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Spikelets Fertility and Quality of Local Upland Rice (*Oryza sativa* L.) Seeds Due to High Temperature at Various Stages

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Abstract: Stress from high temperatures is one of the factors that restrict rice plant growth and development. Rice generative stage sensitivity to high temperatures is well established, but its vegetative stage sensitivity is more uncertain. To identify high-temperature tolerance traits in the vegetative and generative stages, four local upland rice genotypes were tested under high-temperature stress treatment (32-36°C) during the vegetative stage, generative stage and vegetative-generative stage and ambient temperature (27-31°C) during the vegetative-generative stage as a control. The research used a nested randomized block design (RAK) and was repeated in 3 repetitions. SPSS Ver. 20 program was used to analyze the data. The results showed that under stress from high temperature both thegenerative stage and the vegetative-generative stage significantly reduced the quantity of pollen germinated in all genotypes. The application of under stress from the high temperature in the generative stage showed a serious impact on panicle length, spikelets in each panicle, spikelet fertility in each panicle, weight of filled grain in each panicle, weight of 1000 grains and reduction in amylose compared to theunder stress from high temperature both in the vegetative and vegetative-generative stages. In contrast to previous research, the impact of high temperatures in the vegetative stage was able to significantly increase the length of the panicle, quantity of spikelets in each panicle, spikelet fertility in each panicle, weight of filled grain in each panicle and weight of 1000 grains. The Bangkok genotype has a quantity of pollen germinated of 61.21-44.64%, spikelet fertility in each panicle of 74.91-215.52 grains andweight of filled grain in each panicle of 1.43-4.88 g higher as well as a decrease in amylose and an increase in protein lower than the other three genotypes at high-temperature stress at various stage stages, the Bengkok genotype is a potential genetic source for increasing tolerance to under stress from high temperature in the vegetative and generative stages.

Keywords: High Temperatures, Spikelet Fertility, Stage, Tolerance

Introduction

One kind of abiotic environmental stress brought on by climate change is high temperatures, This causes the temperature of the surroundings to rise. By the end of the twenty-first century, the average surface temperature of Earth is expected to rise by 2-4°C, according to the (Allan, 2023). Temperature increases in Indonesia in 2015 ranged from 0.65-1.43°C (BMKG, 2015). When the air temperature exceeds 4°C above the optimum temperature, agricultural yield can decline by more than 20% (Tschirley, 2007; Shah *et al*., 2011).

All plant processes, including germination, growth, development, reproduction and yield, are impacted by heat stress (Mittler and Blumwald, 2010). temperatures exceeding 35°C in the vegetative phase will affect the growth of rice seedlings, causing severe loss of tillers, number of leaves per plant, decreasing leaf elongation, rooting and vigor of seedlings (Bahuguna *et al*., 2015; Hussain *et al*., 2019; Dewi *et al*., 2023). The results of research by Cheabu *et al*. (2018) showed the more significant effects of heat stress on the rate of seeddevelopment, quantity of grains in each panicle and grain yield during the vegetative stage up to harvest compared to the booting stage.

High temperatures during the blooming and grain-filling stages have a substantial impact on grain fertility and rice grain quality. Flowering is one of the times when plants are most susceptible to the negative effects of heat stress. An average temperature above 34°C during the flowering stage of rice will result in floral sterility and decreased yields (CAO *et al*., 2009; Tian *et al*., 2010). Excessive temperatures during anthesis prevent pollen grains from developing in the anther locule, which prevents the anther from dehiscing. Thus, this will affect the likelihood of imperfect pollination (Matsui *et al*., 2000). Even when there is a suitable amount of pollen grains deposited on the stigma, there are circumstances in which poor heat stress results in poor pollen germination and pollen tube expansion (Jagadish *et al*., 2010).

High temperature also directly affects seed development, including grain filling, dry matter production rate in seeds (Kobata and Uemuki, 2004), seed size and amylose content (Yamakawa *et al*., 2007; Yamakawa and Hakata, 2010). Chalky grains are the result of uneven filling and interruptions in storage biosynthesis caused by high-temperature stress during the ripening period. Chalky grains are more likely to break during seed processing, which increases the amount of broken grains and lowers the percentage of head rice (Sreenivasulu *et al*., 2015). Because high temperatures during seed filling limit the enzymatic processes of starch and protein synthesis, they have an adverse effect on the accumulation of various components, particularly starch and protein (Farooq *et al.*, 2017).

