Sensitivity of Acehnese varieties of rice (*Oryza sativa* L.) to high temperature stress during flowering stage

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Abstract. Rice is very sensitive to high temperature stress at the flowering stage. The rice plants were exposed to high temperature condition under green house. Some varieties show tolerance to heat stress, and the others are sensitive. Nine local Acehness varieties: Si Puteh, Itam Tangke Lango, Ramos Tihion Tamping, Sireundeun Semantok, Bo Santeut Seumantok, Pade Cut Krusek, Sipirok, Sigeudop and Cantek Lembayung were observed for high temperature stress during flowering stage. The objective of this research are: to identify the effect of heat stress on reproductive stage of local Acehnese, Indonesian rice; and to find the varieties that sensitive and hopefully have the gene of "temperature genic male sterility" (TGMS) that can be used as restorer varieties for production of hybrid rice. Heat stress significantly decreased pollen viability, plant architecture, and yield components. The pollen viability was observed by stainability and germination rate of the pollen. Based on pollen germination ability showed that one variety of rice, Sigeudop, showed tolerance to high temperature stress. Whereas the sensitive variety was Bo Santeut Seumantok, the local Indonesian rice that is potential to have TGMS gene. Pollen stainability showed that all varieties of pollen involved stainability from plants cultivation at high temperature, and all the varieties had the high rate of pollen stainability. We also found that pollen germination rate showed three varieties of rice cultivated in high temperature involved high resistance of the pollens.
Keywords: rice, heat stress, tolerance, pollen viability.

Introduction

High temperature is becoming one of the significant abiotic stresses limiting plant growth and productivity, especially as the global temperature is probable to increase by 1.8°C–4.0°C at the end of 21st century (IPCC, 2007). High temperature condition results heat stress that causes detrimental effects on growth, yield, and quality of the rice crop by the inhibition of photosynthesis, injuring to cell membrane, senescence, and cell death (Nagai and Makino, 2009). Several studies reported that the rice is very sensitive to high temperature condition at the booting and flowering stages (Satake and Yoshida, 1978; Tan *et al*., 1985; Weerakoon *et al*., 2008). The critical temperature of 33°C for seed set is resulting in spikelet sterility and reduced yield (Nakagawa *et al.*, 2002).

In reproductive stage of rice, the heat stress affects floret sterility, decrease fertility, and the lost of yield. This diminution of rice productivity is to be caused by decreasing activities of pollens, such as reducing germination rate of pollen, limiting growth of pollen tube, low ability of pollen dehiscence, inability of pollen to reach chapiter, and imperfect of pollination (Matsui *et al*., 2001; Matsushima *et al*., 1982; Tang *et al*., 2006). Additionally, Matsui *et al*. (1997) proved that, in some varieties of rice, exposing rice during flowering stage at temperature 30°C is able to induce sterility of pollens. Consequently, this sterility increases the empty grain of rice so that the yield is reducing substantially.

Furthermore, high temperature that caused empty grain in the rice plant became the serious problem mainly for cultivation of rice in tropical area such as Indonesia. Sipayung (2007) reported that the daily temperature has been increase as the highest point in Aceh, one of the province of Indonesia, located on the northwestern tip of the island of Sumatera. The temperature change in Aceh shows the surface temperature increase of about 1.9ºC (CGCM) and 2.1ºC (CSIRO) during period of a hundred years (1900-2000) observations. The province was also affected by disaster of the giant tsunami waves following the 9.1 earthquake on December 26, 2004. Therefore, the impacts of global warming to rice productivity become more serious problem in this region (Efendi, 2011).

Shah *et al*. (2011) explained that the increase in temperature has exposed most of the world's crops to heat stress during some stages of their life cycle. However, the growth responses of rice to high temperature stress are still poorly understood (Nagai and Makino, 2009), especially the Indonesian local variety of rice. Therefore, this research attempts to study the phenotypic performances of rice as response to heat stress in the post tsunami-affected area with the highest temperature increase in Indonesia, using local Acehnese rice as the tropical variety. Addressing the mechanisms conferring heat tolerance during anthesis will help to develop rice germplasm capable of adapting to changing climates.

Materials and Methods

Pot experiment with high temperature treatment

The experiment was conducted in the district of Aceh Besar, Province of Aceh, Indonesia that severe affected by giant tsunami on December 26, 2004. The climate of the district is type C with 26-36°C of minimum and maximum temperature, and 75-90% of humidity. The local Acehnese varieties: *Si Puteh, Itam Tangke Lango, Ramos Tihion Tamping, Sireundeun Semantok, Bo Santeut Seumantok, Pade Cut Krusek, Sipirok, Sigeudop, and Cantek Lembayung* were used in this research to study their response to heat stress. We also used a Chinese variety, *Louhui*, as control of restorer variety. The rice seeds were germinated and sown into a tray containing natural sandy loam soil with 20% of compost. Seedlings then were transplanted into each pot filled with 5 kg of soil containing 4:1 of sandy loam and compost. For basal dressing, nutrient was applied for each pot with 1 g of Urea and 2 g of NPK (15-15-15) fertilizers at one day before transplanting. The top dressing was applied at 30 days after transplanting by using only Urea with the rate 1 g per pot.

