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# Variation in spikelet fertility and grain quality under heat stress during reproductive stage in Thai non-photosensitive rice (*Oryza sativa* L.) cultivars

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**Abstract** The averaged daytime temperature is 40 ℃ during reproductive stage of rice production in dry season, Thailand leading to spikelet sterility and grain yield reduction. The total of 27 non-photosensitive rice cultivars together with a heat tolerant cultivar (M9962) and a susceptible cultivar (Sinlek) were investigated under high temperature (40-45 ℃) during 6 hr daytime for heat tolerance during reproductive stage in dry season in the year of 2017 and 2018. The selected top four cultivars and other 6 lines/cultivars were validated again under high temperature in dry season in the year of 2019. The results of screening and yield trials in 2017-2018 revealed that most of Thai cultivars were ranged from susceptible to high temperatures during the reproductive stage. The top four cultivars showing the greatest heat stress seeds in 2017 and 2018 were SPB2, RD41, Bahng Taen (BT) and SPB60 cultivars. In 2019, these four cultivars showed high performance of seed production under high temperature, and the genetic relationship of SPB2, RD41 and BT were closely related to M9962. However, BT showed good grain quality in heat stress, especially in the chalky trait. In conclusion, the four cultivars can be promoted to grow in dry season as moderately heat tolerant cultivars. Moreover, these cultivars would be useful for breeding programmes to improve heat tolerance in terms of yield and grain quality.

**Keywords:** High temperature, Thai non-photosensitive rice, Spikelet fertility, Grain quality

## Introduction

Over the past decades, rice has become one of the largest food crops worldwide because of the rapid increased in the population both in Asia and Africa, where rice is a staple food (Hope, 2007). Approximately 45% of the rice growing area in Southeast Asia is irrigated, with the largest areas found

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in Indonesia, Viet Nam, the Philippines and Thailand (Mutert and Fairhurst, 2002). Currently, Southeast Asia is expected to be seriously affected by the adverse impacts of climate change, and continued to be a critical factor affecting crop productivity in the regions (Change, 2007). In Indonesia, the Philippines, Thailand and Viet Nam, the annual mean temperatures are projected to rise upto 4.8 ℃ in 2100 (Weiss, 2009).

Most rice is grown in vulnerable regions where current temperatures are already closed to optimum for rice production. Therefore, any further increased in mean temperature or short episodes of high temperature during sensitive stage may be supra-optimal and reduced grain yield and quality (Ceccarelli *et al.,* 2010; Shah *et al.,* 2011). A decrease of 10% in rice yield has been found to be associated with every 1 ℃ increased in temperature, while Peng *et al.* (2004) reported that the yield of dry-season rice crop in the Philippines decreased by 15% for each 1 ℃ increased in the growing season mean temperature. Spikelet sterility induced by high temperature was observed in dry season crops in Asia, including Cambodia, Thailand,

India, Pakistan, Iran, Iraq and Saudi Arabia (Matsui *et al.,* 1997). Thailand has experienced an average daytime temperature up to 40 ℃, especially during April (Organization, 2015). Thus, the flowering stage of rice in the dry season occurs during the period with the highest temperature, which can adversely affect rice yield. The negative effect of high temperature can be shown in the seed setting rate, grain-filling duration, and quality of rice grains (Matsui *et al.,* 1997; Shi *et al.,* 2016).

In the case of heat stress at the flowering stage, the differences in heat tolerance among rice cultivars to alleviate heat stress have been investigated (Cheabu *et al.,* 2018; Cheabu *et al.,* 2019). High temperatures affected to spikelet sterility was occurred during booting and flowering stages (Yoshida and Hara, 1977). It begins to occur when the daily maximum temperature reaches approximately 34 to 36 ℃ (Matsui *et al.,* 2001; Weerakoon *et al.,* 2008). Spikelet fertility decreased under high temperatures because of anther dehiscence inhibition and a reduction in pollen activity (Matsui and Omasa, 2002; Zhang *et al.,* 2014; Coast *et al.,* 2016).

Heat stress is not only affected rice yield but also leaded to a significantly elevated proportion of chalky grains, which in turn alters starch and cooking quality (Cheng *et al.,* 2005; Cooper *et al.,* 2008; Li *et al.,* 2011). High temperature has been reported to reduce grain size (Fitzgerald *et al.,* 2009), which leading to a reduction in the weight of rice grains (Kobata and Uemuki, 2004). For Japonica cultivars of rice, a temperature above 26 °C during the first half of the ripening period adversely affected yield through a decrease in grain size (Tashiro and Wardlaw, 1991; Peng *et al.,* 2004). In addition, high temperature during grain filling affected endosperm quality (Duan *et al.,* 2012) and causing to damaged (chalky) grains, which starch granules are loosely packed round shape (Mitsui *et al.,* 2013), while those of normal translucent endosperm are tightly packed

(Nagato and Ebata, 1965). Thus, grain chalk is one of the major issues under heat stress (Yoshida and Hara, 1977) because chalky grains can reduce the commercial value of rice due to increased grain cracking during polishing (Fitzgerald *et al.*, 2009).

To date, heat tolerance varieties at reproductive stage have been reported (Prasad *et al.*, 2006; Jagadish *et al.*, 2007). The reported tolerant cultivars included six Indica varieties, IR36, IR24, and IR64 (Maruyama *et al.*, 2013), Ciherang, ADT36, and BG90-2 (Shi *et al.*, 2015); two Japonica cultivars, Akitakomachi and Koshihikari (Maruyama *et al.*, 2013); and an Aus cultivar, N22 (Ye *et al.*, 2012; Poli *et al.*, 2013; Manigbas *et al.*, 2014). Cheabu *et al*. (2018) confirmed that N22 was the most heat tolerant genotype and found other heat tolerant genotypes, including AUS17, M9962, SONALEE and AUS16, based on slight decreases in seed-setting rate. In addition, heat stress affected grain quality in most varieties, but the grain quality of SONALEE did not change much (Cheabu *et al.*, 2018). In Thai cultivars, Sukkeoa *et al.* (2017) identified San-pah-tawng 1, Neaw Phrae 1, R258 and Skon Nakhon 1 as sensitive cultivars, and identified RD10, Chai Nat1, and Suphan Buri 1 as tolerant cultivars that showed much less seasonal difference in yield.