Rice plants that are tolerant of stress from high temperatures are able to perform physiological functions during the vegetative and reproductive phases (Lee, 2011). The diversity of rice germplasm collections is the main capital in developing upland rice varieties. The local upland rice used comes from the eastern region of Aceh province, Indonesia. This local upland rice is a source of germplasm that is rich in genetic potential and needs to continue to be explored to be developed. In plant breeding programs for under stress from high temperature resistance, local upland rice that is temperature-resistant can be a valuable genetic source. The level of resistance of rice to stress from high temperatures in the generative stage is well known, while information regarding the level of resistance of rice to stress from high temperatures in the vegetative stage is still less available. This study aims to identify genetic traits that confer tolerance to high temperatures from the germplasm of local upland rice at various stages.

Materials and Methods

Plant Components and Growth Management

The study was carried out in the Brawijaya University Faculty of Agriculture's experimental garden and greenhouse in Malang Regency, East Java Province, Indonesia between June and November 2021. The study was carried out using a nested randomized block design (RAK) consisting of temperature treatment and local upland rice genotype, where the genotype treatment was nested in temperature and the replicates were nested in genotype, the experiment was repeated in 3 replications. The local upland rice genotypes used are Bangkok (G1), Rias Halus (G2), Ramos Gunung Bayeun (G3) and Si Raden (G4). Four pots were used for each genotype in each temperature stress treatment.

The experiment began by carefully selecting healthy seeds for each genotype. These seeds were immersed in saline water and only those that sank were used for further experimentation. Germinated upland rice seeds were chosen based on their healthy growth and uniformity. After that, they were immediately planted in pots that included a 15 kg mixture of soil and manure in a 2:1 ratio. Two seedlings were planted in each pot and after one week, thinning was conducted to maintain one plant per pot. Initial fertilizer application was carried out during planting, consisting of 0.47 of urea, 0.75 of SP-36 and 0.75 g/pot of KCl. Subsequent fertilizer applications of 0.47 g/pot of Urea were provided at 15 Days After Planting (DAP), 35 DAP and at the flower primordial stage at approximately 55 DAP, other management activities following conventional cultivation approaches with high yields.

Temperature Stress Treatments

The temperature stress treatments consisted of 4 stages: (S0) ambient temperature during the vegetative-generative stages, where plants were placed outside the greenhouse and exposed to temperatures ranging from 27-31°C from planting to harvest; (S1) under stress from high temperature the vegetative stage, where plants were placed inside the greenhouse from planting until booting and exposed to temperatures ranging from 32-36°C, after which they were moved outside the greenhouse; (S2) under stress from high temperature the generative stage, where plants were placed inside the greenhouse from booting until harvest and exposed to temperatures ranging from 32-36°C and (S3) under stress from high temperature the vegetative-generative stages, where plants were placed inside the greenhouse and exposed to temperatures ranging from 32-36°C from planting to harvest.

Pollen Germination

Pollen was taken from the panicles that emerged (heading) after 3 days under temperature stress treatment conditions from 4 panicles for each genotype when the panicles bloomed (Das *et al*., 2014). Observations of germinating pollen were carried out using germination media consisting of 1 mL H_3BO_3, 3 mL $Ca(NO_3)_2.4H_2O$, 2 mL $MgSO_4.7H_2O$, 1 mL KNO_3 and 10 g sucrose. After collecting pollen samples, the rice flowers are immediately covered using tracing paper (clear paper) to avoid pollination of other rice.

After collection, it is stored for 1×24 h and then observed under a microscope at a magnification of 4×20. Viable pollen is characterized by the formation of pollen tubes (germinates) at least as long as the diameter of the pollen (Tyuy *et al*., 2020). The percentage of pollen germinatedgrains to total pollen grains is used to calculate the pollen germination rate.

Yield Components

To determine the grain yield we collected samples from four pots of plants, in each treatment once the plants reached maturity. The number of productive tillers, panicle length, spikelets in each panicle, spikelet fertility in each panicle, the weight of filled grain in each panicle, weight of 1000 grains and potential yield per hill are among the yield components that were noted.

Grain Quality

Protein Content (%)

Protein content was analyzed using the Kjeldhal method, where 2 g of the sample was put in a measuring cup, mixed with sulfuric acid and allowed to digest for 2-4 h. Then it was distilled, in the determinator for 5 min to accommodate the N gas in an Erlenmeyer containing 20 mL of 4% boric acid in alkaline conditions with 3 drops of indicator added.

