

Evaluation of Growth and Physiological Responses of Three Rice (*Oryza sativa* L.) Varieties to Elevated Temperatures

Dede Yudo Kurniawan^A, Ahmad Junaedi^{*B}, Iskandar Lubis^B, Titi Candra Sunarti^C

^A Graduate School of Bogor Agricultural University, Bogor, 16680, Indonesia.

^B Department of Agronomy and Horticulture, Bogor Agricultural University, Bogor, 16680, Indonesia.

^C Department of Agroindustrial Technology, Bogor Agricultural University, Bogor 16680, Indonesia.

*Corresponding authors; email: junaedi_agr@yahoo.com

Abstract

Temperature is a primary factor that affects the rate of plant development and has great impacts on plant growth, metabolism, and yield. A study was conducted to analyze the effects of elevated temperature on rice morphological and the physiological growth. The research was arranged in a nested randomized block design consisting of two factors, temperatures and rice varieties. Elevated temperatures were provided through the uses of different materials of plastic roof and walls to have an average and maximum temperature of 27.6 °C and 41.6 °C (T1); 28.1 °C and 43.8 °C (T2), and 29.5 °C and 47.1 °C (T3), respectively. The study used three varieties of rice, "Ciasem", "Ciherang", and "IR64". All rice varieties showed significant increases in tiller number per hill and shoot dry weight, but had a decrease in the stomatal conductance, transpiration rate, and SPAD values at grain filling stage with the increasing temperatures. The number of tiller per hill increased when temperature was elevated from 27.6 to 28.1 and 29.5 °C by about 29.9 and 21.3%, respectively.

Keywords: *Oryza sativa*, high temperatures, dry weight, stomatal conductance

Introduction

Global warming represents another challenge for crop and yield of the plant under abiotic stress (Hedhly et al., 2009). Rising concentrations of CO₂ and other greenhouse gases is likely to affect crop production thus human food supplies (Chakrabarti et al., 2013). The concentration of carbon dioxide (CO₂) increases from 340 to 400 ppm (IPCC, 2014) with global mean surface temperature projected to rise by 1.0–3.7 °C by the end of the century (IPCC, 2014). In addition to a consistent increase in background global mean

temperatures, plants will also experience heat stress via increased frequency, intensity, and duration of heat waves (IPCC, 2014). Barlow et al. (2015) and Prasad et al. (2011) stated in his review for recent years on the effect of temperature extremes that rice crops exposed to excessive heat had a reduced grain number and reduced duration of the grain filling period. Temperature is a major factor affecting plant development. Warmer temperatures as expected with climate change and the potential for more extreme temperature events will have negative impacts on crop productivity (Hatfield and Prueger, 2015).

Rice (*Oryza sativa* L.) is one of the most important food crops that supply carbohydrates for half of the population worldwide, especially in Asia (Teixeira et al., 2013). Rice is becoming increasingly exposed to adverse climatic conditions such as heat and water deficit stress, resulting in significant yield losses. In recent years, abiotic stress recorded in growing season of rice has been mapped in South East Asia (Wassmann et al., 2009). High-temperature stress is defined as the rise in temperature beyond a critical threshold for a period of time sufficient to cause irreversible damage to plant growth and development (Wahid et al., 2007).

High-temperature stress is one of the limiting factors in the environment that could damage growth, metabolism, and yield of a plant, especially those that are very sensitive to high temperature. Plant responses to high temperature vary with the degree and duration of high temperature and the plant type (Hazanuzzaman et al., 2013). The major impact of warmer temperatures was during the reproductive stage of development and in all cases, grain yield in maize was significantly reduced by as much as 80–90% from a normal temperature regime (Hatfield and Prueger, 2015).

Responses to high temperature in rice differ according to the developmental stage, with the highest sensitivity recorded at the reproductive stage (Endo et al., 2009). The timing and duration of developmental phases, including flowering is determined by temperature (Bahuguna and Jagadish, 2015).

High percentage of flower sterility in rice has been shown to be a result of high temperature; temperatures of $>35^{\circ}\text{C}$ for more than one hour at anthesis induced the sterility of female reproductive organ (Jagadish et al., 2008). High-temperature stress could reduce the effective duration of pollens to reach the stigma, hence may lead to the flower sterility in rice. Both the male and female organs of the flower are especially sensitive to temperature fluctuations, both during their development before pollination and during the post-pollination stage (Hedly, 2011). The unfavorable influence of high temperature on cereal crop yields result in the negative impact on the development of morphological units that contribute to harvest index (HI) (Barnabas et al., 2008). Increasing temperatures from 24 to 32°C resulted in reductions in tiller numbers but had no impact on axillary branch numbers per tiller (Harsant et al., 2013). Hence, the aim of this research was to analyze the effects of elevated temperature conditions on growth, morphology and the physiological growth of rice.