The study of heat stress has been conducted in environmentally controlled chambers and open fields in heat-vulnerable regions. In these chamber experiments, heat tolerance at flowering in japonica type is often tested at 37.5 ℃ to 38.0 ℃ (relative humidity of 60%–70%) for a short period (2 h in 15 days) and had been found to have a great contrast in spikelet fertility between susceptible and tolerant genotypes (Matsui and Omasa, 2002; Kobayashi *et al.*, 2011). Thus, the present study aimed to investigate the responses of 33 Thai rice (*Oryza sativa* L.) cultivars to high temperatures, from the booting phase until the harvesting period, in greenhouses with controlled high temperatures between 40-45 ℃ for 6 h during the daytime and compared the responses with field conditions. In addition, this study also aimed to screen for Thai rice cultivars that were more tolerant in terms of seed set and grain quality under high temperatures during the reproductive stage.

## Materials and methods

### *Plant materials and growth conditions*

The experiments were conducted from 2017 to 2019 at the Rice Science Center of Kasetsart University, Nakhonpatum (14° 01′ 16.08″N, 99° 58′ 53.63″ E), Thailand. A total of 27 non-photosensitive cultivars were obtained from the Rice Department, Ministry of Agriculture and Cooperation, Thailand. Two lines from the Rice Science Center, Kasetsart

University, Thailand, were used as positive (M9962) and negative (Sinlex; SL) controls.

***Screening of spikelet fertility under heat stress***

The screenings to investigate seed set under high temperatures were done in dry season (DS) in 2017 (February-May) and in 2018 (February-May). The experiment was set up in a split-plot in Randomized Complete Block Design (RCBD) with 3 replications (five pots per replication). The main plot was the temperature condition including high temperature condition (HC) and natural temperature condition (NC), and the subplot was lines/cultivars. From the seedling (VE) until booting (R2) stages (approximately 60 days) according to Counce *et al.* (2000), the rice plants were grown under natural conditions. After that, each cultivar was moved to the controlled temperature greenhouses (area of 40 x 8 m), when the R2 was shown, which were maintained at high temperatures from 42-43 ℃ with the relative humidity at 50%, and a light intensity of 900-1000 µmoles/m2/s. The greenhouse temperature was gradually increased from 30 ℃ in the morning at 6:00 a.m. to 42-43 ℃ at 10:00 h. The door and windows were opened whenever the indoor temperature was above 43℃ during the daytime. The rice plants were treated with heat stress for six consecutive hours until 4:00 p.m. with gradual adjustment to the greenhouse's temperature down to 28-30 ℃ at night (18:00 to 6:00 p.m.). These conditions were applied to the rice plants until the harvest period. The natural treatments (controls) were planted under field conditions with the same experimental design. The air temperature, relative humidity and light intensity were recorded every 10 min using data loggers (WatchDog 1000 Series Micro Stations, Spectrum Technology, Inc.) at 3 positions in the greenhouse, and the carbon dioxide concentration in the greenhouse, which was approximately 390 µmol-1, was also monitored with a HUATO 653 Series detector. The weather data in the field were measured by a data logger (WatchDog 2000 Series Micro Stations, Spectrum Technology, Inc.)

***Validation of heat tolerant and sensitive cultivars***

Four of the heat tolerant and four of the sensitive cultivars were treated at high temperatures (40-45℃) under the same conditions as in previous work, with M9962 and SL as the controls. The experiment was started in DS 2019 (February-May) and set up in a split-plot in RCBD with 3 replications (5 pots/rep). The natural treatments (controls) were planted in pots under field conditions. Spikelet fertility, grain weight, grain yield and grain quality were investigated.

### *Data collection*

**Spikelet fertility and grain quality**

Three of panicles from main culm at the flowering stage (R4) were collected from each replication, and were tagged in each selected cultivar. These tagged panicles were harvested at the harvesting stage (R9). The spikelet fertility was estimated as the ratio of the number of filled grains to the total number of reproductive sites (florets) and expressed as a percentage (IRRI, 2013). In addition, the grain yield and 100-grain weight were also recorded, and grain quality including grain size, chalkiness degree, amylose content, gel consistency and grain elongation (IRRI, 2013).

**Cross section to investigate chalky grains by scanning electron microscopy (SEM)**

The cultivar with the highest chalk degree in HC was used to investigate starch granule formation in milled grain. Milled rice grains that had chalky grain degrees from 0-5 were cut in cross section and placed onto the sample stub. Finally, the sample was investigated for starch granule formation under an SEM (Hitachi SU8020).

**Phylogenetic analysis based on whole-genome sequencing**

DNA was isolated according to the DNeasy Plant Mini Kit (QIAGEN) protocol. DNA was sequenced on the Illumina Hiseq X by NovogeneAIT, Singapore. Finally, WGS from twenty-nine lines/cultivars was used to analyse the phylogenetic tree by using the MEGA X program.

### *Statistical analysis*

All the data were analysed using the R program for statistical analysis to test the significance of the results for the high-temperature treatments. The means were separated using Tukey’s Least Significant Difference (LSD) test at an alpha level of 0.05. The standard errors of the means were also calculated, and they are presented in the graphs as error bars.

## Results

### *Climatic data in the experiment*

From April to May (booting to harvesting stage) 2017, 2018 and 2019 (DS), the mean temperatures in NC (May-April) for day (10:00–16:00) and night (18:00-06:00) were 36.20/28.23, 36.16/28.67 and 35.86/27.76 ℃, respectively. The day/night temperatures in HC (May-April) were 43.50/29.31, 42.07/29.41 and 43.08/28.12 ℃, respectively (Table 1). Additionally, the mean relative humidity in the day (10:00–16:00 h) /night

(18:00–06:00 h) from April to May 2017, 2018 and 2019 under NC were 38.40/68.92% RH, 45.36/82.08% RH and 43.32/70.45% RH, respectively, while the mean relative humidity day/night in HC was 61.20/89.51% RH, 49.22/82.41% RH and 51.76/81.57% RH, respectively (Table 1). Moreover, the mean daytime light intensity (06:00-18:00 h) under NC and HC from April to May 2017, 2018 and 2019 was 1589.13/897.43 µM/m^2s, 1557.89/849.26 µM/m^2s and 1538.70/997.34 µM/m^2s, respectively (Table 1).