Amylose Content (%)

Using the IRRI method, the material's amylose content was determined by adding 1 mL of 95% ethanol and 9 mL of 1 N NaOH to a test tube containing 100 mg of rice flour. Subsequently, it was brought to a boil in water for about 10 min, during which a gel developed. It was then allowed to cool for an hour before the entire gel was placed into a measuring flask. After adding water to the measuring flask until it reached the 100 mL threshold, the gel was dissolved by shaking it. After pipetting 5 mL of this solution it was put into a 100 mL measuring flask with 60 mL of water. After adding 1 mL of 1 N acetic acid and 2 mL of 2% I_2 and adding water to reach the 100 mL level, the mixture was shaken and allowed to sit for 20 min. After that, absorbance measurements at 620 nm wavelength were made with a UV-Vis spectrophotometer.

Data Analysis

The SPSS Ver.20 application was used to do an analysis of variance. Duncan's Multiple Range Test (DMRT) was used to examine genotype differences in significantly varied temperature stress treatments at the 0.05 level.

Results

Pollen Germination Under Temperature Stress at Various Stages

The analysis of variance showed that different genotypes have a very significant influence on pollen germination under temperature stress at various stages. The Bengkok and Ramos Gunung Bayeun genotypes did not significantly differ in pollen germination under ambient temperature conditions during the vegetative-generative stages andduring the vegetative stages under stress from high temperatures. Nonetheless, a reduction in the quantity of pollen germinated was brought about by stress from high temperatures during the generative stages and during the vegetative-generative stages. Among the three genotypes rias halus, ramos gunung bayeun and si raden there was a higher decrease in pollen germinated under stress from the high temperature in the vegetative-generative stage, with decreases of 54.73, 42.05 and 66.58%, while the Bengkok genotype showed a decrease of only 35.95% when compared to providing ambient temperature during the vegetative-generative stage (Fig. 1a).

Yield Components Under Temperature Stress at Various Stages

The analysis of variance showed that genotypes under different temperature stress stages had a very significant influence on the number of productive tillers, panicle length, spikelets in each panicle, spikelet fertility in each panicle, the weight of filled grain in each panicle, weight of 1000 grains and potential yield per hill. Bengkok, Rias Halus and Si Raden genotypes, when subjected to ambient temperature during the vegetative-generative stage, produced a quantity of spikelet fertility in each panicle that did not provide significant differences under stress from the high temperature in the vegetative-generative stage. Under stress from high temperature the vegetative stage increasing the quantity of spikelet fertility in each panicle by 75.84, 31.07 and 54.33% in the bent Bengkok, Rias Halus and Si Raden genotype, however giving under stress from high temperature the generative stage caused a reduktion the quantity of spikelet fertility in each panicle in the Bengkok genotype was 38.88%, Rias Halus was 49.96% and Si Raden was 55.17%. In the Ramos Gunung Bayeun genotype the quantity of spikelet fertilityin each panicle does not provide a significant difference between temperature stress at various stages (Fig. 1b). High-temperature stress during the generative stage causes low pollen production resulting in a decrease in pollen grains on the stigma which can cause imperfect fertilization (Fig. 2).

