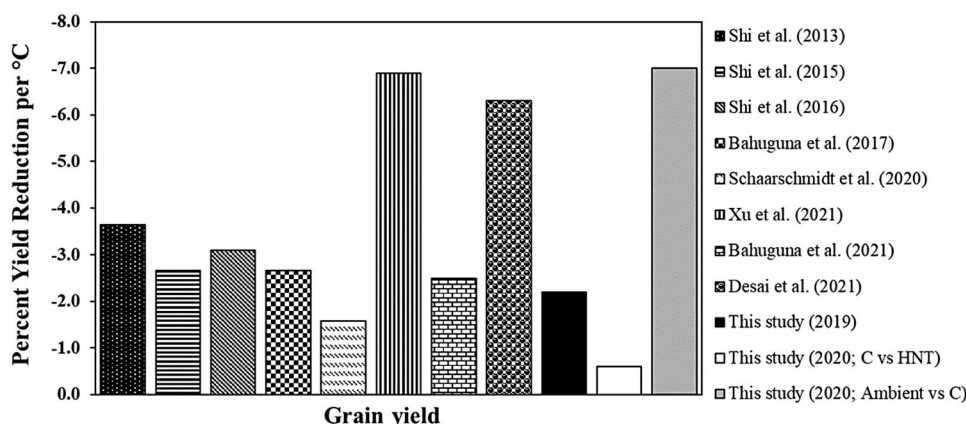


FIGURE 7 Graphical comparison of HNT stress impact on grain yield between nine independent high night air temperature (HNT) field experiments in rice. The data as presented in percent reduction per 1°C of HNT represent the average of all genotypes that showed grain yield decrease within the experiment. Details of independent experiments are described in Table 1. Briefly, most of the rice genotypes were exposed to HNT stress during panicle initiation to maturity (Bahuguna et al., 2017, 2022; Desai et al., 2021; Schaarschmidt et al., 2020; W. Shi et al., 2013, 2015; P. Shi et al., 2016; Xu et al., 2021). In the current study, HNT stress was imposed during the anthesis stage for 2 weeks. Temperature differences between the control/ambient temperature and HNT stress range significantly from 1 to 6°C in different experiments. *Ambient (unhoused/outside); C, Control greenhouses.

conditions. Similarly, LaGrue reported a decrease in grain yield quality when exposed to HNT (>25°C) during R6 and R7 grain filling stages (Ambardekar et al., 2011), while yield of Lemont varied under HNT stress. Our results, together with other studies mentioned above, provide evidence that rice grain yields decreased when exposed to high night air temperature and longer heat stress (Ambardekar et al., 2011; Bahuguna et al., 2017; W. Shi et al., 2013). Clearly, the varying degrees and the extent of impact caused to grain yield by HNT stress underscores the need for comprehensive understanding of rice plant mechanistic tolerance processes to heat stress and such understanding should take into account the actual growing field conditions of paddy rice.

For example, the daily amount of light and daily temperature can be consistently lower under controlled conditions, hence the plant's source: sink dynamics are greatly affected, thus influencing grain yield, physiological processes, and morphology. In contrast, plants under field conditions can grow at higher densities, leading to smaller plants with strong negative effects on tiller or side-shoot formation (Poorter et al., 2016).

3.6 | Grain yield responses of rice under HNT across field-based facilities and systems

HNT stress beyond 23°C imposed during panicle initiation until maturity had a significant impact on the grain yield of rice under field-based heat tent conditions (Figure 7). Reduction in grain yield per 1°C among selected rice accessions screened under the same field heat tents was consistent, ranging from 1.6% to 3.6% (W. Shi et al., 2013, 2015; Bahuguna

et al., 2017; Schaarschmidt et al., 2020; P. Shi et al., 2016), with a study using field heat tents and heat radiators (2.5%) (Bahuguna et al., 2022). A similar grain yield reduction per 1°C was observed in this study from the experiment conducted in 2019, but a lower yield reduction per 1°C was observed in the 2020 season. This is due to a warmer cropping season in 2019 (23.8°C) compared to the 2020 (22.7°C) cropping season. A higher percentage of yield reduction per 1°C was observed from a large population of rice genotypes (6.9%) (Xu et al., 2021), while a 6.3% yield reduction was observed in one rice genotype from the field infrared heating system (T-FACE) (Desai et al., 2021). A high percent yield reduction per 1°C (7%) was observed in this study between ambient (unhoused) and control (housed). These results demonstrate that different genotypes from different geographical sources responded significantly different to HNT, which suggests further analyses.