**Table 1.** The temperature, relative humidity and light intensity under high temperature and natural conditions from booting stage to the ripening stage of rice in DS 2017, DS 2018 and DS 2019

**Temperature (°C)**

| Experiments | Duration | HC | | NC | |
|---|---|---|---|---|---|
| | | Day time (6 hr.) | Night time | Day time (6 hr.) | Night time |
| DS 2017 | April-May 2017 | 43.50±1.90 | 29.31±1.28 | 36.20±1.90 | 28.23±0.87 |
| DS 2018 | April-May 2018 | 42.07±1.15 | 29.41±1.10 | 36.16±1.16 | 28.67±0.98 |
| DS 2019 | April-May 2019 | 43.08±1.62 | 28.12±1.20 | 35.86±1.26 | 27.76±0.95 |
| Average | | 42.88±1.55 | 28.94±1.19 | 36.07±1.44 | 28.22±0.93 |

**Relative humidity (%)**

| Experiments | Duration | HC | | NC | |
|---|---|---|---|---|---|
| | | Day time (6 hr.) | Night time | Day time (6 hr.) | Night time |
| DS 2017 | April-May 2017 | 61.20±4.42 | 89.51±4.90 | 38.40±7.76 | 68.92±6.49 |
| DS 2018 | April-May 2018 | 49.22±4.47 | 82.41±4.85 | 45.36±5.81 | 82.08±7.01 |
| DS 2019 | April-May 2019 | 51.76±3.44 | 81.57±5.86 | 43.32±6.13 | 70.45±8.57 |
| Average | | 59.04±4.11 | 84.36±5.20 | 53.12±6.56 | 83.06±7.35 |

**Light intensity (µM/m^2s) at day time (12 hr.)**

| Experiments | Duration | HC | NC |
|---|---|---|---|
| DS 2017 | April-May 2017 | 897.43 | 1589.13 |
| DS 2018 | April-May 2018 | 849.26 | 1557.89 |
| DS 2019 | April-May 2019 | 997.34 | 1538.70 |
| Average | | 975.92 | 1558.24 |

***Spikelet fertility under high temperature***

During the DS in 2017 and 2018, the twenty-seven cultivars obtained from the Rice Department, Ministry of Agriculture and Cooperation, Thailand, were screened for spikelet fertility under high temperature using M9962 and SL as controls for tolerance and susceptibility to heat stress. The results of both 2017 and 2018 showed significant effects among the high-temperature cultivars, and an interaction between the temperature and cultivars on the spikelet fertility from the booting stage until the harvesting stage. Most Thai cultivars were susceptible to high temperatures during the reproductive stage. The spikelet fertility in HC ranged from 0% to 38% in 2017 and 0% to 53% in 2018. In the parallel trial on 27 Thai rice cultivars grown under NC for the same period (control treatment), the ranges of spikelet fertility percentages in 2017 and 2018 were from 14% to 70% and 24% to 80%, respectively (Figure 1a and 1b).

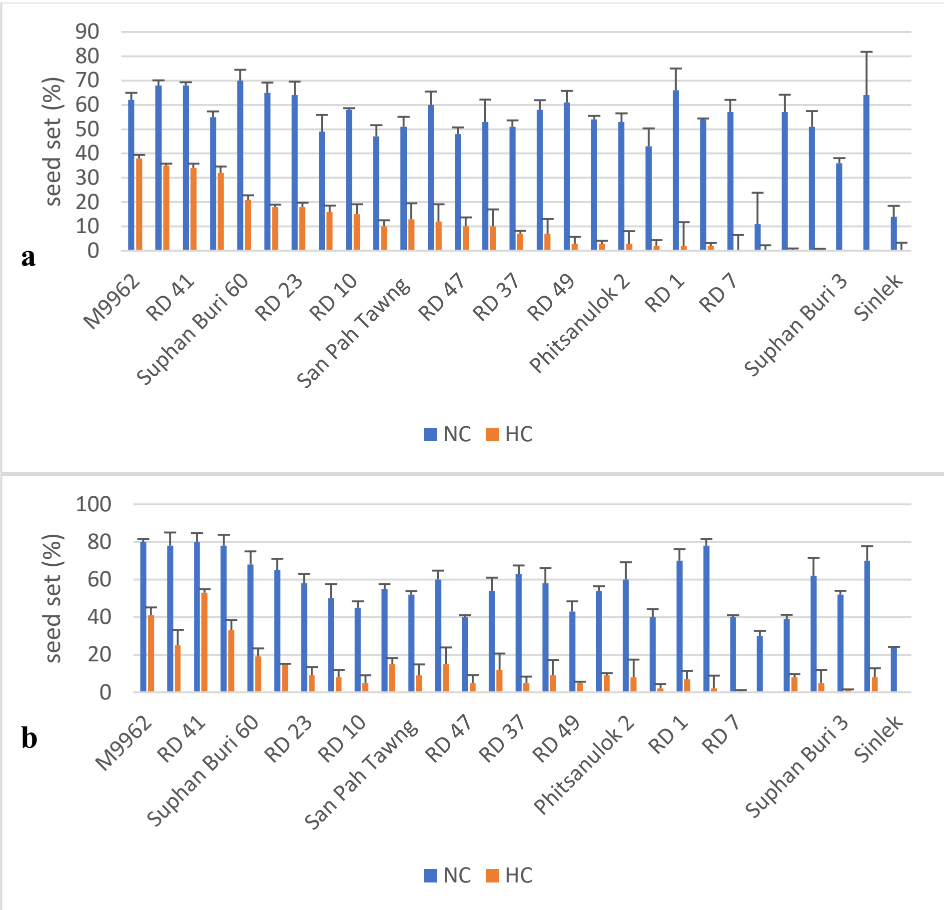


**Figure 1**. Comparison of seed set among twenty-seven Thai rice cultivars plus M9932 and SL (control) under high temperature conditions (HC) and natural conditions (NC) in dry season 2017 (a) and dry season 2018 (b)

The top four cultivars showing the greatest seed set after being treated with high temperatures in both DS 2017 and 2018 were the same cultivars, but rankings were slightly changed. In 2017, the top four cultivars were Suphan Buri 2 (SPB2) (35%), RD41 (34%), Bahng Taen (BT) (32%) and Suphan Buri 60 (SPB60) (21%). However, M9962 had the highest seed set (38%). In 2018, the top four cultivars were RD41 (53%), BT (33%), SPB2 (25%), and SPB60 (19%), while M9962 showed a seed set of 41% (Fig. 1a and 1b). Therefore, the results over two years could confirm that four Thai rice cultivars,  and M9962 showed stable performance of seed set in response to high temperatures in the reproductive stage, although the percentage of seed set was less than 60%.

