RESEARCH ARTICLE

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INHERITANCE OF TOLERANCE TO HIGH TEMPERATURE AT ANTHESIS IN RICE

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SUMMARY

The problem of high temperature in rice in the tropics is exacerbated by climate change. Anthesis is the stage when rice is most sensitive to high temperature stress, understanding genetic control of the tolerance should contribute to efforts to adapt the rice plant to global warming. This study examined how heat tolerance at anthesis of the progeny of a cross between a tolerant and sensitive parent was inherited through the F1, F2 and F3 generations. Raising anthesis temperature from 32 ^{o}C to 38 ^{o}C decreased pollen viability and spikelet fertilization in the sensitive parent but had no effect on pollen viability and less severe depression on the percentage of fertilized spikelets of the tolerant parent and the F1 hybrids, in either of the reciprocal crosses. A pattern of transgressive segregation of spikelet fertilization under high temperature that was skewed towards the tolerant parent was observed in the F2 and F3 populations subjected to 37-38 ^{o}C at anthesis. It is concluded that high temperature tolerance during anthesis is controlled by complete dominance with a complexity of genes. Identification of the relevant genes and molecular markers associated with the tolerance should enable the trait to be deployed in rice breeding programmes. The dominant gene action suggests that the progeny testing would be essential during selection.

Key words: Rice, high temperature, flowering, heat tolerance, global warming

Key findings: This study has shown tolerance to high temperature at anthesis in rice, manifested in pollen viability, pollen germination on the stigma and spikelet fertility, from a tolerant parent can be inherited in its offspring. Hence, selection by these traits would be effective in heat tolerance rice breeding.

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INTRODUCTION

High temperature stress, a common problem in crop production in the tropics, is intensified by climate change, with the global temperature expected to increase by 1-6 ^{o}C by the year 2100 (Thornes, 2002). As rice is grown mostly in the tropics (Maclean *et al*., 2013), the crop is exposed to greater risk from global warming than any of the world's major food crops. In addition to the rise in daytime temperature, rice yield has also been reportedly decreased by the rise in night time temperature, with a yield decline of 10% for every 1 ^{o}C rise in the daily minimum temperature above 22 ^{o}C (Peng *et al*., 2004). The critically low and high temperatures

for rice, normally below 20 °C and above 30 °C, vary among the different growth stages (Yoshida, 1981), with the reproductive stage generally considered to be more sensitive to high temperature than the vegetative stage. Among the different stages of reproductive development, anthesis is the most sensitive to high temperature, followed by microsporogenesis (Wassmann *et al*., 2009). Fertility of the rice spikelets is decreased by high temperature during anthesis, resulting in large percentages of empty glumes (Osada. *et al*., 1973 and Prasad *et al*., 2006).

Rice varieties can be affected differently by high temperature stress in the depression of the number of pollen produced per anther (Prasad *et al*., 2006), pollination, pollen germination and spikelet fertility (Matsui *et al*., 2001). Three Thai rice varieties were shown to be affected differently when anthesis took place at 36 ^{o}C with grain set in only 21-26% of the spikelets in the varieties CNT1 and SPT1 compared with 71% in the SPR1, while anthesis at 34 ^{o}C resulted in grain set in > 90% of the spikelets in all three varieties (Jongjaidee *et al*., 2010). The varietal difference in the adverse effect of high temperature on grain set was shown to be associated with the difference in pollen viability and number of pollen grains that germinated in the stigma. Tolerance to high temperature in rice has been shown to be under the control of multiple genes (Mackill *et al*., 1982, Mackill and Coffman 1983, Maestri *et al*., 2002 and Yang *et al*., 2002). Heat tolerance in wheat was reported to be under the control of additive gene action (Porter *et al*., 1995), while heat tolerance in legumes was a completely dominant trait (Rainey and Griffiths, 2005). However, since plant growth and yield is adversely affected by high temperature stress through many different physiological processes, genetic control of the tolerance is likely to depend on the process under focus. This study therefore set out to examine heritability of the high temperature tolerance in rice, by focusing on pollen viability and the fertilization process.

