



## Effects of Heat Stress at Vegetative and Reproductive Stages on Spikelet Fertility



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**Abstract:** Seed-setting rate, yield components and grain quality traits of 169 accessions from an exotic rice germplasm were tested under high temperatures from 40 °C to 45 °C for 6 h during the daytime at the vegetative and reproductive stages, respectively. The results showed that heat stress significantly decreased the seed-setting rate of all the accessions, but the heat stress effects varied among accessions. Based on the decreases in seed-setting rate at high temperatures, N22 was the most tolerant, followed by AUS17, M9962, SONALEE and AUS16. Moreover, the reductions in seed-setting rate and yield under heat stress were more serious at the vegetative stage (45 d before heading) than at the booting stage (15 d before heading). In addition, heat stress also affected grain quality, especially by conferring chalkiness to most of the accessions, but SONALEE did not change much. The heat-tolerant accessions identified here and the phenotype protocols developed could be used in future genetic studies and breeding programmes focused on heat tolerance.

**Key words:** heat tolerance; high temperature; rice germplasm; spikelet fertility; seed-setting rate

In the past three decades, the earth's surface temperature has become warmer than it was during any preceding decade since 1850, and the worst predicted scenario is that the global mean surface temperature may rise by 4.8 °C by the end of this century relative to the 1986–2005 period (Stocker et al, 2014). Most rice is grown in regions where current temperatures are already close to optimum for rice production. Therefore, any further increases in mean temperatures or short episodes of high temperatures during sensitive stages may be supra-optimal and reduce grain yield. Rice yields have been estimated to be reduced by 41% by the end of the 21st century (Ceccarelli et al, 2010; Shah et al, 2011). Heat stress affects all aspects of plant processes such as germination, growth, development, reproduction and yield (Mittler

and Blumwald, 2010). Some common effects of heat stress (38 °C / 30 °C) have been summarized for chili pepper (*Capsicum annuum*) with respect to its reduced fruit width and fruit weight, and the increased proportion of abnormal seeds per fruit (Cao et al, 2009). Wheat (*Triticum aestivum*) shows a shortened duration of grain filling and maturity and decreases in grain weight and yield under heat stress (37 °C / 28 °C) (Rahman et al, 2009). Sorghum (*Hordeum vulgare*) displays decreased chlorophyll content and ultimately a decreased grain yield at 40 °C / 30 °C (Mohammed and Tarpley, 2010). Maize (*Zea mays*) displays reduced ear expansion, particularly the suppression of cob extensibility by impairing the hemicellulose and cellulose synthesis through reduction in the photosynthate supply under heat stress of 35 °C / 27 °C

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(Yin et al, 2010).

In rice, heat stress at the flowering and grain-filling stages seriously affects the spikelet fertility and grain quality. Rice is the most susceptible to heat stress at the flowering stage, and previous chamber experiments show that temperatures of 32 °C to 36 °C cause high spikelet sterility (Satake and Yoshida, 1978). Heat-induced spikelet sterility at flowering is associated with reduction in the grain yield. High temperatures above 35 °C at the flowering stage inhibit anther dehiscence and thus result in lower pollen shed on the stigma, resulting in incomplete fertilization (Jagadish et al, 2010). Even if a sufficient number of pollen grains are shed on the stigma, in some cases, the pollen germination and pollen tube growth are poor under heat stress (Satake and Yoshida, 1978). Thus, aberrant anther dehiscence is considered as the primary cause of disturbed pollen development and shedding as the secondary cause for heat-induced spikelet sterility at the flowering stage. Exposing flowering spikelet to heat stress for 1 h is sufficient to induce sterility (Jagadish et al, 2007), whereas heat stress for 1 h after flowering does not lead to spikelet sterility (Ishimaru et al, 2010), possibly because the fertilization is completed. Genotypes with better anther dehiscence and flower opening at a cooler time of the day (early-morning flowering) are proposed to be desirable for heat tolerance and heat escape, respectively (Satake and Yoshida, 1978). High temperature stress during the rice ripening period causes damaged (chalky) grains, which have starch granules with a loosely packed round shape (Mitsui et al, 2013), which is one of the major issues that decreases the appearance quality of rice grains (Yoshida and Hara, 1977).

To date, screening for heat tolerance has been conducted in environmentally controlled chambers and open fields in heat-vulnerable regions. In these chamber experiments, heat tolerance at flowering is often tested at 37.5 °C to 38.0 °C (relative humidity of 60%–70%) and found to have great contrast in spikelet fertility between susceptible and tolerant genotypes (Matsui and Omasa, 2002; Kobayashi et al, 2011). N22, an Indian *aus*-type landrace, is identified as one of the most heat-tolerant genotypes in both chamber and open field experiments (Jagadish et al, 2010; Ye et al, 2012; Poli et al, 2013; Manigbas et al, 2014), while the varieties L-204, M-202, Labelle, Italica Livorna, WAB-12, CG-14 and CG-17 are highly susceptible, and M-103, S-102, Koshihikari, IR8 and IR72 are moderately susceptible to high temperatures (Prasad et al, 2006). These varieties can

be used as check varieties in heat tolerance tests (Shi et al, 2015). In *japonica*, Akita-komachi and Nipponbare are classified as considerably heat-tolerant genotypes (Maruyama et al, 2013). In *indica*, IR24 and IR36 (Maruyama et al, 2013), Ciherang, ADT36, BG90-2 (Shi et al, 2015), Dular and Todorokiwase are known as heat-tolerant genotypes at the booting stage, while Milyang 23 and IR2006-P12-12-2-2 are tolerant at the flowering stage. It is notable that Giza 178, an Egyptian variety developed from a *japonica-indica* cross, has considerable heat tolerance at the booting and flowering stages (Tenorio et al, 2013). These heat-tolerant accessions are considered to be useful genotypes for the breeding programme to improve heat resistance in relation to the spikelet sterility. Since the global mean surface temperature may increase in the future, the aim of this study was to identify new genetic donors for heat tolerance from the national rice germplasm, with 169 accessions (from 24 countries) for extreme temperatures (40 °C to 45 °C) at the vegetative and reproductive stages.

## MATERIALS AND METHODS

### Rice materials and growth conditions

The experiments were conducted from 2014 to 2015 at the Rice Science Center at Kasetsart University, Nakhon Pathom (14°01'16" N, 99°58'54" E), Thailand. A total of 169 accessions were used, including 161 international varieties derived from the national rice germplasm gene bank in Thailand, 6 from the Rice Department of Thailand breeding programmes and 2 from the Rice Science Center, Kasetsart University, Thailand (Supplemental Table 1).

All the accessions were seeded in the field nursery. After 30 d, the rice seedlings were transferred into plastic pots (30 cm in height and 25 cm in diameter with 8 kg sieved sandy loam soil) with one plant per pot. Soil containing 5.57% organic matter and total N of 0.332%, available P of 111.56 mg/kg, exchangeable K of 558 mg/kg, exchangeable Ca of 2 882.3 mg/kg, exchangeable Mg of 118 mg/kg and pH of 7.22 was dispensed into each pot before transplanting. The other 0.5 g and 0.6 g urea were applied to each pot at the mid-tillering (45 d) and panicle initiation (65 d) stages. Other managements followed the conventional high-yielding cultivation approach.

### Heat tolerance screening

Preliminary screening to identify heat tolerance started

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