The rankings of the seed set of Thai rice cultivars from the bottom in both DS 2017 and 2018 were nearly the same. Sixteen Thai rice cultivars produced seed sets of lower than 10% at high temperatures in DS 2017. Moreover, six cultivars did not produce seeds (0%), including RD7, RD33, Khao Jow Hawm Khlong Luang1 (KJHKL1), Pathum Thani 1 (PTT1), Suphan Buri 3 (SPB3) and Suphan Buri 90 (SPB90). Furthermore, in DS 2018, twenty-two cultivars showed seed set of less than 10% at high temperatures, and only RD33 did not produce seeds. However, the negative control (SL) did not produce seeds in either DS 2017 or 2018 (Figure 1a and 1b). This could confirm that RD33 and SL were very sensitive to high temperature during the reproductive stage.

Only the grain weight of the top four cultivars with positive control M9962 was considered in DS 2017 and 2018. The results showed that the grain weight of the top four cultivars was significantly decreased under HC, especially in SPB2 and SPB60, while the grain weight of M9962 was not significantly decreased. In addition, BT showed the highest grain weight both in NC and HC (Figure 2).

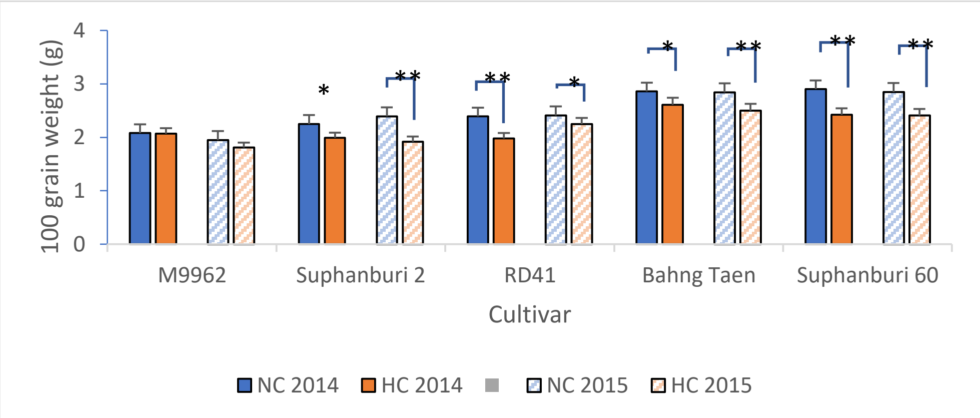


**Figure 2**. Comparison of 100 grain weights among the top four high seed set cultivars plus M9962 (control) under high temperature conditions (HC) and natural conditions (NC) in dry season 2017 and dry season 2018

***Validation of spikelet fertility and grain quality in DS 2019***

Based on the performance of the cultivars in the NC and HC, the four cultivars (BT, RD41, SPB60 and SPB2) with outstanding heat tolerance in DS 2017 and DS 2018 were selected to validate the performance of seed set and grain quality in DS 2019 with four other cultivars. There were famously grown in the dry season in central Thailand, but susceptible to high temperatures including PTT1, Phitsanulok 2 (PSL2), RD31 and SPB1. M9962 and SL were used as positive and negative controls, respectively.

The top four cultivars from DS 2017 and DS 2018 still showed high performance in producing seed set under HC in DS 2019. However, the seed set in HC was not significant among the top four cultivars (54.21-44.45%), but these cultivars produced seed set higher than RD31, SPB1, PTT 1 and PSL2 (28.73-10.67%) in HC. In addition, M9962 showed the highest seed set (71.96%), and SL showed the lowest seed set (3.77%) (Figure 3a). Moreover, the seed set of four cultivars under HC in DS 2019 showed higher performance than in DS 2017 and DS 2018. The grain weight of all cultivars was significantly decreased under high temperature, except for BT, SPB2 and SPB1, while the grain weight of M9962 and SB60 decreased more than the grain weight of other cultivars. In addition, BT showed the highest grain weight both in NC and HC (Figure 3b) in both DS 2017 and DS 2018. Considering the grain yield per plant, all cultivars showed dramatically decreased levels, more than 50%, under HC. However, the top four cultivars showed higher grain yield under HC than other cultivars. In addition, M9962 showed a slight decreased in grain yield under HC (Figure 3c).

The grain qualities of the top four cultivars in DS 2019 were observed both physically and chemically. The grain length in HC in paddy rice was not significantly different between the two conditions, but grain width decreased significantly in HC, except for in M9962 and PTT1 (Table 2). On the other hand, the changed in grain length of milled rice of BT, SPB2, RD41 and SPB1 under NC and HC was not significant, while the grain width of milled rice under HC decreased significantly in all cultivars except PTT1(Table 2).

HC also induced the changed in appearance quality as shown in Table 3. The change in chalky degree under HC at the reproductive stage led to a poor appearance quality in all cultivars except BT, RD41 and PSL2. SPB2 increased in chalky degree by 50.28%, whereas M9962 was slightly increased in chalky degree (10.07%). Thus, the starch granules of SPB2 were investigated under SEM (Figure 4). The results revealed different percentages of chalkiness in milled grains obtained from SPB2 and presented differences in the microstructure between them. SEM images showed that a loosely packed area with round and large compound starch granules was clearly observed in milled rice with a high chalkiness degree (50%, Figure 4b), while tightly packed, polyhedral and small single starch granules were observed in milled rice with lower chalkiness (10%, Figure

4a). In addition, the central part of the rice grains (red square) showed a looser structure than the outer part (Yellow Square).