MATERIALS AND METHODS

Parents, hybridization and evaluation of the F1 hybrids

The parents used in this study, SPR1 and SPT1 were selected from different heat tolerance at anthesis by pollen viability and spikelet fertility as previously identified by Jongjaidee *et al*. (2010). The high temperature tolerant SPR1 (IR25393-57-2-3/RD23//IR27316-96-3-2-2///SPRLR77205-3-2-1-1/SPRLR79134-51-2-2) and sensitive SPT1 (BKNLR75001-BCNT-B-RST-36-2/RD2) rice varieties were hybridized in reciprocal crosses to produce the seed of SPT1 x SPR1 and SPR1 x SPT1 F1 hybrids.

The F1 hybrids and parents were grown in soil in plastic pots (15 cm in diameter and 12 in depth) at 1 plant per pot, 10 plants constituting 1 experimental unit, of a factorial experiment involving the 4 rice genotypes exposed to 2 temperature regimes during anthesis, 32 and 38 ^{o}C, in 5 replicates. The plants were maintained in the greenhouse until anthesis commenced. The first panicle was removed to make for uniform flowering in the remaining panicles and also for determination of pollen viability and pollen germination. The pots were placed in growth cabinets at 32 ^{o}C or 38 ^{o}C with 67.0 ± 3.8% RH from 8:00 to 15:00 hour until flowering was completed, about 5 days. The pollen grain from 3 spikelets in the middle of the main panicle that just began to open was examined under microscope with I_2/KI staining (Cheng *et al*., 1992). Pollen that were round and stained dark blue with iodine were considered viable, those that were misshapen and unstained were considered non-viable (Figure 1a). Three spikelets (i.e. from the middle of the spikelets that had completed anthesis) approximately 3 hours after the glumes had closed, were examined for pollen germination on the spikelets with phenol cotton blue staining of the stigma (Khatun and Flowers, 1995). Germinated pollen was determined by the pollen tube that stained blue under microscope (Figure 1b).

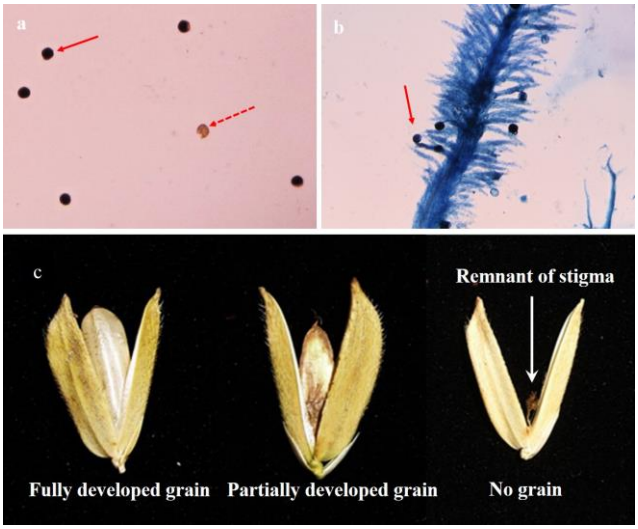

Figure 1. (a) Pollen viability with iodine staining, viable (full arrow) and non-viable (dotted arrow); (b) pollen germination on stigma (farrow); (c) rice spikelet: fertilized and filled (left), fertilized but unfilled (center) and unfertilized and unfilled (right).

At maturity, first two panicles of each plant were harvested to determine the number of spikelets that were fertilized and filled, fertilized but unfilled, and unfertilized (Jongjaidee *et al*., 2010), from which spikelet fertilization percentage was derived. Analysis of variance was computed using Statistix Version 8.0; Analytical Software for the data set. Response to high temperature of parents and F1 hybrids were compared by Fisher's least significant difference (LSD) at $P < 0.05$.

High temperature tolerance in the F2 and F3 generations

Four hundred plants of the SPT1 x SPR1 hybrid were generated by splitting 2 tillers from each of 200 plants grown from the self-fertilized F1 seed and separated into 2 plants at 2-tiller stage, approximately 20 day-old. The 400 F2 plants and 10 plants each of the parents were grown in soil in plastic pots (15 cm in diameter and 12 in depth) at 1 plant per pot at ambient temperature until anthesis. At the beginning of anthesis 200 of the F2 plants and 5 each of the parents were placed in a plastic enclosure from 8:00 to 15:00 hour until anthesis was completed, the other 200 of the F2 plants and 5 each of the parents remained at ambient temperature. Ambient temperature recorded with data logger averaged 31.3 ± 2.0 ^{o}C and 62.8 ± 5.7% RH (designated T31) and in the plastic enclosure at 37.1 ± 2.5 ^{o}C and 65.4 ± 5.3% RH (designated T37). At maturity the seed was harvested for determination of number of spikelets that were fertilized and filled, fertilized but unfilled, and unfertilized (Jongjaidee *et al*., 2010), from which spikelet fertilization percentage was derived. The F3 plants were grown from seed of 157 families, each family deriving from self-fertilized seed of individual F2 plants, with 6 plants from each family grown in a pot (30 cm in diameter and 30 in depth). There were also 3 pots, with 6 plants per pot, of each of the parents. The same high temperature treatment and assessment of the effects were carried out in the same way for the F2 generation.

