Divergent phenotypic response of rice accessions to transient heat stress during early seed development

Puneet Paul
Balpreet K. Dhatt
Jaspreet Sandhu
Waseem Hussain
Larissa Irvin

See next page for additional authors

Follow this and additional works at: https://digitalcommons.unl.edu/agronomyfacpub

Part of the Agricultural Science Commons, Agriculture Commons, Agronomy and Crop Sciences Commons, Botany Commons, Horticulture Commons, Other Plant Sciences Commons, and the Plant Biology Commons

This Article is brought to you for free and open access by the Agronomy and Horticulture Department at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Agronomy & Horticulture -- Faculty Publications by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.
Authors
Puneet Paul, Balpreet K. Dhatt, Jaspreet Sandhu, Waseem Hussain, Larissa Irvin, Gota Morota, Paul E. Staswick, and Harkamal Walia
Divergent phenotypic response of rice accessions to transient heat stress during early seed development

Puneet Paul1 | Balpreet K. Dhatt1 | Jaspreet Sandhu1 | Waseem Hussain1,3 | Larissa Irvin1 | Gota Morota2 | Paul Staswick1 | Harkamal Walia1

1Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE, USA
2Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA
3International Rice Research Institute, Los Banos, Philippines

Correspondence
Harkamal Walia, Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE, USA.
Email: hwalia2@unl.edu

Funding information
This work was funded by National Science Foundation Grant # 1736192 to HW.

Abstract
Increasing global surface temperatures is posing a major food security challenge. Part of the solution to address this problem is to improve crop heat resilience, especially during grain development, along with agronomic decisions such as shift in planting time and increasing crop diversification. Rice is a major food crop consumed by more than 3 billion people. For rice, thermal sensitivity of reproductive development and grain filling is well-documented, while knowledge concerning the impact of heat stress (HS) on early seed development is limited. Here, we aim to study the phenotypic variation in a set of diverse rice accessions for elucidating the HS response during early seed development. To explore the variation in HS sensitivity, we investigated aus (1), indica (2), temperate japonica (2), and tropical japonica (4) accessions for their HS (39/35°C) response during early seed development that accounts for transition of endosperm from syncytial to cellularization, which broadly corresponds to 24 and 96 hr after fertilization (HAF), respectively, in rice. The two indica and one of the tropical japonica accessions exhibited severe heat sensitivity with increased seed abortion; three tropical japonicas and an aus accession showed moderate heat tolerance, while temperate japonicas exhibited strong heat tolerance. The accessions exhibiting extreme heat sensitivity maintain seed size at the expense of number of fully developed mature seeds, while the accessions showing relative resilience to the transient HS maintained number of fully developed seeds but compromised on seed size, especially seed length. Further, histochemical analysis revealed that all the tested accessions have delayed endosperm cellularization upon exposure to the transient HS by 96 HAF; however, the rate of cellularization was different among the accessions. These findings were further corroborated by upregulation of cellularization-associated marker genes in the developing seeds from the heat-stressed samples.

Keywords
endosperm, genetic diversity, heat stress, rice, seed development, syncytial
INTRODUCTION

Present-day agriculture is facing multifaceted challenges. The constant shifts in global food demands associated with exponential growth in world population, shrinking arable land, and increased frequency of extreme events need to be addressed urgently to mitigate future global food security crisis (Godfray et al., 2010; Foley et al., 2011; Röth, Paul, & Fragkostefanakis, 2016). Among the climatic variables, rising temperature is one of the most detrimental for crop yields (Porter & Gawith, 1999; Zhao et al., 2017). The average global temperature is anticipated to increase by 0.2°C per decade over the next few decades (Lobell, Schlenker, & Costa-Roberts, 2011). In this context, a recent study estimates a 3%–8% decline in the yields of rice, wheat, and maize, for each degree-Celsius increase in temperature (Zhao et al., 2017). These three cereal crops are a major food source worldwide; thus, a decline in yields will likely cause increasing malnutrition and socio-economic unrest.

Rice provides 50% of the dietary caloric supply and is the primary source of income for a billion households globally, the majority of which are small landholders in Asia and Africa (Jagadish, Murt, & Quick 2015; Khush, 2005; Khush & Jena, 2009; Muthayya, Sugimoto, Montgomery, & Maberly, 2014; Van Nguyen & Ferrero, 2006). Rice grain yield, which is determined by the number of panicles per plant, number of grains per panicle, ratio of filled grains, and their weight (Sakamoto & Matsuoka, 2008; Xing & Zhang, 2010), is sensitive to high temperatures. The heat stress (HS) sensitivity of rice, particularly during reproductive development, pollen development, pollination, or anthesis, can dramatically reduce spikelet fertility (Ali et al., 2019; Arshad et al., 2017; Jagadish, Craufurd, & Wheeler, 2007; Prasad, Boote, Allen, Sheehy, & Thomas, 2006). On the other hand, HS during grain filling can negatively impact the grain quality (Sreenivasulu et al., 2015). HS exposure during early to mid-grain filling period impairs starch biosynthesis, triggering non-uniform filling of starch granules that lead to a chalky grain appearance (Fitzgerald & Resurreccion, 2009; Kadan, Bryant, & Miller, 2008; Lisle, Martin, & Fitzgerald, 2000). These grains are more prone to breakage during the milling process (Sreenivasulu et al., 2015). Despite our understanding of HS response of rice during vegetative, reproductive, and grain filling stages, information on impact of HS on early seed development in rice is sparse.

Endosperm is the dominant tissue and the primary determinant of seed size in the monocot crops rice, wheat, and maize (Chaudhury et al., 2001; Gao, Xu, Shen, & Wang, 2013). Postfertilization, endosperm undergoes rapid nuclear division without cytokinesis (syncytial phase). The free nuclear divisions are followed by cell wall formation (cellularization), initially around the periphery of a central vacuole and eventually filling the entire inner cavity. Afterward, endosperm development enters the grain filling phase, where it accumulates storage compounds such as starch, proteins, and lipids, thus providing a major food source worldwide (Xing & Zhang, 2010; Zuo & Li, 2014). Previous reports have shown that HS alters the timing of syncytium–cellularization transition, where moderate HS (35/30°C) accelerates the cellularization event, while severe HS (39/35°C) delays cellularization (Chen et al., 2016; Folsom, Begcy, Hao, Wang, & Walia, 2014). Historically, the majority of rice-producing regions prefer specific sub-populations, for example, indica in South Asia, temperate japonica in North Asia, and tropical japonica in South America (Wang et al., 2016); however, with the current climate scenario, we need to explore the diverse germplasm to breed climate resilient rice. In this context, we aimed to evaluate the effect of transient HS on early seed development in a diverse set of rice accessions and determine the outcomes of this stress at seed maturity. Our subset of rice accessions represented the four sub-populations (aus, indica, temperate japonica, and tropical japonica). Based on their response, we categorized the accessions into highly sensitive, moderately, and strongly heat tolerant. Our work provides an important component for the knowledgebase needed to utilize a diverse gene pool to develop heat-resilient rice.