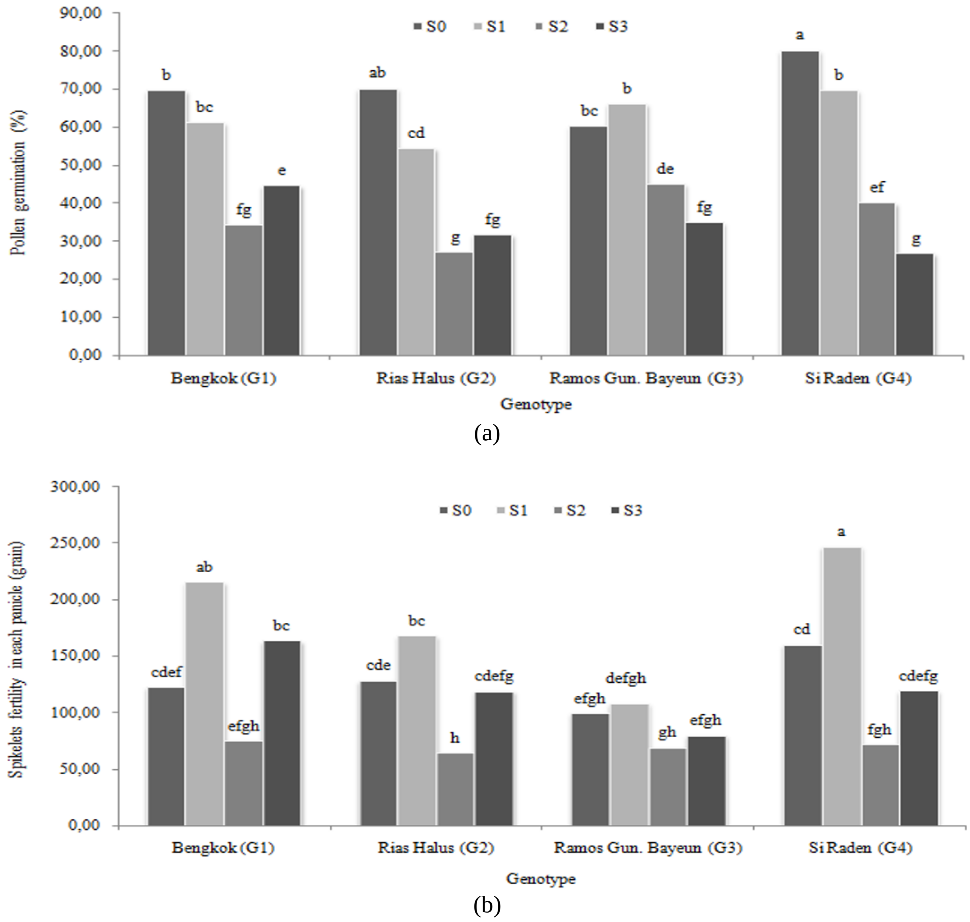


Fig. 1: Temperature stress treatment at various stages on four local upland rice genotypes on; (a) Pollen germination and (b) Spikelet fertility in each panicle. Not substantially different at the 0.05 level is indicated by the same letter

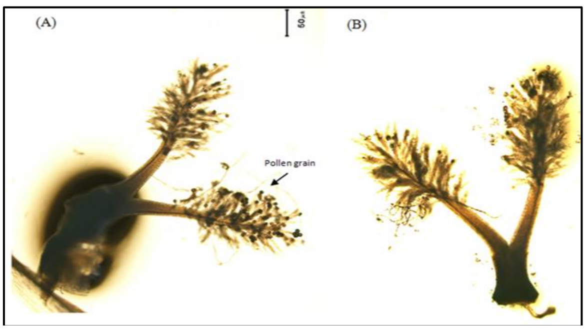


Fig. 2: Pollen on the stigma of the Bengkok genotype due to; (a) Ambient temperature during the vegetative-generative stage (S0) and (b) Under stress from high temperature during the generative stage (S2)

The number of productive tillers was dramatically decreased in the four genotypes when under stress from high temperature was applied in the early growth stage (Table 1). The highest quantity of productive tillers in the four real genotypes was obtained as a result of providing an early stage of growth with ambient temperature. The genotypes Bengkok, Rias Halus, Ramos Gunung Bayeun and Si Raden when given ambient temperature during the vegetative-generative stage and when given under stress from the high temperature the generative stage produced a higher quantity of productive tillers and did not provide significant differences. Given under stress from the high temperature the vegetative-generative stage caused a reduction in the number of productive tillers of the Bengkok genotype by 65.18, Rias Halus by 55.50, Ramos Gunung Bayeun by 41.12 and Si Raden by 63.22% when compared with giving ambient temperature during the vegetative-generative stage.

There was no significant difference in panicle length between various stages of temperature stress in the Rias Halus, Ramos Gunung Bayeun and Si Raden genotypes, however in the Bengkok genotype there was a significant increase in panicle length due to under stress from the high temperature the vegetative-generative stage of 10.08%, but giving under stress from the high temperature the generative stage caused a decrease of 8.26% when compared with giving ambient temperature during the vegetative-generative stage (Table 1 and Fig. 3).

Table 1: Average number of productive tillers and panicle length of four local upland rice genotypes under various stages of temperature stress treatment

Genotype	number of productive tillers (stems)				Panicle length (cm)			
	S0	S1	S2	S3	S0	S1	S2	S3
Bengkok (G1)	24.89^{bc}	9.67^{d}	24.94^{bc}	08.67^{d}	28.80^{b}	30.88^{ab}	26.42^{c}	31.70^{a}
Rias Halus (G2)	24.22^{bc}	10.56^{d}	26.00^{bc}	10.78^{d}	25.36^{cd}	25.21^{cde}	23.56^{def}	25.18^{cde}
Ramos Gun. Bayeun (G3)	45.67^{a}	28.11^{bc}	48.67^{a}	26.89^{bc}	23.94^{def}	24.79^{cde}	23.14^{ef}	24.11^{def}
Si Raden (G4)	29.00^{b}	11.56^{d}	23.00^{c}	10.67^{d}	24.48^{cdef}	24.45^{cdef}	22.45^{f}	23.70^{def}
Average	30.94^{a}	14.97^{b}	30.65^{a}	14.25^{b}	25.65^{a}	26.33^{a}	23.89^{b}	26.17^{a}
Temperature	**				**			
Genotype (Temperature)	**				**			