Means, ranges and standard deviation of spikelet fertilization percentage were determined for the F2 and F3 plant compared with the parents. Heritability (h^2) is estimated by the regression of performance of the offspring on its parent, with the h^2 value quantified by the slope of the regression (Kempthorne and Tandon, 1953).

RESULTS

High temperature tolerance in the F1 generation

Tolerance to high temperature during anthesis of the F1 progeny of the cross between high temperature tolerant and sensitive parents was indicated by pollen viability, pollen germination on the stigma, spikelets fertilization and grain filling (Figure 2). No difference was observed in these parameters between the reciprocal crosses. At 32 ^{o}C pollen viability of the parents and the F1s were close to 100%, at 38 ^{o}C the pollen of SPR1 and the F1s was only slightly affected while pollen viability of SPT1 was decreased to 80%. At 32 ^{o}C both parents and F1s has similar number of pollen germinating on the stigma, at 38 ^{o}C the number of pollen germinating in SPT1 was significantly lower than at 31^{o}C, while the effect of temperature did not differ in SPR1, and while the number of germinating pollen of the F1s at 38 ^{o}C was between the two parents. Percentage of spikelets successfully fertilized at 32 ^{o}C were similar among the parents and F1s at 93-94%, but at 38^{o}C fertilization in SPT1 was decreased to 50% compared with significantly higher percentage of fertilized spikelets of 69% in SPR1 and 67% in the F1s. At 32 ^{o}C approximately 90% filled grain was found for the parents and F1s, but at 38 ^{o}C grain filling in SPT1 was decreased to 49% compared with significantly higher percentage of filled grain in the F1s that was similar to SPR1 at 68%.

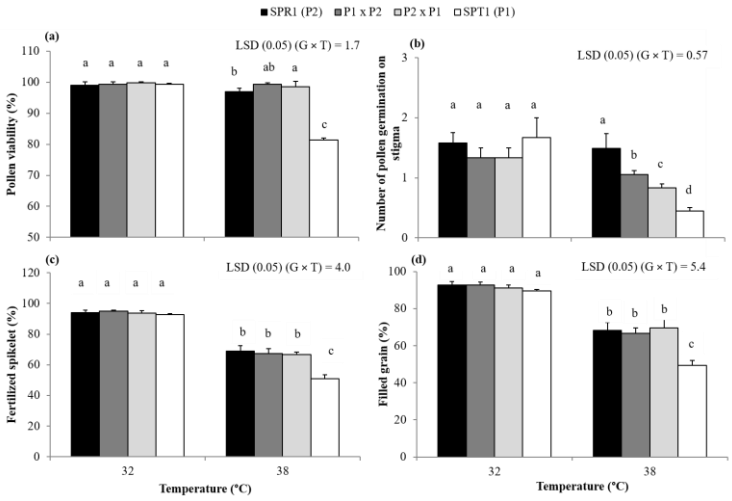

Figure 2. Pollen viability (a); number of pollen germinated on stigma (b); percentage of fertilized spikelets (c); and percentage of filled grain (d), of SPR1, SPT1 and the F1 hybrids from their reciprocal crosses at 32°C and 38°C. Significant differences between means at $P < 0.05$ were indicated by different lowercase letters.

High temperature tolerance in the F2 and F3 generations

Since no difference was found between the reciprocal crosses in their tolerance to high temperature in the F1s, self-fertilized seed from the SPT1 x SPR1 cross was used in the evaluation of the F2 and F3 generations. The effect of high temperature on the frequency distribution of spikelet fertilization was found to vary among the parents and F2s (Figure 3). At 31^{o}C (T31) percentage of fertilized spikelets of both parents and the F2 varied within a narrow range of 80-97%, and standard deviation of 1-3%. At 37 ^{o}C (T37) percentage fertilized spikelets ranged from 91-95% for the tolerant parent SPR1, and from 69-79% for the sensitive parent SPT1 and standard deviation of 1-3%.