MATERIALS AND METHODS

Plant material and growth conditions

Nine rice accessions from Rice Diversity Panel 1 (RDP1; Zhao et al., 2011) were selected based on their relatively similar flowering time (<120 days) to ensure that the accessions flowered under similar light conditions in the glasshouse. Genetic and geographical diversity of the accessions was also considered (Table 1). Mature seeds were dehusked using a Kett TR-130, sterilized with water and bleach (40%), and germinated in dark on half-strength Murashige and Skoog media. After 5 days, germinated seedlings were transplanted to soil in 4-inch square pots, and plants were grown in control glasshouse conditions: 16-hr light and 8-hr dark at 28 ± 1°C and 23 ± 1°C, respectively and relative humidity of 55%–60% was consistently maintained throughout the plants' life cycle (Sandhu et al., 2019).

Heat stress treatments

Plants were grown in control conditions until flowering (Figure 1a). To track developing seeds, florets were marked at the time of fertilization. Twenty-four hours after fertilization (HAF), plants were kept in either control conditions (16-hr light and 8-hr dark at 28 ± 1°C and 23 ± 1°C) or moved to a reach-in growth chamber for heat stress (HS) treatment (16-hr light and 8-hr dark at 28 ± 1°C and 35 ± 1°C). The HS treatment was applied for either 1 (HS1), 2 (HS2), or 3 (HS3) days corresponding to 48, 72, and 96 HAF, respectively (Figure 2a). Afterward, plants were moved back to the control condition until maturity for evaluating the effect of HS on mature seed traits. Three independent HS experiments (in 2016, 2017, and 2018) were conducted with 10–20 plants per treatment per accession in each experiment.

Analysis of developing and mature seeds

To accurately assess the effect of HS on early seed development, only the florets marked at the time of fertilization were considered for...
downstream analysis. For mature seed phenotyping, seeds harvested at maturity were examined. Total number of fully developed and unfilled or completely sterile seeds were scored to calculate percentage of fully developed seeds. For morphometric analysis, marked mature seeds were dehusked and scanned using Epson Expression 12,000 XL scanner. The images were analyzed using ImageJ (Abramoff, Magalhães, & Sunanda, 2004) to extract the length of developing seeds. For developing seed analysis, 15–20 marked seeds from 3 to 4 plants were evaluated.

### 2.4 | Histochemical analysis

The control and HS developing seeds (72 and 96 HAF) were harvested and fixed in 1 ml of formaldehyde (2%), acetic acid (5%), and ethanol (60%; FAE solution) for 16 hr. Tissue was then washed with 70% ethanol and stored overnight at 4°C, followed by dehydration with an ethanol series (85%, 95%, and 100%) for 1 hr each at room temperature. Samples were transferred to xylene (100%) for 2 hr, followed by transfer to 500 µl of a 1:1 mixture of xylene and paraplast tablets and stored at 60°C. Finally, samples were transferred to and embedded in paraplast (Paul et al., 2020). Cross sections (10 µm) were obtained using a rotary microtome (Leica RM2125 RTS). Images from the cross sections were observed using bright-field microscope (Leica DM-2500). For the experiment, six to eight developing seeds for each developmental stage from two independent experiments were considered for cross sections.

### 2.5 | RNA Extraction and RT-qPCR

Total RNA was extracted from developing seeds (48 and 96 HAF) using the RNeasy Plant mini kit (Qiagen), followed by DNase treatment. One microgram of total RNA was used for cDNA synthesis using the SuperScript VILO cDNA synthesis kit (Invitrogen). The RT-qPCR (10 µl) comprising gene-specific primers, SYBR Green Master Mix (Bio-Rad) and template, was conducted using LightCycler 480 Real-Time PCR System (Roche). A ubiquitin (UBQ5) gene was used as an endogenous control (Jain, Nijhawan, Tyagi, & Khurana, 2006). Data were analyzed using standard methods (Livak & Schmittgen, 2001) and represented as log, fold changes (Fragkostefanakis et al., 2015). For all RT-qPCR assays, a minimum of two independent biological and three technical replicates was evaluated. The primers used to analyze the syncytial and cellularization-associated genes are listed in Table S1.

### 2.6 | Statistical analysis

Descriptive statistics and analysis of variance (ANOVA) for mature seed traits were performed in statistical software R version 3.5.3 (R Core Team 2018). Principle component analysis for traits derived from mature seeds was performed using R package FactoMineR and factoextra (Lê, Josse, Rennes, & Husson, 2008). Trait correlations between mature seed traits in control and HS treatments were performed using functions cor.matrix() and corplot() from the corrplot R package (Wei et al., 2017). Statistical analysis used for analyzing individual experiments is explained in the figure legends.

### 3 | RESULTS

#### 3.1 | Genotypic level heat sensitivity of fully developed mature seeds is conversely related to single-grain weight

To understand the impact of HS on early seed development, selected rice accessions (Table 1) were subjected to transient HS for three days, that is, 24–96 hr after fertilization (HAF; Figure 1a). Only the florets marked at time of fertilization were considered for downstream

---

**Table 1** Genetic and geographical information of the rice accessions used in the current study

| Accessions | GSOR Id  | NSFTV Id | IRGC Id | Common name         | Sub-population | Country of origin |
|------------|----------|----------|---------|---------------------|----------------|------------------|
| AUS-1      | 301129   | 228      | 117673  | CA 902/B/2/1        | aus            | Chad             |
| IND-1      | 301163   | 172      | 117944  | Zhenshan 2          | indica         | China            |
| IND-2      | 301221   | 231      | 117753  | Hunan Early Dwarf 3 | indica         | China            |
| TEJ-1      | 301110   | 118      | 117828  | Oro                 | temp. jap.     | Chile            |
| TEJ-2      | 301195   | 204      | 117860  | Razza 77            | temp. jap.     | Italy            |
| TRJ-1      | 301141   | 150      | 117897  | Sultani             | trop. jap.     | Egypt            |
| TRJ-3      | 301217   | 226      | 117762  | IRAT 44             | trop. jap.     | Burkina Faso     |
| TRJ-4      | 301374   | 391      | 117706  | Palmyra             | trop. jap.     | United States    |
| TRJ-4      | 301374   | 391      | 117706  | Della               | trop. jap.     | United States    |