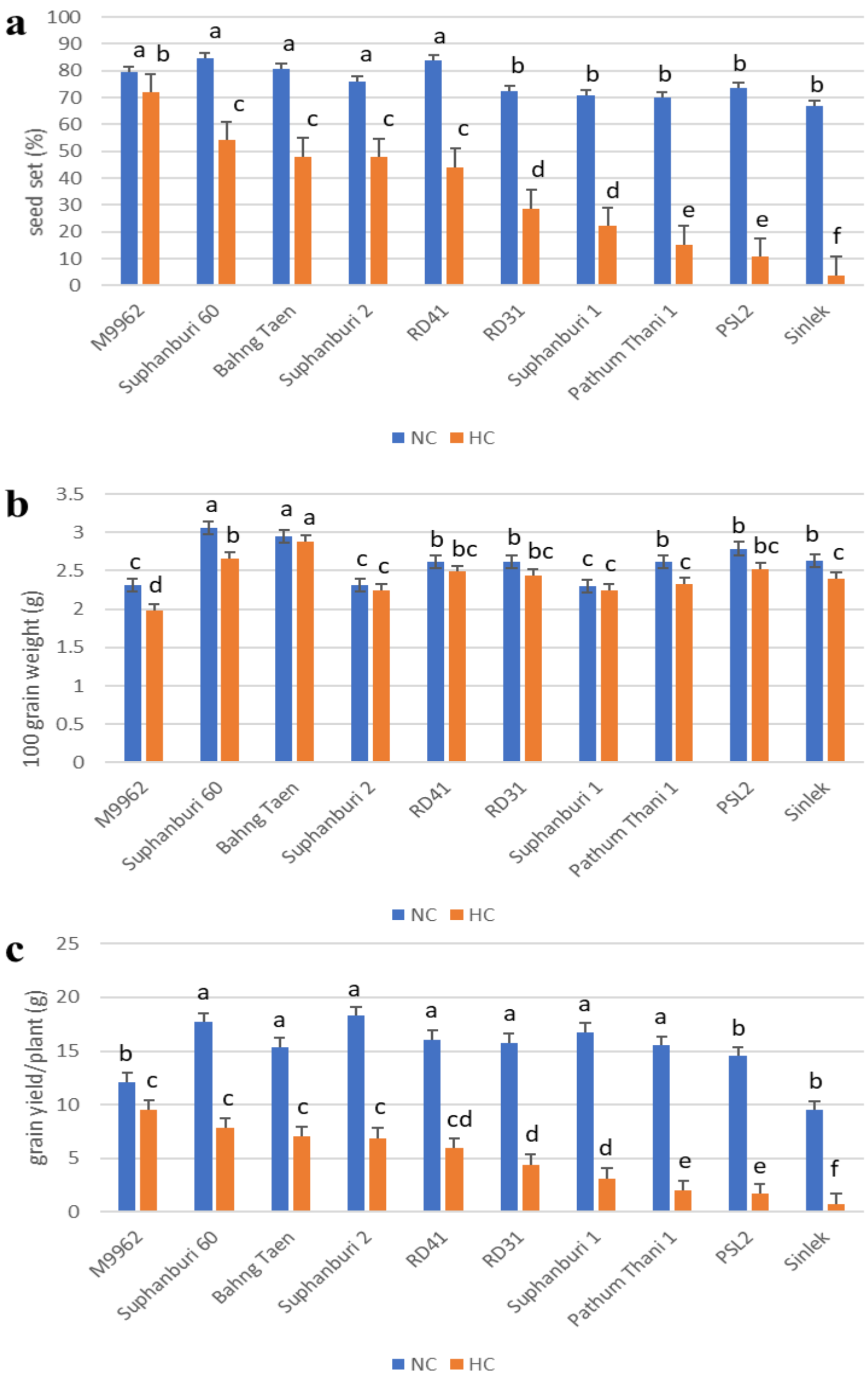


**Figure 3.** Effects of high temperature at the reproductive stage (R2-R9) on seed set (a), 100 grain weight (b) and grain yield per plant (c) of ten cultivars compared with natural conditions in DS 2019