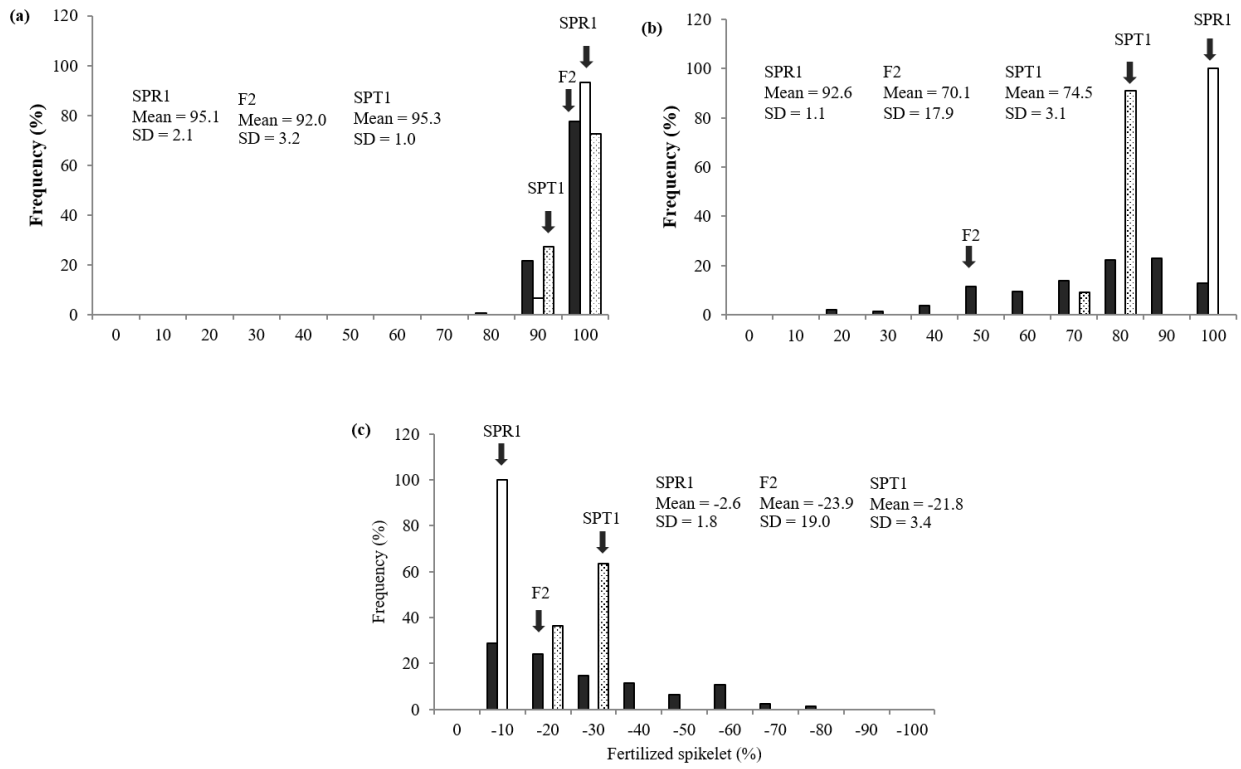

Figure 3. Frequency distributions of percent fertilized spikelets of F2s and their parents at 31 ^{o}C (a); 37^{o}C (b); and the percentage of percent fertilized spikelet reduction at 37 ^{o}C compared to 31 ^{o}C, (T37-T31)/T31×100 (c).

The F2s at T37, however, segregated into a broad range of spikelet fertilization that ranged from 21% to 96% with standard deviation of 18%, and 30% of the population had lower spikelet fertilization than the sensitive parent SPT1. This same pattern of segregation of the F2s was observed with percentage of reduction of spikelet fertilization at T37 compared to T31 (Figure 3c), fertilized spikelet reduction (%) of the sensitive parent SPT1 was significant higher than the tolerant parent SPR1 (22% in SPT1 and 2% in SPR1) while F2s ranged from 1 - 78%, and there were more sensitive progeny about 30% of the population which reduction of fertilized spikelet were larger than the sensitive parent SPT1. Close relationship between percentage of fertilized spikelets of F2s at T37/T31 and at T37 was observed (y = 0.93x-0.83, r = 0.98, P < 0.001) thus the F3s were tested at 37 ^{o}C during anthesis without the control at 31 ^{o}C.