Materials and Methods

Experimental Site

This research was conducted from September 2017 until January 2018 in the Cikabayan experimental field at the Bogor Agriculture University, Bogor (106.717575 ; -6.548433). Plant physiological observations were conducted in the Postharvest Laboratory, Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University.

Experimental Design

This experiment used a nested randomized complete block design (RCBD) with two factors, i.e. growing temperature and rice varieties. The experimental unit was replicated three times, nested in the main factors. The present study used three levels of elevated temperatures, (T1) with the maximum and average air temperature of $41.6/27.6^{\circ}\text{C}$, (T2) with the maximum and average air temperature of $43.8/28.1^{\circ}\text{C}$, and (T3) with the maximum and average air temperature of $47.1/29.5^{\circ}\text{C}$. The three rice varieties used were "Ciasem", "Ciherang", and "IR64".

Fertilizer Application

The fertilizer application was based on the standard dosage for rice which is $300\text{ kg Urea ha}^{-1}$, $120\text{ kg SP36 ha}^{-1}$, and $120\text{ kg KCl ha}^{-1}$, and applied at 2 g Urea , 0.8 g SP36 , and 0.8 g KCl per plant.

Temperature Treatments

Rice was grown in polybags and maintained under a lowland system where the water level was kept at 20 mm above the soil surface. The elevated temperature treatment was created through the use of different types of growing structures, i.e. polyethylene roof with insect screen walls with ventilation (T1; Figure 1 A); polyethylene roof and half of the walls are from polyethylene with ventilation (T2; Figure 1 B); roof and walls are fully covered with polyethylene, without ventilation (T3; Figure 1 C). The different materials used for the growing structures elevated the temperature inside the structure. Rice crops were exposed at 14 days after sowing until harvesting. The air temperature inside the plastic house during the rice growing season was periodically recorded using a thermal recorder (TR-71U, T and D, Japan). The rice crops inside the T1, T2, and T3 plastic houses were exposed to high temperatures daily from $10:00$ to $14:00$, $09:30$ to $14:00$, and $09:00$ to $14:00$, respectively.



Figure 1. Rice growing structures T1 (polyethylene roof with insect screen walls, A); T2 (polyethylene roof and half of the walls from polyethylene; B) and T3 (roof and walls are fully covered with polyethylene; C)

The measurement of rice growth is described below:

1. Tiller number per hill was measured at the maximum vegetative stage or about 55 days after sowing
2. Number of productive tiller was measured at the generative phase, or around 65 days after sowing
3. The dry weight of rice shoots at harvest
4. SPAD value of the flag leaf was measured at anthesis using a SPAD-502 plus.
5. Photosynthesis rate, stomatal conductance, and transpiration of the flag leaf were measured from five plants per rice variety at generative phase using a Portable Photosynthesis (Li-Cor 6400XT).

great impact on the number of tillers as compared to the normal ambient temperature. Rice tillers started to develop within 30 days after transplanting (Makarim and Suhartatik, 2009). The formation of tillers for all rice varieties in this current study increased under elevated temperature during the 3rd and 4th weeks and all varieties seem to have the similar responses.

There was no significant interaction between elevated temperature and rice varieties in affecting the tiller numbers at maximum vegetative phase. The number of tillers per hill at the maximum vegetative phase was significantly affected by the elevated temperature at 28.1 and 29.5°C, where there was an increase by about 29.9 and 21.3%, respectively, when compared to 27.6 °C (Figure 3 a).