Note: According to the Duncan test at the 0.05 level, there is no significant difference between numbers that are followed by the same letter in the same row

Table 2: Average spikelets in each panicle and weight of filled grain in each panicle of four local upland rice genotypes under various stages of temperature stress treatment

Genotype	Spikelet in each panicle (grain)				Weight of filled grain in each panicle (g)			
	S0	S1	S2	S3	S0	S1	S2	S3
Bengkok (G1)	151.20^{ef}	282.74^{ab}	122.27^{f}	250.94^{bc}	2.74^{cde}	4.88^{a}	1.43^{fg}	3.96^{ab}
Rias Halus (G2)	153.07^{ef}	211.20^{cd}	109.88^{f}	197.76^{cde}	2.73^{cde}	3.66^{bc}	1.23^{g}	2.38^{def}
Ramos Gun. Bayeun (G3)	122.87^{f}	135.63^{f}	103.58^{f}	118.27^{f}	1.85^{efg}	2.03^{defg}	1.21^{g}	1.42^{fg}
Si Raden (G4)	189.64^{de}	306.10^{a}	107.70^{f}	206.40^{cd}	3.10^{bcd}	4.96^{a}	1.27^{g} 2.24^{defg}	
Average	154.20^{c}	233.92^{a}	110.86^{d}	193.34^{b}	2.60^{b}	3.88^{a}	1.28^{c}	2.50^{b}
Temperature	**				**			
Genotype (Temperature)	**				**			

Note: According to the Duncan test at the 0.05 level, there is no significant difference between numbers that are followed by the same letter in the same row

Fig. 3: Appearance of panicles of the Bengkok genotype (G1) due to temperature stress at various stages: (S0) Ambient temperature during the vegetative-generative stage, (S1) Under stress from the high temperature during the vegetative stage, (S2) Under stress from the high temperature the generative stage and (S3) Under stress from the high temperature the vegetative-generative stage

The number of spikelets in each panicle of the Bengkok and Rias Halus genotypes when applying ambient temperature during the vegetative-generative stage did not provide a significant difference with high-temperature stress during the generative stage ranged from 109.88-153.07 grains. When applied under stress from the high temperature during the vegetative stage there was an improvement in the number of spikelets in each panicle was 87.00% in Bengkok and 37.97% in Rias Halus, while under stress from the high temperature during the vegetative-generative stage, there was an increase of 65.96 in Bengkok and 29.19% in Rias Halus when compared by providing ambient temperature during the vegetative-generative stage. Applied ambient temperature during the vegetative-generative stage and under stress from the high temperature the vegetative-generative stage does not significantly differ in the number of spikelets in each panicle for the Si Raden genotype.

There was, however, an increase of 61.41% when applying under stress from high temperatures in the vegetative stage. In the Ramos Gunung Bayeun genotype, the number of spikelets in each panicle did not provide a significant difference between the various stages of temperature stress. The improvement in the number of spikelets in each panicle when applying stress from the high temperature during the vegetative stage was higher than when applying stress from the high temperature during the vegetative-generative stage (Table 2).

The reduction in the weight of filled grains in each panicle and the weight of 1000 grains in temperature stress treatment during the generative stage was relatively higher than in other stages of stress treatment. The weight of filled grain in each panicle of the Bengkok, Rias Halus and Si Raden genotypes when compared with the provision of ambient temperature during the vegetative-generative stage, there was an increase of 78.28, 33.98 and 60.14% respectively, in under stress from high temperature the vegetative stage. The application of stress from the high temperature in the vegetative-generative stage resulted in a decrease in the weight of filled grain by only 18.84, 34.91 and 54.85% respectively, but under stress from the high temperature, the generative stage caused a decrease of 70.75, 66.31 and 74.48% (Table 2). The Bengkok genotype showed the lowest reduction in filled grain weight and did not provide a significant difference between under stress from high temperature in the vegetative-generative stage and under stress from high temperature during the vegetative stage. In Ramos Gunung Bayeun genotype, the weight of the filled grain in each panicle did not provide a significant difference between temperature stress at various stages.