Note: First column refers to the names used in this study for the respective accessions. Genetic Stocks Oryza collection identification number (GSOR Id); National Science Foundation “Exploring the genetic basis of transgressive variation in rice” accessions; identification number (NSFTV Id); International Rice Genebank Collection identification number (IRGC).
analysis, which ensured accurate assessment of HS impact on early seed development. We detected genotypic differences in response to transient HS during early seed development as measured by the percentage of fully developed marked seeds (Figure 1 and Table 2). In this context, 3/9 tested accessions corresponding to indica (IND-1 and 2) and a tropical japonica (TRJ-3) showed drastic reduction in number of fully developed seeds (Figure 1b). One indica accession (IND-2) showed complete sterility under HS treatment, as we did not recover any developed mature seed, so the respective accession was not part of the downstream mature seed measurements (Figure 1b and c). On
the other hand, *aus* (AUS-1), one of the temperate japonicas (TEJ-2), and three tropical japonicas (TRJ-1, −2, and −4) also showed significant reductions; however, reductions were relatively less severe (Figure 1b). One of the temperate japonicas (TEJ-1) exhibited strong resilience to transient HS, as number of fully developed seeds recovered in the heat-stressed plants was comparable to the respective control plants (Figure 1b). We also measured seed parameters for the seeds that did fully develop for each accession. The indica accession that showed a steep decline for fully developed seeds (IND-1) was not affected in single-grain weight for the fully developed seeds at maturity. Contrarily, the four accessions maintaining the relatively higher mature seed numbers under stress (TEJ-1, TEJ-2, TRJ-1, and TRJ-4) showed a significant reduction with respect to single-grain weight (Figure 1c).

Given the phenotypic variability for the fully developed seeds (Figure 1d), we next dissected the impact of HS on early developing seeds in a temporal context by imposing HS for either 1 (HS1; 24 to 48 HAF), 2 (HS2; 24 to 72 HAF), or 3 (HS3; 24 to 96 HAF) days (Figure 2a).

In general, the severity with respect to fully developed seeds increased with increase in duration of the HS (HS3 > HS2 > HS1 > Control; Figure 2b). Interestingly, the *aus* accession (AUS-1), which showed a significant decrease in fully developed seeds on exposure to three days of HS (Figure 1b), was not significantly affected after 1 and 2 days of HS (HS1 and HS2, respectively; Figure 2b). In contrast, the *indica* accession (IND-2) that exhibited complete sterility following 3 days of stress (Figure 1b) also showed a sharp decline in fully developed seeds after only one day of HS (HS1) treatment (Figure 2b). A similar trend was observed for one of the tropical japonica accessions (TRJ-3), while the others (TRJ-1, TRJ-2, and TRJ-4) exhibited a decrease in fully developed seeds after two days of HS (HS2) treatment (Figure 2b). The temperate japonica accession TEJ-1 did not show an inflection point for the respective HS treatments, maintaining near control level of mature seeds throughout (Figure 2b). The other temperate japonica accession TEJ-2 had fewer fully developed seeds under HS2 treatment but was not further affected after an additional day of stress (HS3; Figure 2b).

### 3.2 Heat stress penalizes seed length of the relatively resilient accessions

To elucidate the impact of the three-day HS during early seed development on seed size, morphometric measurements on fully developed control and heat-stressed seeds were performed. In this context, area, perimeter, length, width, and length-to-width ratio of

---

**TABLE 2** Analysis of variance of the nine accessions

| Sources of variation | DF | F-value |
|----------------------|----|---------|
|                      |    | FDS     | SGW  | Area   | Perimeter | Length | Width | L/W   |
| Accession            | 8  | 2.64*   | 13.94$ | 29.87$ | 3.84*     | 2.92   | 30.19$ | 5.43$ |
| Treatment (T)        | 1  | 90.74^  | 21.96$ | 17.24$ | 0.14      | 5.74   | 9.17$  | 0.27  |
| Environment (E)      | 2  | 0.15    | 0.40   | 5.27*  | 11.88     | 7.05   | 3.10   | 1.67  |
| T × G                | 8  | 1.94    | 2.65   | 2.05   | 2.30      | 1.70   | 0.54   | 0.53  |
| E × G                | 16 | 0.30    | 1.088  | 1.30   | 1.94      | 1.34   | 0.82   | 0.90  |
| Residuals            | 18 |         |        |        |           |        |        |       |

Note: DF represents degrees of freedom; FDS, fully developed seeds (%); SGW, single-grain weight; L/W, length/width.  
* 5 Significant level at 5%, 1%, and 0.1% level, respectively.
the individual seeds were evaluated. A significant difference between control and HS treatments was observed for all measured parameters for certain accessions except for length-to-width ratio, indicating the prevalent treatment effect (Table 3). Interestingly, both temperate japonica accessions (TEJ-1 and TEJ-2) showed a significant reduction in seed length under HS. Further, seed area and width were also reduced for TEJ-2, while these traits were significantly heat-tolerant for TEJ-1 (Table 3). Among the tropical japonica accessions, only TRJ-2 showed a significant reduction in seed area, length, width, and perimeter. Another tropical japonica accession, TRJ-3, showed marginal reduction in seed width under HS. It is notable that the indica accession IND-1 maintained seed size parameters, while the aus accession AUS-1 showed reduction only in seed area (Table 3). A significant positive correlation for several examined mature seed traits was detected for both control and HS treatments. Most of these were common to both treatments, indicating possible involvement of the same genetic loci in controlling these traits or perhaps that the respective traits are under pleiotropic influence (Figure 3). As expected, seed area is significantly \((p < .001)\) correlated with perimeter, length, width, and single-grain weight under control conditions, suggesting the interdependency of the analyzed traits; however, area was significantly \((p < .001)\) correlated only with seed width and single-grain weight under HS (Figure 3). Likewise, we did not detect a significant positive correlation of fully developed seeds with other traits under control, indicating that the respective trait is independent. A significant correlation was detected for single-grain weight with seed width under control as well as HS treatments (Figure 3). Single-grain weight was weakly correlated with seed length under control, while no correlation existed between the respective traits under HS (Figure 3).