With anthesis temperature at 37 ^{o}C the F3s exhibited a pattern of segregation outside the range of both parents with percentage of fertilized spikelets of that ranged from 21% to 98%, compared with spikelet fertilization at 72-94% in the tolerant parent SPR1 and 42-74% in the sensitive parent SPT1 (Figure 4). Spikelet fertilization at high temperature of the offspring in F3 was related (r = 0.51, P < 0.001) positively with that of its F2 parent with the slope (b) of the linear regression of 0.37 (Figure 5).

DISCUSSION

Percentage of fertilized spikelet under high temperature in growth chamber (response of F1s) was lower than in plastic enclosure (response of F2s and F3s) observed by parental means, SPR1 and SPT1. This was similar to the study of Xiao (2010) which reported that

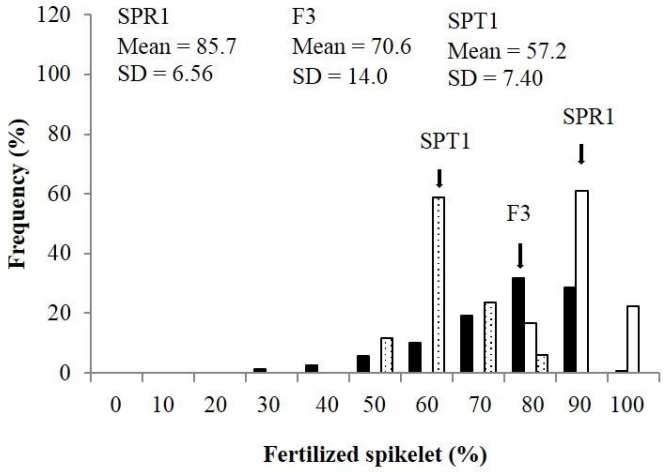

Figure 4. Frequency distributions of percent fertilized spikelets of F2-derived F3 families and their parents grown under high temperature at flowering.

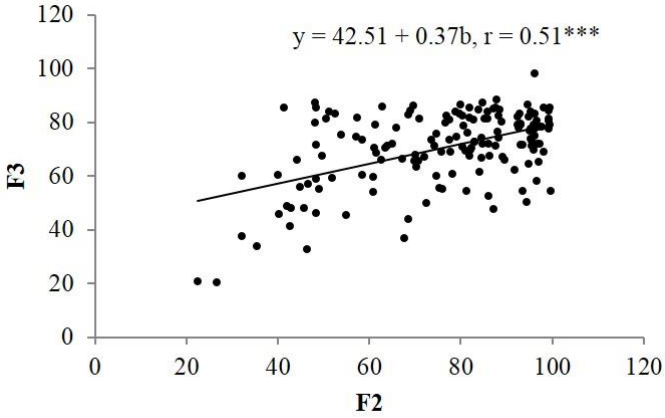

Figure 5. The regression of spikelet fertilization at high temperature of the offspring (F3) on the parent (F2).

different in relative humidity (%) may be the important reason in difference of grain set between conditions. Matsui (1997) reported that increasing of relative humidity (%) reduced spikelet fertility by reducing the number of pollen grain on stigma. However regardless of these possible differences in the condition between F1s and F2, F3s tests, the consistency in the heat stress in this study was confirmed by the relative tolerance of the parents, with percent fertilized spikelet of the sensitive parent (SPT1) was always significantly lower than the tolerant parent (SPR1). Screening for heat tolerance in segregating populations especially with a large number of plants under the plastic enclosure is simpler and more convenient than in growth chamber.