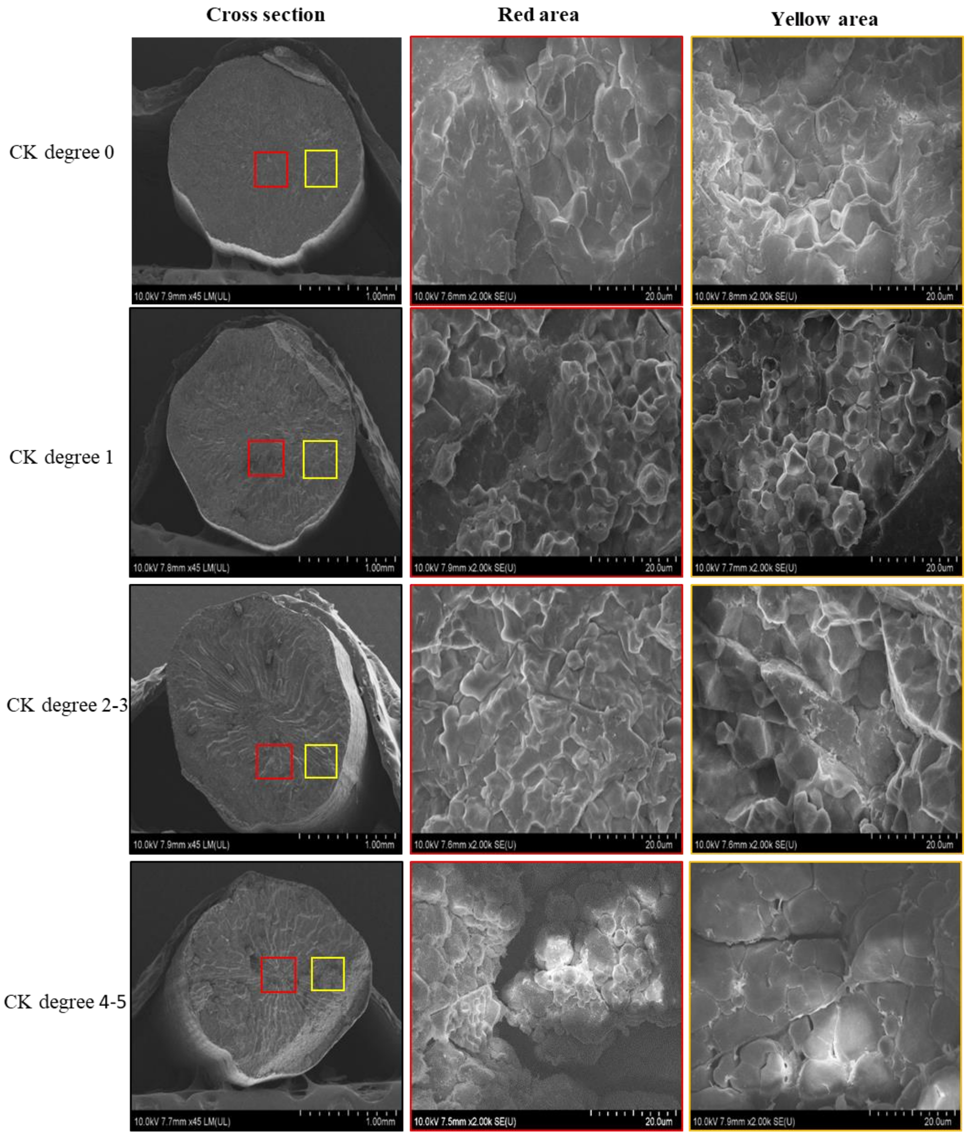


**Figure 4.** Investigation by SEM of the starch granule formation under high temperature that caused chalky grain in different degrees in SPB2

In addition, HC affected the amylose content of all cultivars, with the exception of RD31. However, each cultivar was different in the change in amylose content. The amylose content in some cultivars increased under HC, including M9962, SPB1, and PSL2, while the other cultivars decreased in amylose content. The gel consistency in the top four cultivars significantly decreased in BT and RD41, while SPB60 and SPB2 were not significantly different between NC and HC. In the case of grain elongation, all cultivars did not significantly differ between NC and HC (Table 3).

*Phylogenetic relationships among 27 Thai rice cultivars*

According to the results obtained for the WGS of twenty-seven cultivars, plus M9962 and SL, a phylogenetic tree showing the relationships among the twenty-nine lines/cultivars was constructed (Figure 5). The phylogenetic tree in this experiment was divided into three groups. Group I contained thirteen cultivars that had moderate tolerance and tolerance to heat stress, including BT, SPB2, RD41 and M9962. In addition, group I was subdivided into two subgroups, which indicated that BT and RD41 had close relationships and that these cultivars had SPB60 (moderate tolerance in group II) as a co-parent in the pedigree (Table supp. 1), while SPB2 and M9962 were classified in another subgroup. Group II contained twelve cultivars and was divided into two subgroups that contained one moderate tolerance (SPB60) and two susceptible cultivars, including SL and RD3. Interestingly, SL and RD33 showed close relationships in their genetic background. However, SPB60 was not classified in the same subgroups as SL and RD33. In group III, the genetic relationships of four cultivars, RD1, RD7, RD11 and RD37, were separated from the other cultivars.

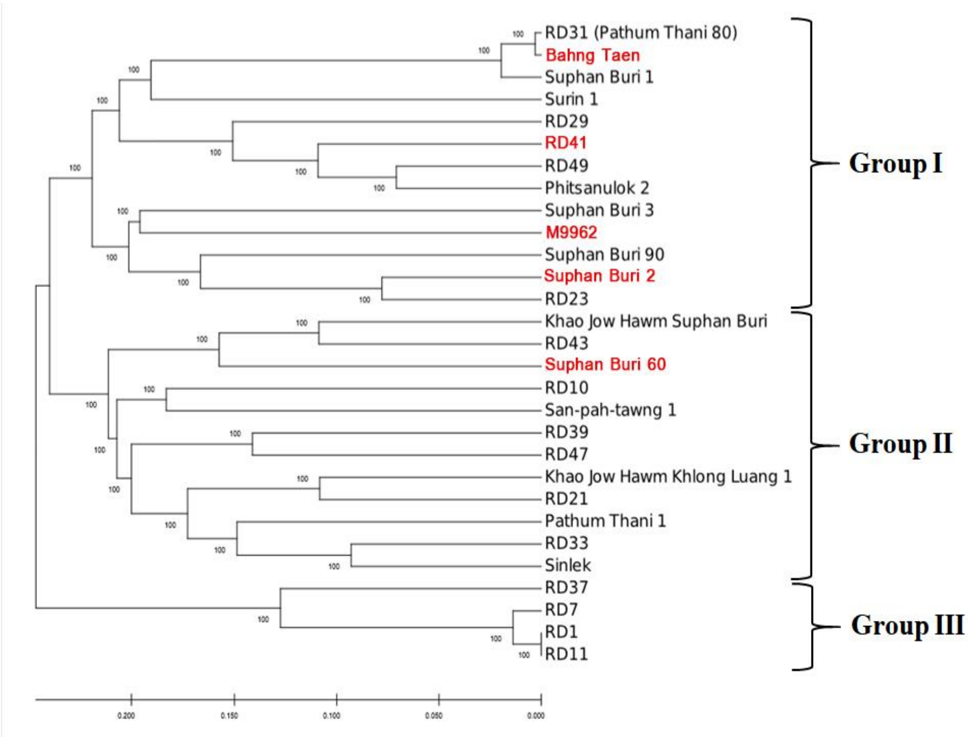


**Figure 5.** A phylogenetic tree of 29 lines/cultivars based on whole genome sequencing. The phylogenetic tree was classified into 3 classes, and the moderate tolerance cultivars (red colour) were in group I except Suphan Buri 60