### 3.3 | Principle component analysis

To assess phenotypic diversity among accessions under control and HS treatments, we performed principle component analysis (PCA; Figure 4) using all mature seed traits. The first two principle components PCs (1 and 2) explained 87% of total variation. PCA effectively explained the variation in the severely susceptible accessions (IND-1, TRJ-3, IND-2) due to HS. Also, these accessions were clustered together under control, first quadrant, Q-1, and HS (Q-2; Figure 4). On the other hand, control and HS for TRJ-1 (Q-1), TRJ-4 and AUS-1 (Q-2), TEJ-2 and TRJ-2 (Q-3), and TEJ-1 (Q-4) exhibited relatively closer associations (Figure 4). PCA provided a broader context for phenotypic diversity by showing that the sensitive accessions exhibited distinct responses to control and HS treatments, while the relatively tolerant accessions showed comparable phenotypes in the two treatments.

### 3.4 | Resilient accessions sense heat stress prior to the sensitive accessions

Because we observed differences in seed length at maturity for four of the relatively heat-tolerant accessions (TEJ-1, TEJ-2, TRJ-1,

---

**TABLE 3** Morphometric analysis of mature seeds.

| Accession | Area (mm^2) | Perimeter (mm) | Length (mm) | Width (mm) | Length/Width |
|-----------|-------------|----------------|-------------|------------|--------------|
| AUS-1 Control | 11.48 ± 1.0^1 | 15.49 ± 1.6 | 6.13 ± 0.6 | 2.50 ± 0.1 | 2.56 ± 0.4 |
| AUS-1 HS | 9.45 ± 1.9 | 14.53 ± 2.0 | 5.68 ± 0.8 | 2.33 ± 0.2 | 2.71 ± 0.8 |
| IND-1 Control | 12.38 ± 0.7 | 15.08 ± 0.4 | 5.74 ± 0.1 | 2.78 ± 0.1 | 2.16 ± 0.2 |
| IND-1 HS | 11.69 ± 1.3 | 16.70 ± 3.9 | 6.40 ± 1.7 | 2.68 ± 0.2 | 3.52 ± 2.9 |
| IND-2 Control | 12.68 ± 0.5 | 15.17 ± 0.4 | 5.80 ± 0.1 | 2.79 ± 0.2 | 2.12 ± 0.2 |
| IND-2 HS | NA | NA | NA | NA | NA |
| TEJ-1 Control | 14.28 ± 0.4 | 15.64 ± 0.4 | 5.72 ± 0.1 | 3.22 ± 0.1 | 1.80 ± 0.1 |
| TEJ-1 HS | 13.64 ± 0.8 | 15.39 ± 0.5 | 5.56 ± 0.2* | 3.15 ± 0.1 | 1.78 ± 0.1 |
| TEJ-2 Control | 17.18 ± 0.9 | 17.87 ± 1.15 | 6.86 ± 0.3 | 3.26 ± 0.1 | 2.19 ± 0.4 |
| TEJ-2 HS | 15.23 ± 2.2^1 | 17.05 ± 0.9 | 6.50 ± 0.4* | 3.02 ± 0.3^1 | 2.22 ± 0.2 |
| TRJ-1 Control | 11.64 ± 0.8 | 14.69 ± 0.6 | 5.77 ± 0.2 | 2.58 ± 0.1 | 2.25 ± 0.1 |
| TRJ-1 HS | 11.00 ± 1.8 | 14.31 ± 0.8 | 5.53 ± 0.3* | 2.57 ± 0.3 | 2.18 ± 0.2 |
| TRJ-2 Control | 17.40 ± 0.7 | 18.10 ± 0.5 | 7.10 ± 0.2 | 3.10 ± 0.1 | 2.3 ± 0.1 |
| TRJ-2 HS | 13.90 ± 2.3^3 | 16.00 ± 1.6^5 | 6.14 ± 0.8^5 | 2.90 ± 0.3* | 2.10 ± 0.3 |
| TRJ-3 Control | 12.00 ± 0.6 | 15.10 ± 0.6 | 5.93 ± 2.6 | 2.61 ± 0.1 | 2.33 ± 0.1 |
| TRJ-3 HS | 11.10 ± 0.7 | 17.00 ± 2.7 | 6.80 ± 1.5 | 2.47 ± 0.2* | 3.8 ± 2.3 |
| TRJ-4 Control | 11.80 ± 1.0 | 16.00 ± 0.9 | 6.61 ± 0.4 | 2.22 ± 0.1 | 3.0 ± 0.11 |
| TRJ-4 HS | 11.80 ± 0.3 | 15.94 ± 0.3 | 6.50 ± 0.1 | 2.21 ± 0.1 | 2.90 ± 0.1 |

Average morphometric measurements from mature seeds of control plants and heat stressed (HS) plants. \(n = 700-1000\) marked seeds from 25 to 40 plants. Paired \(t\)-test was used for statistical analyses (Control vs HS for the respective accession). NA not available. ± indicate standard deviation, ^ indicates \(p\)-value < .001, * .01, * .05.
and TRJ-2), we next aimed to capture the genotypic differences in growth of the developing seeds exposed to control and HS treatments. Like mature seed phenotypes, varied responses among accessions were observed. From the analysis, 7/9 accessions exhibited a significant variation in the developing seed length by 72 HAF, whereas an indica (IND-1) and a temperate japonica (TRJ-3) showed significant changes only by 96 HAF (Figure 5). However, one of the tropical japonica (TRJ-4) started showing HS effect from 48 HAF, that is, 24 hr after the HS treatment. These results suggest that developing grain response in context of grain length is predictive of lengths at maturity (Table 3) and measuring the grain length increase daily provides further insight into genotypic differences to HSR (Figure 5).