Likewise, the weight of 1000 grains in the four genotypes did not provide a real difference between ambient temperature in the vegetative-generative stage and under stress from high temperature in the vegetative stage. An application under stress from the high temperature the vegetative-generative stage of the Bengkok, Rias Halus, Ramos Gunung Bayeun and Si Raden genotypes experienced a decrease in the weight of 1000 grains by 5.55, 5.71, 3.57 and 8.47% respectively. However, under stress from the high temperature, the generative stage decreased to 15.24, 9.70, 5.32 and 12.68% respectively when compared with under stress from the high-temperature vegetative stage. The lowest 1000-grain weight reduction was obtained in the Ramos Gunung Bayeun genotype (Table 3).

The highest yield potential per hillin the 4 genotypes was obtained when ambient temperature was applied during the vegetative-generative stage, there was a significant decrease in yield potential at various other stages of temperature stress and the Si Raden genotype produced the highest yield potential of 94.42 g, while under stress from high temperature the vegetative-generative stage the highest yield potential was obtained in the Bengkok genotype at 39.74 g compared to other genotypes (Table 3). Yield potential is related to increased productive tillers under early growth stage ambient temperature conditions.

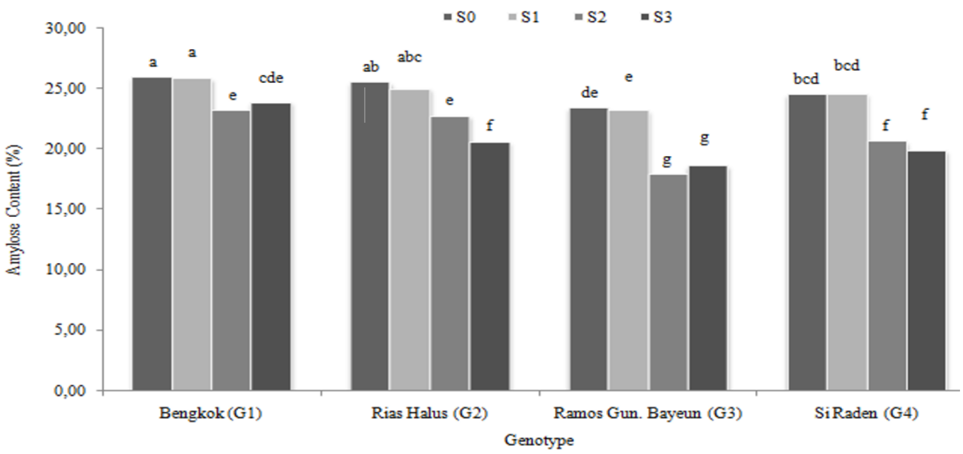
Grains Quality Under Temperature Stress at Various Stages

The analysis of variance showed that genotypes under different temperature stress stages had a significant effect on amylose content and protein content. Amylose and protein contents in the four genotypes due to ambient temperature in the vegetative-generative stage did not show a significant difference compared to under-stress from high temperature in the vegetative stage. Amylose content in the grains of Bengkok, Rias Halus, Ramos Gunung Bayeun and Si Raden genotypes due to under stress from high temperatures the generative stage and the vegetative-generative stage was lower, ranging from 7.9-10.48, 10.9-19.30, 20.40-23.49 and 15.84-18.90% respectively compared to ambient temperature during the vegetative-generative stage (Fig. 4a). While the protein content in the grains of Bengkok, Rias Halus, Ramos Gunung Bayeun and Si Raden genotypes due to under stress from the high temperature the generative stage and the vegetative-generative stage was higher, ranging from 19.25-30.00, 18.35-26.53, 33.20-44.36 and 18.25-31.96% respectively compared to ambient temperature during the vegetative-generative stage (Fig. 4b) among these genotypes, under stress from high-temperature conditions at this stage the Bengkok and Rias Halus genotypes produced the decrease in amylose and increase in protein lowest.