**Table 2.** The grain length and grain width of ten cultivars compared under HC and NC in DS 2019

| Cultivars | Classification of heat tolerance | Grain length (mm) | | | | | | Grain width (mm) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Paddy rice | | | Milled rice | | | Paddy rice | | | Milled rice | | |
| | | NC | HC | % changed | NC | HC | % changed | NC | HC | % changed | NC | HC | % changed |
| M9962 | T | 9.18 | 8.98 | -2.18 | 6.31 | 6.09 | -3.49 | 2.26 | 2.24 | -0.88 | 1.90 | 1.87 | -1.58 |
| Suphan Buri 60 | MT | 10.05 | 9.72 | -3.28 | 6.79 | 6.52 | -3.98 | 2.52 | 2.41 | -4.36 | 2.12 | 2.08 | -1.88 |
| Bahng Taen | MT | 10.18 | 10.22 | +0.39 | 6.88 | 6.89 | +0.14 | 2.42 | 2.33 | -3.72 | 2.05 | 1.98 | -3.41 |
| Suphan Buri 2 | MT | 9.17 | 9.28 | +1.19 | 6.48 | 6.50 | +0.30 | 2.39 | 2.34 | -2.09 | 2.04 | 1.87 | -8.33 |
| RD41 | MT | 9.79 | 10.05 | +1.69 | 7.01 | 7.05 | +0.57 | 2.39 | 2.30 | -3.77 | 2.02 | 1.93 | -4.46 |
| RD31 | S | 9.73 | 9.61 | -1.23 | 6.68 | 6.49 | -2.84 | 2.51 | 2.39 | -4.78 | 2.15 | 2.02 | -6.05 |
| Suphan Buri 1 | S | 9.46 | 9.62 | +1.69 | 6.42 | 6.39 | -0.47 | 2.58 | 2.50 | -3.10 | 2.20 | 2.15 | -2.27 |
| Pathum Thani 1 | S | 10.16 | 10.14 | -0.20 | 6.90 | 6.38 | -7.54 | 2.42 | 2.43 | - | 1.91 | 1.91 | - |
| Phitsanulok 2 | S | 10.02 | 9.92 | -1.00 | 6.98 | 6.96 | -0.29 | 2.47 | 2.31 | -6.47 | 2.03 | 1.92 | -5.42 |
| Sinlex | S | 10.20 | 10.38 | +1.76 | 6.72 | 6.63 | -1.34 | 2.48 | 2.37 | -4.44 | 2.04 | 1.93 | -5.39 |
| Mean | | 9.79 | 9.8 | | 6.72 | 6.61 | | 2.45 | 2.37 | | 2.05 | 1.96 | |
| C.V. (%) | | 1.59 | | | 1.86 | | | 1.94 | | | 2.24 | | |
| LSD .05 (T) | | 0.21ns | | | 0.06** | | | 0.02** | | | 0.02** | | |
| LSD .05 (G) | | 0.18** | | | 0.14** | | | 0.05** | | | 0.05** | | |
| LSD .05 (GxT) | | 0.26** | | | 0.20** | | | 0.08** | | | 0.07** | | |

**Table 3.** The chalky degree, amylose content, gel consistency and grain elongation of ten cultivars compared under HC and NC in DS 2019

| Cultivars | Classification of heat tolerance | Chalky degree (1-5) | | | Amylose content (%) | | | Gel consistency (mm) | | | Grain elongation (mm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | NC | HC | % changed | NC | HC | % changed | NC | HC | % changed | NC | HC | % Changed |
| M9962 | T | 2.59 | 2.88 | +10.07 | 46.10 | 49.52 | +7.42 | 6.30 | 4.93 | -21.75 | 1.47 | 1.39 | -5.44 |
| Suphan Buri 60 | MT | 1.01 | 1.26 | +19.84 | 30.60 | 27.11 | -11.41 | 7.87 | 8.00 | +1.65 | 1.38 | 1.42 | +2.90 |
| Bahng Taen | MT | 0.98 | 1.00 | +2.00 | 48.59 | 46.02 | -5.29 | 5.17 | 3.40 | -34.24 | 1.35 | 1.4 | +3.70 |
| Suphan Buri 2 | MT | 1.77 | 3.56 | +50.28 | 25.78 | 25.06 | -2.79 | 8.70 | 8.43 | -3.10 | 1.37 | 1.35 | -1.46 |
| RD41 | MT | 0.93 | 0.98 | +5.10 | 46.75 | 44.7 | -4.39 | 5.83 | 3.50 | -39.97 | 1.37 | 1.35 | -1.45 |
| RD31 | S | 0.96 | 1.42 | +32.39 | 47.79 | 48.31 | +1.09 | 5.93 | 4.90 | -17.37 | 1.46 | 1.52 | +4.11 |
| Suphan Buri 1 | S | 1.52 | 2.23 | +31.84 | 45.70 | 48.43 | +5.97 | 4.73 | 5.07 | +7.19 | 1.46 | 1.43 | +2.05 |
| Pathum Thani 1 | S | 1.1 | 2.11 | +47.87 | 20.84 | 18.07 | -13.29 | 8.80 | 9.27 | +5.34 | 1.32 | 1.35 | +2.22 |
| Phitsanulok 2 | S | 0.37 | 0.37 | +0.00 | 49.40 | 50.60 | +2.43 | 7.83 | 7.30 | -6.77 | 1.51 | 1.57 | +3.97 |
| Sinlex | S | 1.33 | 2.24 | +40.63 | -* | -* | -* | -* | -* | -* | -* | -* | -* |
| Mean | | 1.34 | 1.89 | 29.10 | 39.09 | 38.35 | 1.89 | 6.909 | 6.19 | 10.41 | 1.41 | 1.42 | -0.71 |
| C.V. (%) | | 6.89 | | | 2.75 | | | 19.51 | | | 3.71 | | |
| LSD.05 (T) | | 0.05** | | | 0.55** | | | 0.66** | | | 0.09ns | | |
| LSD.05 (G) | | 0.13** | | | 1.22** | | | 1.47** | | | 0.06** | | |
| LSD.05 (GxT) | | 0.18** | | | 1.73** | | | 2.08ns | | | 0.09ns | | |

*/ not enough grains for testin

## Discussion

In this study, the high temperature and relative humidity in the greenhouse were uniform in the three years, with a high temperature of 42.88 ℃ for 6 h during the daytime, which was higher than that of NC by 6 ℃. The relative humidity of HC was higher than that of NC by 6% RH during the day and 1% RH at night. The light intensity in HC was lower than in NC due to the plastic sheeting on the greenhouse. However, the light intensity in the greenhouse (975.92 µM/m^2s) was higher than in Jagadish *et al*. (2007), who set the light intensity at 650 µM/m^2s in the controlled temperature chamber.