3.5 Histochemical analysis reveals differential rate of endosperm cellularization

The observed differences in seed morphology at maturity and length of developing seed prompted us to explore potential genotypic variation in the cellular biology of early seed development under heat stress. Previous reports have shown that the developing endosperm is at the syncytial stage at or around 48 HAF and completes cellularization around 96 HAF in a model rice variety, Kitaake (Chen et al., 2016; Folsom et al., 2014). We observed that the cellularization timing was affected not only by HS, but also varied among accessions even under control conditions (Figure 6 and Figure S2). For instance, the aus accession (AUS-1) completed endosperm cellularization by 72 HAF under control conditions, whereas all other accessions were either showing the radial microtubule system (RMS; tropical japonicas: TRJ-2, TRJ-3, and TRJ-4) or were initiating cell wall formation (indicas: IND-1 and IND-2, temperate japonicas: TEJ-1 and TEJ-2) by 72 HAF. Except for one of the temperate japonicas (TRJ-2), all other accessions completed endosperm cellularization by 96 HAF in the control conditions (Figure 6 and Figure S2). Under the impact of transient HS, endosperm cellularization was delayed in all the tested accessions. The RMS, which precedes cell wall formation, was initiated in AUS-1, TEJ-2, and TRJ-1 by 72 HAF in the HS treatment, while TEJ-1 and TRJ-3 exhibited RMS by 96 HAF in the HS-treated samples (Figure 6 and Figure S2). We did not detect RMS at either 72 or 96 HAF in the heat-stressed samples for the two indica accessions, IND-1 and IND-2 (Figure 6 and Figure S2). A similar pattern was detected for the two tropical japonicas (TRJ-2 and TRJ-4; Figure 6 and Figure S2).
3.6 | Gene expression of syncytial- and cellularization-specific marker genes corroborates the histochemical analysis

The transition of endosperm from the syncytial phase to cellularization is a major event during seed development as it defines the extent of nuclear proliferation and thereby determines final seed size (Brown, Lemmon, & Olsen, 1996; Li, Xu, Duan, & Li, 2018; Olsen, 2004). The event is finely orchestrated at the genetic and epigenetic levels (Folsom et al., 2014). CMT3 (Folsom et al., 2014; Lindroth et al., 2001), DRM3 (Sharma et al., 2009), KRP3 (Mizutani, Naganuma, Tsutsumi, & Saitoh, 2010), and several type-I MADS-box transcription factors, MADS78, MADS79, MADS87, and MADS89 (Chen et al., 2016; Folsom et al., 2014; Kang, Steffen, Portereiko, Lloyd, & Drews, 2008; Paul et al., 2020; Steffen, Kang, Portereiko, Lloyd, & Drews, 2008), show expression specific to the syncytial phase, and decline with the progression of cellularization. Therefore, we referred these as syncytial-associated genes (Figure 7). On the other hand, genes that are upregulated with the progression of endosperm cellularization relative to the syncytial phase, for example, OSTF1 (Yang, Chung, Tu, & Leu, 2002), SSIIa (Folsom et al., 2014), ZmEBE-1 (French, Abu-Zaitoon, Uddin, Bennett, & Nonhebel, 2014), and others reported by Chen et al (Chen et al., 2016), are referred to as cellularization-associated (Figure 7). For these syncytial and cellularization marker genes, RT-qPCR analysis on developing seeds (48 and 96 HAF) was performed for 4 accessions: AUS-1, IND-1, TEJ-1, and TRJ-2 representing each of the sub-populations, aus, indica, temperate, and tropical japonicas, respectively. Developing seed samples at 48 and 96 HAF from control and heat-stressed samples were chosen because they reflected syncytial and cellularization stages for most of the accessed accessions (Figure 6). The primary objective of the gene expression analysis was to validate marker gene expression at 48 and 96 HAF under control conditions (Figure 6). As expected, we observed
down- and upregulation of syncytial- and cellularization-associated
genes, respectively, by 96 HAF relative to 48 HAF under control
conditions (Figure 7a). However, the aus accession (AUS-1) showed
exceptions, as DRM3 corresponding to syncytial-associated genes
showed upregulation, while two cellularization-associated genes
(OSTF1 and dirigent) were downregulated by 96 HAF (Figure 7a).

Next, we examined the expression of the selected genes at 48 HAF
under HS relative to 48 HAF control (Figure 7b). Three syncytial-
associated genes (CMT3, KRP3, and MADS89) were downregulated
in all the tested accession for the heat-stressed samples from 48
HAF, wherein DRM3 and MADS79 were significantly upregulated in
at least one of the accessions (Figure 7b). Two of the cellularization-
associated genes (expressed protein: LOC_Do05g34310 and OSTF1)
were upregulated in all the tested accession, while other cellulari-
zation-associated genes (SSIIa, TPP8, ZmEBE-1, CSLA9, and dirigent)
were downregulated in at least one of the tested accessions for the
heat-stressed samples from 48 HAF (Figure 7b). We also examined
the expression of these genes at 96 HAF under HS relative to 96
HAF control (Figure 7c). In this context, 5/7 syncytial-associated
(except CMT3 and KRP3) and 6/7 cellularization-associated (ex-
cept dirigent) genes were up- and downregulated, respectively, in
at least one of the tested accessions (Figure 7c). Taken together,
the gene expression level responses support the observation of
developmental disruption in initiation and/or progression of en-
dosperm cellularization under HS (Figure 6), as four of the cellu-
larization-specific marker genes (SSIIa, TPP8, ZmEBE-1, and OSTF1)
were downregulated in all the four tested accessions at 96 HAF in
the heat-stressed samples relative to the 96 HAF control samples
(Figure 7c). While cellularization-associated marker gene response
to HS provides a clear case for endosperm development, there are
no clear expression level responses that enable broad prediction
of individual seed-level tolerance or sensitivity to HS imposed in
this study.

4 | DISCUSSION

The global mean temperature rise over the last century has nega-
tively influenced crop yields (Battisti & Science, 2009; Challinor et
al., 2014; Gourdji, Sibley, & Lobell, 2013; Lobell et al., 2011; Zhao
et al., 2017). The situation is expected to worsen given the pre-
dicted increase in global temperatures (Gourdji et al., 2013; Zhao et
al., 2017). Extensive work has been carried out regarding the im-
 pact of HS on different phases of plant development (Bokszczanin
& Fragkostefanakis, 2013; Cheng, Sakai, Yagi, & Hasegawa, 2009;
Hasanuzzaman, Nahar, Alam, Roychowdhury, & Fujita, 2013;
Jagadish et al., 2015; Prasad, Bheemanahalli, & Jagadish, 2017). Both
reproductive development and grain filling phases in rice have been
documented as sensitive to environmental perturbations (Prasad et
al., 2006; Jagadish et al., 2007; Prasad et al., 2008; Jagadish et al.,
2010; Coast, Ellis, Murdoch, Quiñones, & Jagadish, 2015; Arshad et
al., 2017; Ali et al., 2019; Dhatt et al., 2019). However, genotypic
variation in rice germplasm for heat stress responses during early
seed development remains largely unexplored. In the present study,
we systematically investigated the impact of HS on the early seed
development that corresponds to the transition phase of endosperm
from syncytial to cellularized. The rate and timing of this transition
is one of the main drivers for determining final seed size (Brown et