As others have previously shown (Jongjaidee *et al*., 2010) high temperature decreased grain set in the rice variety SPT1 more than SPR1 by depressing pollen viability and pollen tube germination on the stigma. However, this study has also shown that the tolerance to high temperature of the tolerant parent was transferred to the F1 hybrid so that its pollen viability, grain set and grain filling under high

temperature was similar to the tolerant parent SPR1, whereas the number of pollen grains germinating on the stigma was between the parents. In addition it was also shown that the F1 from the reciprocal crosses were affected by high temperature in the same way. Three important features of the inheritance of the heat tolerance were indicated. Firstly, the different effects of high temperature on pollen viability and the number of germinating pollen grains on stigma suggested possible differential responses to high temperature between pollen development and the fertilization process. Secondly, similarity between the F1 hybrid and the tolerant parent SPR1 in their pollen viability and percentage of fertilized spikelets under high temperature during anthesis indicated a complete dominance of the heat tolerance trait, this was similar to other studies of genetic control in stress tolerance, with responses of the F1 hybrids that depended on the severity of stress (Jamjod *et al*., 2004, Knight, 1973 and Paull *et al*., 1991) whereas the number of germinating pollen grains on the stigma that was between the parents suggested an additive gene action. Different gene actions for heat tolerance at different stages of reproductive development in rice have been previously reported, with additive to over-dominant gene action for spikelet fertilization and additive to dominant gene action for grain filling (Mackill *et al*., 1982). Thirdly, the lack of difference between the hybrids from the reciprocal crosses in their tolerance to high temperature indicated chromosomal inheritance without the involvement of extrachromosomal genetics.

The percentage of fertilized spikelets combines the effect of high temperature on pollen viability and the fertilization process that begins with germination of the pollen grain on the stigma. Fertility of the spikelets under high temperature has been widely used as a screening index for heat tolerance at reproductive stage (Prasad *et al*., 2006). The heritability of heat tolerance was therefore further explained by segregation of spikelet fertilization under high temperature of the F2s and F3s. A complexity of genes involved in the tolerance to high temperature at anthesis was clearly indicated in the percentage of fertilized spikelets of the F2 and F3s that varied widely beyond the parents, but skewing towards the tolerant parent. These suggested a transgressive segregation of a trait involving a complexity of genes, similar to earlier reports (Ye *et al*., 2015, Xiao *et al*., 2010 and Xiao *et al*., 2011). Heat tolerance during flowering stage has been identified in numerous populations of rice and the relevant quantitative trait loci (QTLs) mapped on almost all of the rice chromosomes (Ye *et al*., 2015). This study has demonstrated that heat tolerance in the tolerance parent SPR1 could be transferred to its progeny through the F2 and F3 generations, with the h^2 value for the tolerance to high temperature during anthesis estimated at 37% by the slope of the regression of spikelet fertilization at high temperature of the offspring (F3) on the parent (F2). This is comparable to a published h^2 value of 48% (Mackill and Coffman, 1983). This means that partially of genetic controlling heat tolerance at anthesis in F2 generation could be transferred to F3 generation, therefore the F3 generation will be in selection further.

In conclusion, this study has shown tolerance to high temperature at anthesis in rice, manifested in pollen viability, pollen germination on the stigma and spikelet fertilization, from a tolerant parent can be inherited in its offspring, with the heritability value estimated at 37%. Segregation of the tolerance in the F2 and F3 generations suggested the involvement of a complementary of genes. It is nevertheless possible that heat tolerance can be selected for, but the dominant nature of the heat tolerance trait means that progeny testing would be necessary. Breeding for heat tolerance with marker-assisted selection (MAS) could benefit from identification of the QTL controlling heat tolerance in these segregating populations. The selection process would be much aided by identification of the relevant molecular markers, which could be used for screening in the field.

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REFERENCES

Cheng C, McComb JA, Rerkasem B (1992). Techniques to study the anther in wheat. In: C.E. Mann, B. Rerkasem, eds., Boron deficiency in wheat, Wheat spec, Rep. 11, CIMMYT, Mexico. pp. 32-33.

Jamjod S, Niruntrayagul S, Rerkasem B (2004). Genetic control of boron efficiency in wheat (*Triticum aestivum* L.). *Euphytica* 135: 21-27.

Jongjaidee J, Lordkaew S, Konsaeng S, Jamjod S, Rerkasem B, (2010) Effects of high temperature on pollen viability and fertilization in Thai rice varieties. *J. Agric. (CMU)* 26: 29-35.

Kempthorne O, Tandon OB (1953). The estimation of heritability by regression of offspring on parent. *Biometrics* 9: 90-100.

Khatun S, Flowers TJ (1995). The estimation of pollen viability in rice. *Journal of Experimental Botany* 46: 151-154.

Knight R (1973). The relation between hybrid vigour and genotype-environment interactions. *Theoretical and Applied Genetics* 43: 311-318.