Results and Discussion

Rice Growth

There was a significant interaction between elevated temperature and rice varieties in affecting the tiller numbers on the 3rd and 4th weeks (Figure 2). The increasing temperature of the environment had a

According to Jumiatur et al. (2016) the increasing of tiller numbers is an adaptation mechanism of plants to decrease temperature. The variable number of productive tillers (Figure 3 B) did not show a significant effect on elevated temperature and varieties. The productive number of tiller at 27.6, 28.1, and 29.5 °C were 15.1, 16.5, and 16.3 respectively (Figure 3 B) and there were no significant differences

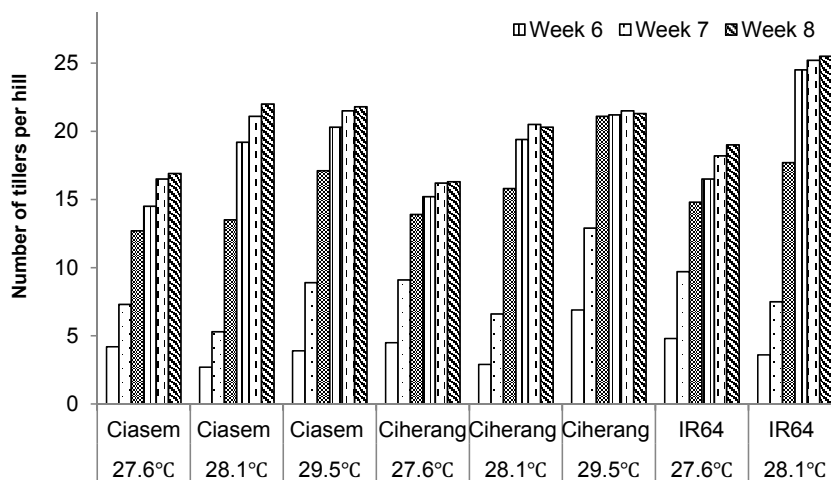


Figure 2. Number of rice tillers per hill at different temperatures

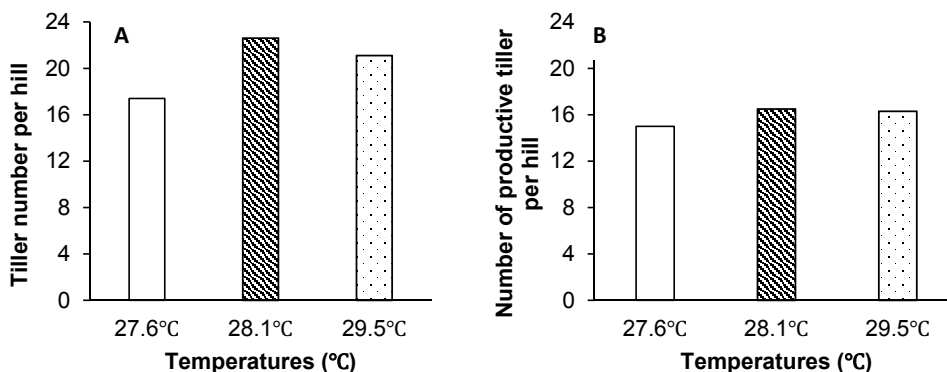


Figure 3. The effects of elevated temperatures on the number of tillers (a) and number of productive tillers per hill (b) at the maximum vegetative phase

between rice varieties in response to the elevated temperatures. Thus, the more tillers produced at elevated temperature treatment did not lead to more productive tillers. The increase in the number of tillers per hill in this study did not have significant correlations with the increase of shoot dry weight in all varieties.

The rice shoot dry weight at 29.5°C increased by 24.8 and 15.1% when compared to 27.6 and 28.1°C, respectively (Figure 4). The results in this present study was in line with Oh-e et al. (2007), which showed that the dry weight increased by 12.8-16.4% at high temperature (30.9°C) compared to the optimal temperature (control). The increase of hill dry weight under temperature stress had resulted in a change in the assimilate partitioning; assimilates for seed production was diverted into vegetative components or biomass. Suwa et al. (2010) reported that with high temperature (35/27°C) there was an increase in the vegetative biomass and a decrease in yield of corn.