3.7 | Considerations, challenges, and system improvement

The results of this study are promising because the field-based infrastructure can control air temperature at the target threshold for growing conditions inside the greenhouses with a high degree of accuracy. The heat stress implementation was sustained even under extreme weather conditions such as flooded fields, heavy rains, strong winds, and thunderstorms. Similar greenhouses have been successfully used for upland crops such as wheat (Hein et al., 2020). In the case of applying this system to upland crops, one consideration is managing humidity inside the greenhouse. Other studies suggest the use of

dehumidifiers to balance the air humidity during enclosure and avoid excessive vapor pressure, as the accumulation of transpiration water inside the greenhouse can result in reduced photosynthetic activity and lead to condensation. Condensate on plants facilitates infection by viruses and fungi and thus increases the risk of disease outbreaks and the establishment of algae and other unwanted organisms (Germer et al., 2011). Additionally, the other important role of these greenhouses is to serve as a rain-out shelter. This type of use is highly suitable for water stress studies where greenhouses are placed in the field. Rain-out shelters can control rainfall capture, canopy wetting, and rain splash, which facilitate the implementation of water stress thresholds at a field scale. The strong and solid structures of the high-tunnel greenhouse can effectively be used year-round, even in regions with common occurrences of heavy rain, destructive wind, hurricanes, and tornadoes.

While our study shows that the cyber-physical greenhouse system accurately maintained an elevated air temperature during heat treatment, we propose following improvements in the system to better provide controlled growing conditions:

1. adding more temperature with RH data sensors and randomly distributing them inside the greenhouse at different heights, above and below rice canopies, will help attain more accurate heating accuracy. Adding other microclimatic sensors, such as light intensity sensors, inside the greenhouse will improve the accuracy of estimating both organ and canopy temperatures during the experimental period;
2. adding more robust Raspberry Pi sensors will help attain fast and accurate heating data and at the same time, can be used for two to four cropping seasons. Precise Raspberry Pi sensors are needed to run both day and night and to track the environmental factors that affect crop growth throughout the cropping season. More precise and fast response sensors that can read temperatures more frequently are needed to modulate the system more efficiently;
3. adding an additional weather station outside of the greenhouses to validate the interior and exterior climatic conditions in comparison to Raspberry Pi sensors, and adding micro-climatic or micrometeorology sensors (i.e., micrometeorological instrument for the near-canopy environment in rice [MINCER]) (Fukuoka et al., 2012) to measure and further validate microclimatic factors affecting grain yield and physiological processes in rice for in-depth studies;
4. adding an automated system that operates the closing and opening of sidewalls and roofs of the greenhouse. Automatic closing of the roofs and walls at the same time will help attain a fast and uniform start and end time of heat treatment imposition in all greenhouses with fewer staff running the program.

4 | CONCLUSIONS

This study established the infrastructure and logistics for a field-based system equipped with automated air temperature sensors and controls for applying heat stress to hundreds of rice selections under field conditions. To understand the rice plant response to heat stress using 320 rice selections, high night air temperature was implemented only during the flowering stage of rice. Nighttime air temperatures in the HNT greenhouses had an average of 4°C temperature difference relative to control greenhouses during the heat stress treatment in 2019 and 2020. Air temperature inside the HNT greenhouses immediately increased within 1 h of heat treatment initiation and declined to ambient temperature within 1–2 h after heat stress treatment. The cyber-physical system was able to efficiently impose HNT stress on rice plants during the flowering stage while automatically sensing subsequent changes in the outside field environment. The rice cultivars Lemont and Carolina Gold 12033 showed yield reductions following HNT stress, while Cocodrie, Kaybonnet, LaGrue, and L-202 cultivars showed consistent reduction of grain yield for two consecutive croppings.

Growing plants under controlled field experiments is often challenging because of variability in environmental factors; this field infrastructure HNT system is one step forward to achieve controlled abiotic and/or biotic stress under varying conditions in the field environment. With the system's alterations and improvements, this infrastructure can be adapted for phenotyping other crops growing, whether fully flooded or under limited water conditions, high day temperature stress, and/or combination with HNT. This phenotyping tool will help to further elucidate mechanisms, physiological biomarkers, and other traits to assist crop breeding to improve the resilience of crops to different abiotic stresses.

AUTHOR CONTRIBUTIONS

Cherryl Quiñones: Data curation; formal analysis; investigation; methodology; resources; software; validation; visualization; writing—original draft; writing—review and editing. **Maria Arlene Adviento-Borbe:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; resources; supervision; writing—original draft; writing—review and editing. **Wenceslao Larazo:** Conceptualization; data curation; formal analysis; investigation; methodology; resources; software; supervision; writing—review and editing. **Rodney Shea Harris:** Conceptualization; data curation; formal analysis; investigation; methodology; resources; supervision; writing—review and editing. **Kharla Mendez:** Conceptualization; data curation; formal analysis; investigation; methodology; resources; writing—review and editing. **Shannon S.**

Cunningham: Data curation; formal analysis; investigation; methodology; resources; writing—review and editing. **Zachary C. Campbell:** Data curation; investigation; methodology; project administration; resources; writing—review and editing. **Karina Medina-Jimenez:** Data curation; formal analysis; investigation; methodology; resources; software; writing—review and editing. **Nathan T. Hein:** Conceptualization; data curation; investigation; methodology; resources; writing—review editing. **Dan Wagner:** Data curation; investigation; methodology; resources; software; writing—review and editing. **Brian Ottis:** Data curation; funding acquisition; investigation; methodology; resources; writing—review and editing. **Harkamal Walia:** Conceptualization; data curation; funding acquisition; investigation; methodology; project administration; resources; writing—review and editing. **Argelia Lorence:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; visualization; writing—original draft; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ORCID

Argelia Lorence  <https://orcid.org/0000-0001-9844-8820>

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