FIGURE 6 Transient heat stress during early seed development delays
endosperm cellularization. (a) Pictorial representation depicting different stages
of rice endosperm from syncytial to cellularization. (b) Based on the stages
defined in (a), progression of endosperm development (at 72 and 96 HAF) under
control and heat stress in the nine rice accessions. This assessment is based on
representative cross-section images of developing seed (Figure S2). rms, radial
microtubule system; HAF, hours after fertilization.
Here, we imposed transient HS (39/35°C) during early seed development (24–96 hr after fertilization; HAF) on nine rice accessions corresponding to different sub-populations (Table 1, Figures 1 and 2). Given the HS sensitivity of pollination and/or fertilization (Zinn, Tunc-Ozdemir, & Harper, 2010), HS was initiated 24 HAF so that it did not interfere with either event. Furthermore, we only considered seeds that were marked at the time of fertilization. This approach helped us to (i) avoid overestimation of final yield penalty caused by HS and (ii) precisely track the early seed developmental stages with respect to syncytial and endosperm cellularization and their response to HS. Thus, our analysis does not necessarily reflect whole plant yield-level sensitivity or tolerance but rather provides a comprehensive and explicit view of the HS impact on early seed developmental stages. This approach is useful for future work that aims to identify the genetic basis of variation with respect to heat tolerance in the rice germplasm. Having developmental and temporal context will enhance prioritization of genes that increase resilience to transient heat stress during early seed development.

Heat stress response of the nine rice accessions varied widely. The two indica accessions, IND-1shs and IND-2shs (“shs” in the superscript refers to severely heat sensitive), and one of the tropical japonica accessions (TRJ-3shs) showed similar heat stress response with respect to proportion of fully developed seeds (Figures 1 and 2). Also, these accessions showed clear separation of the mature seed traits derived from the two treatments, control (Q-1) and HS (Q-2), depicting an overall sensitivity of these accessions (Figure 4). On the other hand, aus (AUS-1), two temperate japonicas (TEJ-1 and TEJ-2), and three tropical japonicas (TRJ-1, TRJ-2, and TRJ-4) showed relatively stronger association between the control and HS treatments (Figure 4). However, because of the limited number of accessions evaluated, we cannot generalize that tolerance or sensitivity to HS during early seed development is related to place of origin or sub-population of the accessions. Further, based on the heat stress
response, the relatively tolerant accessions can be sub-categorized as moderately and strongly heat tolerant (Figure 2b). The four accessions, AUS-1 mht, TRJ-1 mht, TRJ-2 mht, and TRJ-3 mht (*mht* in the superscript refers to moderately heat tolerant), showed drastic reduction in fully developed seeds by either two or three days of HS (HS2 or HS3, respectively) treatment, whereas TEJ-1 sht and TEJ-2 sht (*sht* in the superscript refers to the strongly heat tolerant) showed mild reductions in fully developed seeds in response to HS2 or HS3 (Figure 2b). Moreover, 4/6 tolerant accessions showed a significant reduction in the mature seed lengths on exposure to three days of HS (Table 3). These reductions are possible repercussions of the significant decline in the developing seed lengths as early as 72 HAF for all the relatively tolerant accessions (Figure 5). Contrarily, the sensitive accessions did not show any reduction with respect to mature seed length; however, single-grain weight and seed width were significantly compromised for TRJ-3 shs (Table 3). Likewise, developing seed lengths for 2/3 relatively sensitive accessions (IND-1 shs and TRJ-3 shs) did not show any alteration until 96 HAF in the HS-treated samples (Figure 5). Thus, we hypothesize that the heat-tolerant and the sensitive accessions (defined based on the individual seed level analysis) follow two different HSR mechanisms: the tolerant accessions, on exposure to HS, invest in maintaining the number of fully developed seeds but compromise seed size, whereas the sensitive accessions maintain mature seed size at the expense of the number of fully developed seeds. It is pertinent to state that the whole plant tolerance or sensitive response (as measured by long-term heat stress and its impact on seed weight per plant) may be different from how sensitivity and tolerance to the imposed HS is defined in current study.

Furthermore, histochemical investigation of the accessions during early seed developmental phases revealed that all three sensitive accessions either possessed radial microtubule systems (RMS; TRJ-3 shs) or initiated the process of endosperm cellularization (IND-1 shs and IND-2 shs) by 72 HAF and completed cellularization by 96 HAF under control conditions (Figure 6). The RMS defines the nuclear-cytoplasmic domains, thus facilitating the process of endosperm cellularization (Olsen, 2004; Sabelli & Larkins, 2009). In contrast, under HS conditions, the sensitive accessions did not possess RMS by 72 HAF, suggesting substantial delay in the cellularization process (Figure 6 and Figure S2). On the other hand, 4/6 relatively tolerant accessions (AUS-1 mht, TRJ-1 mht, TEJ-1 sht, and TEJ-2 sht) either possessed RMS or commenced the cell wall formation procedure under HS by 96 HAF (Figure 6 and Figure S2). The difference in the rate of cellularization in the different accessions might be correlated with their adaptation strategies to HS. Gene expression analysis further validated the findings from the histochemical analysis. The syncytial-specific marker genes were downregulated, while the cellularization-specific marker genes were upregulated at 96 HAF in the control samples (Figure 7a). This observation is in accordance with the developing seed cross sections as a majority (8/9, except TRJ-2 mht) of the tested accessions underwent complete endosperm cellularization by 96 HAF (Figure 6 and Figure S2). Similarly, under HS conditions at 96 HAF, 5/7 syncytial-associated and 6/7 cellularization-associated genes were up- and downregulated, respectively (Figure 7c). These results align well with the histochemical findings where all the tested accessions show delay in endosperm cellularization upon exposure to HS (Figure 6). However, further investigation of gene expression in context of the rate of cellularization will require testing of marker genes corresponding to RMS or cell wall initiation.

In summary, this study provides detailed insight into the divergence of heat stress response mechanisms of a subset of rice accessions on exposure to the transient HS during early seed development. This work also emphasizes the challenges in developing a simple sampling approach that would account for these differences in developmental transitions and impact of HS in a duration-dependent manner across accessions. Further validation of the sensitive and tolerant accessions through -omic analysis will be helpful in molecular dissection. This work will also be useful in developing future phenotyping strategies for large-scale screening and mining allelic variants for heat resilience rice.

**ACKNOWLEDGMENTS**

We would like to thank Martha Rowe for help with the histochemical analysis.

**CONFLICT OF INTEREST**

The authors declare no conflicts of interest.