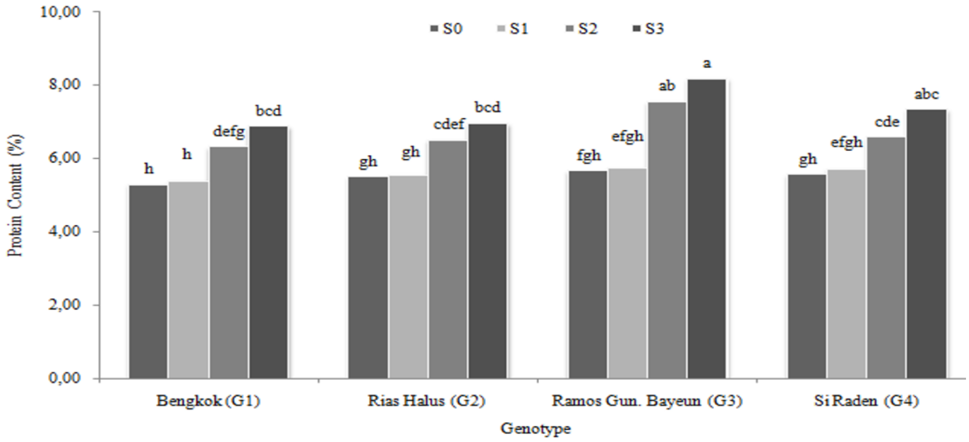
Table 3: Average weight of 1000 grains and potential yield per hill of four local upland rice genotypes under various stages of temperature stress treatment

	Weight of 1000 grains (g)				Potential yield per hill (g)			
Genotype	S0	S1	S2	S3	S0	S1	S2	S3
Bengkok (G1)	22.49^{a}	22.52^{a}	19.09^{g}	21.27^{bcd}	71.57^{b}	53.80^{cd}	34.89^{ef}	39.74^{def}
Rias Halus (G2)	21.48^{abc}	21.73^{ab}	19.62^{efg}	20.49^{cde}	68.33^{bc}	40.31^{de}	34.11^{ef}	30.24^{ef}
Ramos Gun. Bayeun (G3)	18.71^{gh}	18.60^{gh}	17.61^{i}	17.94^{h}	89.85^{a}	58.70^{bc}	58.27^{bc}	37.81^{ef}
Si Raden (G4)	19.40^{fg}	20.35^{def}	17.77^{h}	18.62^{gh}	94.42^{a}	64.42^{bc}	30.18^{ef}	24.91^{f}
Average	20.52^{a}	20.80^{a}	18.52^{c}	19.58^{b}	81.04^{a}	54.31^{b}	39.36^{c}	33.17^{c}
Temperature	**				**			
Genotype (Temperature)	**				**			

Note: According to the Duncan test at the 0.05 level, there is no significant difference between numbers that are followed by the same letter in the same row



(a)



(b)

Fig. 4: Temperature stress treatment at various stages in four local upland rice genotypes on; (a) Amylose content and (b) Protein content. Not substantially different at the 0.05 level is indicated by the same letter

Discussion

Based on observational data, it is known that exposing both the generative and vegetative-generative stages under stress from high temperatures (32-36°C) significantly decreased the amount of pollen germinated in all genotypes that were studied.These results are supported by previous research conducted by Tyuy *et al*. (2020), where the percentage of pollen viability at an average temperature of 33°C was significantly reduced compared to lower temperatures. High temperatures prior to the spikelets flowering have a greater influence on pollen activity. Pollen fertility can be utilized as an indicator of under-stress from high-temperature tolerance in rice because it significantly influences the grain's degree of emptiness. The Bengkok genotype can be classified as a tolerant genotype because it has the lowest reduction in the number of pollen germinated 35.95%.

The disruption of the tapetum's function, which is involved in anther rupture and pollen germination on the stigma, is the reason for the loss in pollen fertility brought on by high-temperature stress (Jagadish *et al*., 2010). Stressful environments reduce pollen protein and viability. High temperatures cause damage to proteinand lipid-based cell membranes, altering their shape and integrity and drastically lowering the viability of rice pollen (Das *et al*., 2014) and causing pollen to lose water content (Fahad *et al*., 2018).

The application of stress from high temperature in the vegetative-generative stage in the Bengkok genotype results in a longer panicle shape and higher panicle density (Fig. 3). The Bengkok genotype is also suspected to have the ability to optimize assimilate filling in grains at the tip position of the primary branch panicle under high-temperature stress conditions, leading to a higher quantity of spikelets in each panicle, spikelet fertility in each panicle and weight of filled grain in each panicle. According to Noviandy (2012), tolerant varieties show a better grain-filling response at the tip of the secondary branches which is better than grain-filling at the base of the panicle on secondary branches. Furthermore, Mohammed and Tarpleys (2010) study reported that high-temperature tolerant varieties tend to have a good grain-filling response at the tip of the panicle.