Rice is the most susceptible to heat stress at the flowering stage, and previous chamber experiments showed that high temperatures (32 ℃ to 36 ℃) cause a reduced seed-setting rate. Extremely high temperatures of 40 ℃ to 45 ℃ (10:00–16:00) in the greenhouse in this experiment significantly decreased the seed-setting rate and grain yield of all Thai rice cultivars. However, the seed set in all cultivars in DS 2017 was lower than in other seasons because the relative humidity in the daytime was higher (61.20% RH) than in other seasons (49.22 and 51.76% RH in 2018 and 2019, respectively). Weerakoon *et al.* (2008) and Shah *et al*. (2011) reported that increasing both air temperature and RH significantly increased spikelet sterility, while high temperature-induced sterility decreased significantly with decreasing RH.

In general, the popular Thai rice cultivars in Thailand have high yields and good grain quality. However, they are susceptible to heat stress (Manigbas *et al.*, 2014). Prasad *et al.* (2006) confirmed that the seed set rate is an important component of the yield that is sensitive to high temperatures. This effect was mainly ascribed to the inhibition of anther dehiscence (Matsui *et al*., 2001; Matsui and Omasa, 2002), pollen viability and pollen germination (Jagadish *et al*., 2007). Thus, the yield reduction in the heat-sensitive cultivars is significantly higher than in heat-tolerant cultivars, which primarily results from the poor fertilization and low seed-setting rate in the heat-sensitive cultivars (Yun-Ying *et al*., 2008). In this experiment, the seed set of all Thai cultivars under HC decreased dramatically due to the extremely high temperatures in the greenhouse. In addition, most Thai cultivars in NC did not produce a high seed set because the air temperature in the field was approximately 36 ℃. Thus, this temperature is critical for the reproductive stage in rice (Prasad *et al*., 2006). Moreover, the screening of Thai rice cultivars in DS 2017 and DS 2018 was practised on a large scale in different cultivars. Thus, the screening did not account for factors such as interactions between cropping and night temperature, genetic variation within genotype and the few samples in each replication. However, we could classify four Thai rice cultivars, including SPB60, BT, SPB2 and RD41 in DS2019, as moderate tolerance, able to produce seed set rates between 44.45-54.21%, while the other cultivars were susceptible. However, this result was different from the results of Sukkeoa *et al*. (2017), who identified SBP1 and RD10 as tolerant cultivars. However, our experiment was set up in a controlled greenhouse, while the study of Sukkeoa *et al*. (2017) was performed in summer field conditions. Moreover, M9962 produced the highest seed set under HC, which was consistently a heat tolerant genotype, as reported by Cheabu *et al*. (2019). However, the grain quality of this line, including the chalky degree, amylose content and gel consistency, was poor under HC. HC also occur during the period of seed development and decrease the grain size in rice (Yun-Ying *et al*., 2008; Chaturvedi *et al*., 2017). The early stage of seed development is highly sensitive to HC (Folsom *et al.,* 2014). In addition, the duration of the syncytial phase and the timing of cellularization are critical to HC because they are correlated with the extent of nuclear proliferation and may influence grain filling and grain weight (Mizutani *et al*., 2009). Previous reports indicated that HC from the R6 to R8 stages resulted in decreased fixed C availability from vegetable organs (leaves and culms) into grains and competition for the absorption of nutrients in grains due to rapid maturation (Nagato *et al*., 1966; Kobata and Uemuki, 2004). HC significantly increased the grain-filling rate but decreased the grain-filling duration more than proportionally, leading to a reduction in the final grain weight (Liu *et al.*, 2013).

Moreover, many reports have demonstrated that the environmental temperature at the ripening stage apparently changes the starch composition in rice grains (Lisle *et al*., 2000; Cheng *et al.,* 2005; Yamakawa *et al.*, 2007). Chalky grain caused by HC during the ripening stage is one of the major issues that decrease the appearance quality of rice grain (Yoshida and Hara, 1977). High temperature during rice grain filling rapidly accelerate endosperm cell development and cause the inconsistent formation of starch granules and protein bodies, resulting in loosely arranged voids and white opaque parts (Tang *et al.,* 2019). In the present experiment, HC significantly increased the percentage of chalkiness in all cultivars, especially in SPB2 (50.28% increase). Interestingly, BT, which was classified as moderate tolerance, did not change significantly in chalky degree between NC and HC. Thus, this cultivar may be used as a donor for grain quality in heat tolerance breeding programmes.The amylose content of rice grains is an important factor determining rice hardness and taste. Rice with low amylose content usually has a soft texture and good taste (Zhu *et al.,* 2001). However, eating quality is not necessarily related to amylose content for rice cultivars with similar AC (Patindol and Wang, 2002). Zhong *et al.* (2005) reported that the effects of HC on the amylose content of grains in Indica rice are variety-dependent; amylose content increases for varieties with higher amylose content, while varieties with lower amylose content have contrary performance. On the other hand, Asaoka *et al.* (1984) and Inouchi *et al.* (2000) reported that HC stress decreased the amylose content and the weight ratio of A + short B chains to long B chains of amylopectin in grain, while the opposite direction of changes in A- and B-fractions were observed at lower temperatures. In this study, we found that the amylose content in milled grains of the top four cultivars showed significantly increased and decreased amylose content under HC compared with NC. However, the amylose content in BT did not change significantly between HC and NC, similar to the chalky degree in BT.

In HC, gel consistency in some cultivars, including M9962, BT and RD41, decreased significantly, but some cultivars, including RD31, SPB1 and PSL2, were not changed. On the other hand, the cultivars with amylose content of less than 30% were not significantly different in gel consistency between NC and HC. Zhong *et al.* (2005) reported that HC reduced or maintained gel consistency values for cultivars with higher amylose content and increased gel consistency values for those with lower amylose content.

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