All of the plants were growth in the ambient temperature until the early flowering stage. When plant reached 20% of flowering, than the plants were transferred Green House with high temperature condition during five days. The panicles that emerged after five days of high temperature treatment were tagged, and then pollens were collected as sample for counting of pollen stainability and pollen germination. The pollens stainability was tested immediately after collecting of the pollens, whereas pollens germination rate were examined at the day after the collecting. When a variety has less than 40% of sterile pollen and more than 50% of filled grain, it was considered that the variety is tolerant to heat stress. Whereas, for the varieties that have more than 95% of sterile pollen and below 4% of filled grains, it was concluded that the variety is sensitive to heat stress.

Pollen stainability analysis

Pollen stainability was analyzed by using the procedure of dying as explained by Khatun and Flowers (1995) and Fernandez-Da Silva and Menéndez-Yu (2006). Pollens from each rice variety that has been exposed to the heat stress were collected immediately after anthesis and treated in the medium containing tetrazolium 3-(4,5-dimethylithyazolyl)-2,5 diphenyl monotetrazolium bromida (MTT) with the concentration of 0.9% w/v in sucrose (54% w/v) during 15 minutes at room temperature (±28°C). Under light microscopy, we observed the stainability of 300 pollens from three panicles of each variety. Analysis of pollen stainability was also conducted by fixation of spikelets with 70% (v/v) ethanol about one hour before pollination. Then, the pollens were dyed by using a solution containing 0.18% (w/v) iodine and 1% (w/v) potassium iodine. Pollens that expressed dark green color were considered as fertile pollen. We analyzed about 300 pollens per spikelet and determined the status of pollen fertility that gathered from four spikelets of each variety of the rice plant (Komari *et al*., 2005).

Pollen germination analysis

Analysis of pollens germination was conducted by germinating the pollens under *in vitro condition*. Prior to germination, the pollens were released from the anther and were placed them in germination medium (1% w/v agar, 20% w/v sucrose and 20 ppm w/v boric acid as explained by Kariya (1989). Then, the pollens were incubated at temperature 20°C during 30 minutes. We observed 300 pollens that released from four panicles of each variety under light microscope.

Results and Discussion

Pollen stainability from heat stressed-plant

Results of study showed a phenotypic variation in pollen stainability as plant responses to heat stress at high temperature condition. We found that the pollens of all varieties involved stainability in different rate (from 90.5% to 100%). The pollen stainability of Sipirok is lower than Louhui variety as control restorer variety. The lowest rate of pollen stainability found at Sipirok (90.5%) of Acehnese variety. Meanwhile, the *Ramos Tihion Tamping* of local Acehnese variety produced the highest rate of pollen stainability (100%). The others varieties showed pollen stainability rates more than 94% (*Si Puteh, Itam Tangke Lango, Bo Santet Seumantok, Pade Cut Krusek, Sigeudop,* and *Cantek Lembayung*. Changing pattern of pollen stainability from the rice plants cultivated in high temperature of Green House showed at Figure 1.