The reduction in panicle length, the number of spikelets in each panicle,spikelet fertility in each panicle weight of filled grain in each panicle and weight of 1000 grains when given under stress from the high temperature the generative stage was relatively higher than when given under stress from the high temperature the vegetative-generative stage in the four genotypes tested. These results are also supported by research by Shah *et al*. (2011); Cheabu *et al*. (2019) where a sharp decline from the booting stage to harvest was observed as a result of intense heat stress.Providing under stress from high temperatures the generative stage is thought to cause plants to be unable to control their adaptability and tend to divert energy and photosynthate to overcome high-temperature stress. According to Kim *et al.* (2011), high temperatures can stress grains and lead them to stop filling prematurely and further research by Mohammed and Tarpley (2010) proposes that stress from high temperatures causes disruption and competition in assimilate transport within rice kernels.

Chalky grains are the product of uneven filling and disturbance in storage biosynthesis caused by high-temperature stress during the ripening period. Chalky grains hence break readily when processing, increasing the proportion of fragmented grains and reducing the head rice percentage (Sreenivasulu *et al*., 2015). In addition, stress from high temperatures direct effect on seed development, including seed filling and the rate at which seeds produce dry matter (Kobata and Uemuki, 2004).

The Bengkok genotype has a number of spikelet fertility that is not significantly different but shows lower reduction and produces a higher weight of the weight of filled grain in each panicle than the other three genotypes when subjected to stress from the high temperature of the generative stage and under stress from the high temperature the vegetative-generative stage. This is one indication of how this genotype may withstand the stress caused by high temperatures. Spikelet fertility was primarily used in earlier research to screen rice germplasm that could withstand heat during the reproductive period (Tenorio *et al*., 2013; Huang *et al*., 2016; Prasanth *et al*., 2016; Sukkeo *et al*., 2017; Cheabu *et al*., 2019). A tolerant genotype can have results that are no different in optimum and stressful environments, but can also have higher results in stressful environmental conditions (Fernandez, 1992).

When the four genotypes were subjected to stress from high temperatures during their final stages of growth, the amylose content decreased and the protein content increased, among the four genotypes tested at high-temperature conditions, the Bengkok and Rias Halus genotypes resulted in a decrease in amylose and increase in protein lowest. This conclusion is reinforced by earlier research carried out by Liu *et al*. (2013), rice grains with high and low amylose contents saw a significant drop in amylose content under stress from high-temperature conditions, while protein content increased by 21.5% when compared to natural temperature conditions. Furthermore, due to high temperatures, Pravallika *et al.* (2020) also reported a decrease in amylose content in the endosperm of rice varieties by 26.93%.

The ripening phase is particularly susceptible to decreasing yield when the temperature is excessively high, resulting in elevated energy usage and seed respiration. Various seed quality traits are adversely affected by abiotic stress caused by stress from high temperatures, as demonstrated by Sreenivasulu *et al*., (2015). High temperatures can reduce starch content, alter starch structure and decrease amylose content (Counce *et al*., 2005).

Conclusion

The adverse effect of heat stress is more adverse the under stress from high temperatures the generative stage compared to the under stress from high temperatures the vegetative stage and the vegetative-generative stage which causes changes in panicle length, quantity of spikelets in each panicle, spikelet fertility in each panicle,the weight of filled grain in each panicle, the weight of 1000 grains, decreased amylose content and increased protein content, but not for the number of productive tillers. The quantity of pollen germinated decreased both at the stage of under stress from the high temperature the generative stage and the vegetative-generative stage.

Given under stress from the high temperature the vegetative stage can increase panicle length, quantity of spikelets in each panicle, spikelet fertility in each panicle andweight of filled grain in each panicle. The Bengkok genotype can be said to be the most tolerant genotype compared to the other three genotypes because it has the lowest reduction in germinating pollen, increase in panicle length, quantity of spikelets in each panicle, spikelet fertility in each panicle, the weight of filled grain in each panicle, decrease in amylose and increase in protein is lower under high-temperature stress various stages. This genotype may serve as a prospective genetic resource for future studies aimed at enhancing thevegetative and generative stages' tolerance to high temperatures. It may also serve as a source material for investigations into the physiology and morphology of high-temperature tolerance mechanisms.

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Author's Contributions

Risky Ridha: Responsible for conducting research, collecting data and preparing journal manuscripts.

Ellis Nihayati: Supervise research implementation, revise and perfect journal manuscripts.

Ariffin: Supervise research implementation, revise and perfect journal manuscripts.

Setyono Yudo Tyasmoro: Supervise research implementation, revise and perfect journal manuscripts.

Ethics

This article contains research results that have not yet been published. The corresponding author declare that there are no ethical issues and that the other authors have read and agreed the manuscript for publication.

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