**AUTHOR CONTRIBUTIONS**

PP and HW conceived and designed the experiments. PP performed all the heat stress experiments. BKD/JS/LI assisted in collection of the developing seed tissue for the downstream analysis. BKD performed histochemical analysis and RT-qPCRs. WH and GM performed the statistical analysis. PP analyzed the results from all the experiments and wrote the manuscript. PS critically reviewed and analyzed the work. All authors read and approved the manuscript.

**REFERENCES**

Abramoff, M., Magalhães, P., & Sunanda, R. J. (2004). Image Processing with ImageJ. Biophotonics Int, 11, 36–42.

Ali, F., Waters, D. L. E., Ovenden, B., Bundock, P., Raymond, C. A., & Rose, T. J. (2019). Australian rice varieties vary in grain yield response to heat stress during reproductive and grain filling stages. Journal of Agronomy and Crop Science, 205, 179–187. https://doi.org/10.1111/jac.12312

Arshad, M. S., Farooq, M., Asch, F., Krishna, J. S. V., Prasad, P. V. V., & Siddique, K. H. M. (2017). Thermal stress impacts reproductive development and grain yield in rice. Plant Physiology and Biochemistry, 115, 57–72. https://doi.org/10.1016/j.plaphy.2017.03.011

Battisti, D., & Science, R. N. (2009). Historical warnings of future food insecurity with unprecedented seasonal heat. Science 322, 240–244. https://doi.org/10.1126/science.1164363

Bokszczanin, K. L., & Fragiostefanakis, S. (2013). Perspectives on deciphering mechanisms underlying plant heat stress response and thermotolerance. Frontiers in Plant Science, 4, 1–20.

Brown, R. C., Lemmon, B. E., & Olsen, O. A. (1996). Development of the endosperm in rice (Oryza sativa L.): Cellularization. Journal of Plant Research, 109, 301–313. https://doi.org/10.1007/BF02344477

Challinor, A. J., Watson, J., Lobell, D. B., Howden, S. M., Smith, D. R., & Chhetri, N. (2014). A meta-analysis of crop yield under climate
Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Gao, Y., Xu, H., Shen, Y., & Wang, J. (2013). Transcriptomic analysis of rice.

Hasanuzzaman, M., Nahar, K., Alam, M., Roychowdhury, R., & Fujita, M. (2013). Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. International Journal of Molecular Sciences, 14, 9643–9684. https://doi.org/10.3390/ijms14096543

Jagadish, S. V. K., Cairns, J., Lafitte, R., Wheeler, T. R., Price, A. H., & Craufurd, P. Q. (2010). Genetic analysis of heat tolerance at anthesis in rice. Crop Science, 50, 1633. https://doi.org/10.2135/cropsci2009.09.0516

Jagadish, S., Craufurd, P., & Wheeler, T. (2007). High temperature stress and spikelet fertility in rice (Oryza sativa L.). Journal of Experimental Botany, 58, 1627–1635. https://doi.org/10.1093/jxb/erm003

Jagadish, S. V. K., Murt, M. V. R., & Quick, W. P. (2015). Rice responses to rising temperatures – challenges, perspectives and future directions. Plant, Cell and Environment, 38, 1686–1698. https://doi.org/10.1111/ pce.12430

Jain, M., Nijhawan, A., Tyagi, A. K., & Khurana, J. P. (2006). Validation of housekeeping genes as internal control for studying gene expression in rice by quantitative real-time PCR. Biochemical and Biophysical Research Communications, 345, 646–651. https://doi.org/10.1016/j. bbr.2006.04.140

Kadan, R. S., Bryant, R. J., & Miller, J. A. (2008). Effects of milling on functional properties of rice flour. Journal of Food Science, 73, E151–E154. https://doi.org/10.1111/j.1750-3841.2008.00720.x

Kang, I.-H., Steffen, J. G., Portereiko, M. F., Lloyd, A., & Drews, G. N. (2008). The AGL62 MADS domain protein regulates cellularization during endosperm development in Arabidopsis. Plant Cell Online, 20, 63–67.

Khush, G. S. (2005). What will it take to Feed 5.0 Billion Rice consumers in 2030. Plant Molecular Biology, 59, 1–6. https://doi.org/10.1007/s11103-005-2159-5

Khush, G. S., & Jena, K. K. (2009) Current status and future prospects for research on blast resistance in rice (Oryza sativa L.). In G. L. Wang & B. Dakwet (Eds.), Advances in Genetics, Genomics and Control of Rice Blast Disease (pp. 1–10). Netherlands, Dordrecht: Springer.

Lé, S., Josse, J., Rennes, A., & Hussen, F. (2008). FacToMineR: An R package for multivariate analysis. Journal of Statistical Software 25.

Li, N., Xu, R., Duan, P., & Li, Y. (2018). Control of grain size under heat high night-time temperature stress. Plant Reproduct, 31, 237–251. https://doi.org/10.1007/s00497-018-0333-6

Lindroth, A. M., Cao, X., Jackson, J. P., Zilberman, D., McCallum, C. M., Henikoff, S., & Jacobsen, S. E. (2001). Requirement of CHROMOMETHYLASE3 for maintenance of CpXpG methylation. Science, 292, 2077–2080. https://doi.org/10.1126/science.1059745

Lisle, A. J., Martin, M., & Fitzgerald, M. A. (2000). Chalky and translucent rice grains differ in starch composition and structure and cooking properties. Cereal Chemistry Journal, 77, 627–632. https://doi.org/10.1094/CCHEM.2000.77.5.627

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods, 25, 402–408. https://doi.org/10.1006/meth.2001.1262

Lobell, D. B., Schlenker, W., & Costa-Roberts, J. (2011). Climate trends and global crop production since 1980. Science, 333, 616–620. https://doi.org/10.1126/science.1204531

Mizutani, M., Naganuma, T., Tsutsui, K. I., & Saitoh, Y. (2010). The syncytium-specific expression of the Osrysa;KRP3 CDK inhibitor: Implication of its involvement in the cell cycle control in the rice (Oryza sativa L.) syncytial endosperm. Journal of Experimental Botany, 61, 791–798. https://doi.org/10.1038/jxb.erp343

Muthayya, S., Sugimoto, J. D., Montgomery, S., & Maberly, G. F. (2014). An overview of global rice production, supply, trade, and consumption. Annals of the New York Academy of Sciences, 1324, 7–14.

Olsen, O.-A. (2004). Nuclear Endosperm Development in Cereals and Arabidopsis thaliana. Plant Cell Online, 16, S214–S227.