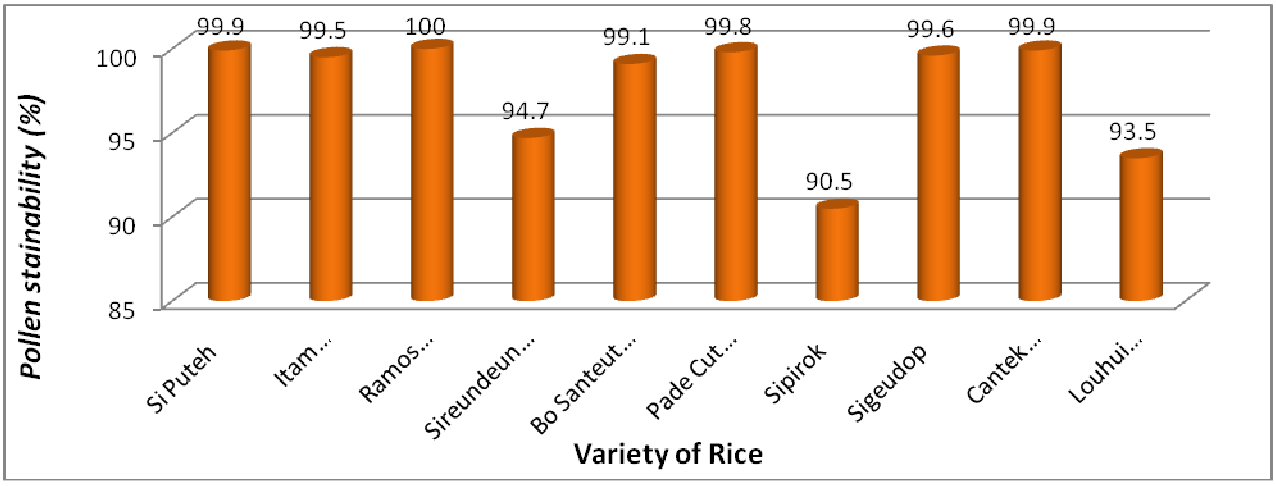


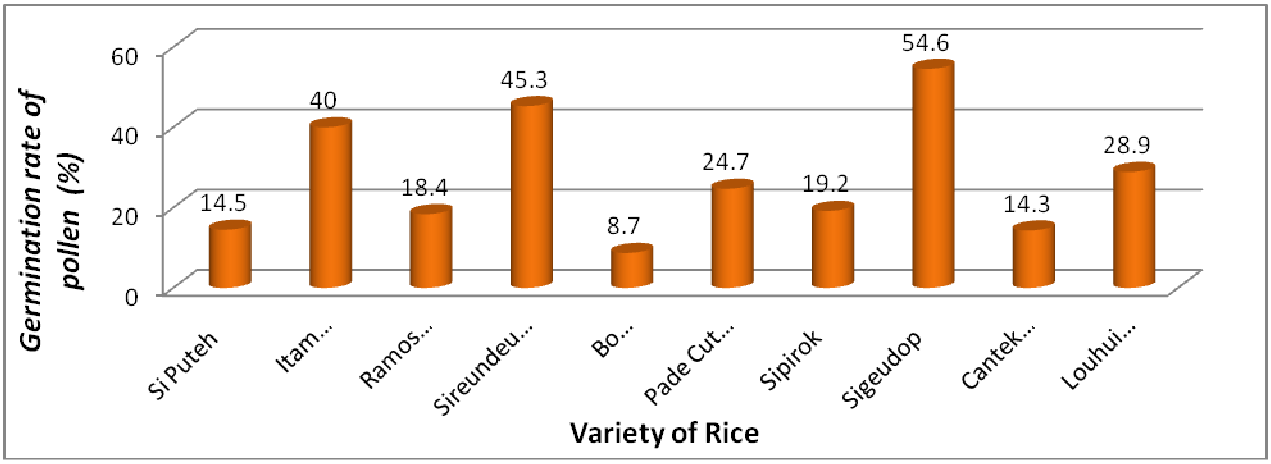
Figure 1. Pattern of pollen stainability from plants of Acehnese rice cultivated in Green House with high temperature condition

Regarding to the results of this research, we concluded that the high temperature condition during flowering stage in the Green House results heat stress that causes damages on pollen activity. This condition of heat stress have the similar result with others research. Some studies showed that high temperature can cause detrimental effects on growth, yield, and quality of the rice crop by the inhibition of photosynthesis, injuring to cell membrane, senescence, and cell death (Xu *et al*., 2006; Nagai and Makino, 2009). Additionally, in the same condition, several researches also reported that the flowering stage of rice is very sensitive to high temperature stress during reproductive phase (Satake and Yoshida, 1978; Tan *et al*., 1985; Weerakoon *et al*., 2008). Nakagawa *et al.* (2002) revealed that the critical temperature of 33°C during flowering stage for seed set is resulting in spikelet sterility and reduced yield of rice plant significantly.

Germination rate of pollen from heat stressed-plant

According to the germination rate of pollens, the results also showed a phenotype variation in germination rate of pollens by way of plant responses to high temperature

condition. We found that germination rate of three varieties of Acehnese rice was significantly decreased by heat stress. The three lowest germination rate of pollen found at variety of *Bo Santeut Seumantok* (8.7%), *Cantek Lembayung* (14.3%) and *Si Puteh* (14.5%). The others variety showed moderately tolerance to the heat stress. Variety of *Sigeudop* of Acehnese rice had the highest rate of pollen germination (54.6%). Furthermore, *Bo Santeut Seumantok* of Acehnese variety showed the lowest rate of pollen germination (8.7%). Variation of pollen germination rates from Acehnese rice plants exposed to heat stress showed at Figure 2.



Based on this study, we found that the Acehnese varieties of rice have respond to heat stress. Another research was carried out in line with what has been investigated by Matsui *et al*. (2001) and Shahid *et al.* (2010) who concluded that in reproductive stage of rice, the heat stress affects floret sterility, decrease fertility, and the lost of yield. This diminution of rice productivity is to be caused by decreasing activities of pollens, such as reducing germination rate of pollen, limiting growth of pollen tube, low ability of pollen dehiscence, inability of pollen to reach chapiter, and imperfect of pollination. In addition, Matsui *et al*. (1997) reported that exposing rice during flowering stage at temperature 30°C in some varieties of rice is able to induce sterility of pollens. Consequently, this sterility increases the empty grain of rice so that the yield is reducing substantially.

Conclusions

Results of study showed a phenotypic variation in pollen stainability and germination rate as plant responses to heat stress at high temperature condition. Although with high temperature condition, almost all of varieties still have high pollen stainability. The lowest percentage of pollen stainability was founded in variety Sipirok (90.5 %). The *Ramos Tihion Tamping* of local Acehnese variety produced the highest rate of pollen stainability (100%). We found that a variety of rice that showed high resistance of pollen under high temperature condition was variety Sigeudop (54.6%). The lowest germination rate of pollen found at variety of *Bo Santeut Seumantok* (8.7%).

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