Mackill DJ, Coffman WR, (1983). Inheritance of high temperature tolerance and pollen shedding in a rice cross. *Zeitschrift fur Pflanzenzuchtung* 91: 61-69.

Mackill DJ, Coffman WR, Rutger JN (1982). Pollen shedding and combining ability for high temperature tolerance in rice. *Crop Science* 22: 730-733.

Maclean J, Hardy B, Hettel G (2013). Rice almanac. 4th edition. Source book for one of the most important economic activities on earth. International Rice Research Institute, Los Baños, Philippines. pp. 283.

Maestri E, Klueva N, Perrotta C, Gulli M, Nguyen H T, Marmiroli N (2002). Molecular genetics of heat tolerance and heat shock proteins in cereals. *Plant Molecular Biology* 48: 667-681.

Matsui T, Omasa K, Horie T (1997). High temperature-induced spikelet sterility of Japonica rice at flowering in relation to air temperature, humidity and wind velocity conditions. *Japanese Journal of Crop Science* 66: 449-455.

Matsui T, Omasa K, Horie T (2001). The difference in sterility due to high temperatures during the flowering period among japonica rice varieties. *Plant Production Science* 4: 90-93.

Osada A, Sasiprapa V, Rahong M, Dhammanuvong S, Chakrabondho H (1973). Abnormal occurrence of empty grains of indica rice plants in the dry, hot season in Thailand. *Proc. Crop Sci. Soc. Jpn.* 42: 103-109.

Paull JG, Rathjen AJ, Cartwright B (1991). Major gene control of tolerance of bread wheat (*Triticum aestivum* L.) to high concentrations of soil boron. *Euphytica*. 55: 217-228.

Peng S, Huang J, Sheehy JE, Laza, RC, Visperas RM, Zhong X, Centeno GS, Khush GS, Cassman KG (2004). Rice yields decline with higher night temperature from global warming. *Proceedings of the National Academy of Sciences of the United States of America* 101: 9971-9975.

Porter DR, Nguyen HT, Burke JJ (1995). Genetic control of acquired high temperature tolerance in winter wheat. *Euphytica* 83: 153-157.

Prasad PVV, Boote KJ, Allen JLH, Sheehy JE, Thomas JMG (2006b). 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.

Rainey KM, Griffiths PD (2005). Differential response of common bean genotypes to high temperature. *Journal of the American Society for Horticultural Science* 130: 18-23.

Thornes JE (2002). IPCC, 2001: Climate change 2001: impacts, adaptation and vulnerability, contribution of working group II to the third assessment report of the intergovernmental panel on climate change. In: J.J. McCarthy, O.F. Canziani, N.A. Leary, D.J. Dokken and K.S. White, eds., Cambridge University Press, Cambridge, UK, and New York, USA, 2001. *International Journal of Climatology* 22: 1285-1286.

Wassmann R, Jagadish SVK, Heuer S, Ismail A, Redona E, Serraj R, Singh R K, Howell G, Pathak H, Sumfleth K, Donald LS (2009). Chapter 2 climate change affecting rice production: The physiological and agronomic basis for possible adaptation strategies. In: L. Donald and Sparks, eds., Advances in agronomy. Academic Press, 101: 59-122.

Xiao Y-H, Pan Y, Luo L-H, Deng H-B, Zhang G-L, Tang W-B, Chen L-Y (2011). Quantitative trait loci associated with pollen fertility

under high temperature stress at flowering stage in rice (*Oryza sativa* L.). *Rice Science* 18: 204-209.

Xiao Y, Pan Y, Luo L, Zhang G, Deng H, Dai L, Liu X, Tang W, Chen L, Wang G-L (2010). Quantitative trait loci associated with seed set under high temperature stress at the flowering stage in rice (*Oryza sativa* L.). *Euphytica* 178: 331-338.

Yang J, Sears RG, Gill BS, Paulsen GM (2002). Quantitative and molecular characterization of heat tolerance in hexaploid wheat. *Euphytica* 126: 275-282.

Ye C, Tenorio FA, Argayoso MA, Laza MA, Koh H-J, Redoña ED, Jagadish KS, Gregorio GB (2015). Identifying and confirming quantitative trait loci associated with heat tolerance at flowering stage in different rice populations. *BMC Genetics* 16: 1-10.

Yoshida S, (1981). Fundamentals of rice crop science, IRRI, Los Banos, Philippines.