Paul, P., Dhatt, B. K., Miller, M., Folsom, J. F., Wang, Z., Krassovskaya, I., ... Walia, H. (2020). MAD578 and MAD579 are essential regulators of early seed development in rice. Plant Physiology. https://doi.org/10.1104/pp.19.00917

Porter, J. R., & Gwathm, M. (1999). Temperatures and the growth and development of wheat: A review. European Journal of Agronomy, 10, 23–36. https://doi.org/10.1016/S1161-0301(98)00047-1

Prasad, P. V. V., Bheemanahalli, R., & Jagadish, S. V. K. (2017). Field crops and the fear of heat stress—Opportunities, challenges and future directions. Field Crops Research, 200, 114–121. https://doi.org/10.1016/j.fcr.2016.09.024

Prasad, P. V. V., Boote, K. J., Allen, L. H., Sheehy, E. J., & Thomas, J. M. G. (2006). Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature.
stress. *Field Crops Research*, 95, 398–411. https://doi.org/10.1016/j.fcrr.2005.04.008

Prasad, P. V. V., Staggenborg, S. A., Ristic, Z., Ahuja, L. R., Reddy, V. R., Saseendran, S. A., & Yu, Q. (2008) Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants. In L. R. Ahuja, V. R. Reddy, S. A. Saseendran & Q. Yu (Eds.), *Response of Crops to Limited Water: Understanding and Modelling Water Stress Effects on Plant Growth Processes* (pp. 301–355). Adv. Agric. Syst. Model. Madison, WI: American Society of Agronomy. Crop Science Society of America, Soil Science Society of America.

Röth, S., Paul, P., & Frągkostefanakis, S. (2016). Plant heat stress response and thermotolerance. In P. Jaiwal, R. Singh & O. Dhankher (Eds.), *Genetic Manipulation in Plants for Mitigation of Climate Change* (pp. 15–41). New Delhi: Springer. https://doi.org/10.1007/978-81-322-2662-8_2

Sabelli, P. A., & Larkins, B. A. (2009). The development of endosperm in grasses. *Plant Physiology*, 149, 14–26. https://doi.org/10.1104/pp.108.129437

Sandhu, J., Zhu, F., Paul, P., Gao, T., Dhatt, B. K., Ge, Y., ... Walia, H. (2019). Pli-Plat: a high-resolution image-based 3D reconstruction method to estimate growth dynamics of rice inflorescence traits. *Plant Methods*, 15, 162. https://doi.org/10.1186/s13007-019-0545-2

Sakamoto, T., & Matsuoka, M. (2008). Identifying and exploiting grain yield genes in rice. *Current Opinion in Plant Biology*, 11, 209–214. https://doi.org/10.1016/j.pbi.2008.01.009

Sharma, R., Mohan Singh, R. K., Malik, G., Deveshwar, P., Tyagi, A. K., Kapoor, S., & Kapoor, M. (2009). Rice cytosine DNA methyltransferases – gene expression profiling during reproductive development and abiotic stress. *FEBS Journal*, 276, 6301–6311. https://doi.org/10.1111/j.1742-4658.2009.07338.x

Sreenivasulu, N., Butardo, V. M., Misra, G., Cuevas, R. P., Anacleto, R., & Kishor, P. B. K. (2015). Designing climate-resilient rice with ideal grain quality suited for high-temperature stress. *Journal of Experimental Botany*, 66, 1737–1748. https://doi.org/10.1038/jxb.2015.44

Steffen, J. G., Kang, I.-H., Portereiko, M. F., Lloyd, A., & Drews, G. N. (2008). AGL61 interacts with AGL80 and is required for central cell development in Arabidopsis. *Plant Physiology*, 148, 259–268. https://doi.org/10.1104/pp.108.119404

Tanabata, T., Shibaya, T., Hori, K., Ebana, K., & Yano, M. (2012). SmartGrain: High-throughput phenotyping software for measuring seed shape through image analysis. *Plant Physiology*, 160, 1871–1880.

Van Nguyen, N., & Ferrero, A. (2006). Meeting the challenges of global rice production. *Paddy and Water Environment*, 4, 1–9. https://doi.org/10.1007/s10333-005-0031-5

Wang, D. R., Bunce, J. A., Tomecek, M. B., Gealy, D., McClung, A., McCouch, S. R., & Ziska, L. H. (2016). Evidence for divergence of response in Indica, Japonica, and wild rice to high CO2 × temperature interaction. *Global Change Biology*, 22, 2620–2632.

Wei, T., Simko, V., Levy, M., Xie, Y., Jin, Y., & Zemla, J. (2017). Visualization of a correlation matrix. *Statistica*, 56, 316–324.

Xing, Y., & Zhang, Q. (2010). Genetic and molecular bases of rice yield. *Annual Review of Plant Biology*, 61, 421–442. https://doi.org/10.1146/annurev-plant-042809-112209

Yang, J.-Y., Chung, M.-C., Tu, C.-Y., & Leu, W.-M. (2002). OSTF1: A HD-GL2 family homeobox gene is developmentally regulated during early embryogenesis in rice. *Plant and Cell Physiology*, 43, 628–638. https://doi.org/10.1093/pcp/pcf076

Zhao, C., Liu, B., Piao, S., Wang, X., Lobell, D. B., Huang, Y., ... Asseng, S. (2017). Temperature increase reduces global yields of major crops in four independent estimates. *PNAS*, 114, 9326–9331. https://doi.org/10.1073/pnas.1701762114

Zhao, K., Tung, C.-W., Eizenga, G. C., Wright, M. H., Ali, M. L., Price, A. H., ... McCouch, S. R. (2011). Genome-wide association mapping reveals a rich genetic architecture of complex traits in Oryza sativa. *Nature Communications*, 2(1). https://doi.org/10.1038/ncomms1467

Zinn, K. E., Tunc-Ozdemir, M., & Harper, J. F. (2010). Temperature stress and plant sexual reproduction: Uncovering the weakest links. *Journal of Experimental Botany*, 61, 1959–1968. https://doi.org/10.1093/jxb/erq053

Zuo, J., & Li, J. (2014). Molecular genetic dissection of quantitative trait loci regulating rice grain size. *Annual Review of Genetics*, 48, 99–118. https://doi.org/10.1146/annurev-genet-120213-092138

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

---

**How to cite this article:** Paul P, Dhatt BK, Sandhu J, et al. Divergent phenotypic response of rice accessions to transient heat stress during early seed development. *Plant Direct*. 2020;4:1–13. https://doi.org/10.1002/pld3.196