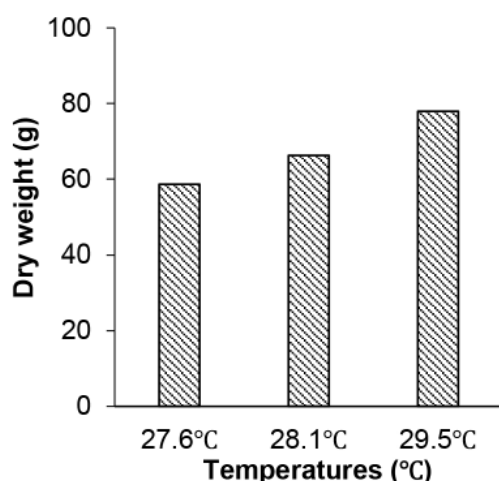


Figure 4. The effects of elevated temperatures on rice shoot dry weight

Correlation test between dry weight and percentage of filled grain showed a significant difference and has a negative value with the coefficient correlation ($r = -0.5^*$), which means the increase in dry weight may lead to decrease in the percentage of filled grain (Kurniawan, 2018). The assimilation from photosynthesis which should be translocated to the sink, namely grain, is inhibited due to the influence of the temperature which causes disruption of enzyme activity. Enzymes play important roles in the life cycle of plants to provide energy from the process of catalyzing carbohydrates into simple sugars such as hexose invertase enzymes. Cheikh and Jones (1995) reported that heat stress that occurs causes a decrease in the activity of vacuolar invertase enzyme (VIN) in converting sucrose to hexose causing a decrease in starch formation in seeds. According to

Ruan et al. (2010) invertase enzymes and sugars play significant roles in regulating the crop responses to drought and heat stress, which in turn affects the formation of grains. Correlation test between photosynthesis rate and canopy dry weight showed significant results with a correlation coefficient of $r = 0.4$ (Kurniawan, 2018). These results showed that the assimilates produced from photosynthesis was collected in the vegetative organs, hence increased plant biomass. The reduced the rice crop harvest index under high temperatures was due to the greater weight of biomass compared to the yield.

Photosynthesis Rate, Stomatal Conductance and Transpiration Rate

SPAD values/chlorophyll content of the rice flag leaf is presented in Table 1. The interaction between temperature and varieties shows no significant differences in affecting the photosynthesis rate, stomatal conductance and transpiration rate (Table 1). Variations in the SPAD values during the grain filling phase indicated the existence of tangible results from the influence of the ambient temperature that decreases the SPAD value at increased temperature environment. The highest SPAD value was at 27.6°C, which has a higher SPAD value around 19.9 and 16.8% when compared with temperature treatment of 28.1 and 29.5°C. The highest SPAD value was in the "Ciasem" variety. SPAD value scoring was aimed at measuring the chlorophyll content in the leaves, and these values are affected by light (Xie et al, 2011). Correlation test in the study of Xie et al. (2011) reported that there was a strong correlation between the chlorophyll content and the rice leaf SPAD values. Leaves exposed to excessive heat at certain times can cause changes in the chlorophyll content (Shaheen et al., 2015). The results for the scoring of the SPAD values indicate the ability of the leaves to continue producing assimilates during an exposure to an increase in ambient temperature. The SPAD values in this study, however, did not show correlation with physiological characters of rice varieties, including photosynthesis rate, stomatal conductance, and transpiration rate.

Another crop response that should be considered as a result of the increase in ambient temperature is the rate of photosynthesis, which affects the production of assimilates to be translocated to all parts of the plant. The change in temperature did not affect photosynthesis rate in this study, and rice varieties showed similar rates of photosynthesis at all elevated temperatures. Photosynthesis rate was determined by the temperature of the plant organ (leaves) when the ambient temperature reached $\pm 32.5^\circ\text{C}$ at the time of measurement). The rice crops have the ability to

Table 1. The photosynthesis rate, stomatal conductance and transpiration rate of rice varieties at elevated temperatures

Treatment	SPAD values	Photosynthesis rate ($\mu\text{mol.m}^{-2}.\text{s}^{-1}$)	Stomatal conductance ($\text{mmol.m}^{-2}.\text{s}^{-1}$)	Transpiration ($\text{mmol H}_2\text{O.m}^{-2}.\text{s}^{-1}$)
Temperature				
27.6°C	39.86a	22.45	0.4668	9.15
28.1°C	31.93b	24.80	0.4601	9.59
29.5°C	33.18b	22.84	0.3688	7.82
P-value	0.04	0.63	0.08	0.08
Rice varieties				
“Ciasem”	36.50a	23.63	0.4204	8.83
“Ciherang”	34.29b	24.35	0.4615	8.09
“IR64”	34.18b	22.11	0.4138	8.64
P-value	0.04	0.12	0.52	0.83
Temperature x rice varieties	ns	ns	ns	ns

Note: values followed by different letters within one column show significant difference according to Least Significant Difference (LSD) test at $P < 0.05$.

reduce the internal temperature (flag leaf) to $\pm 25.5^\circ\text{C}$. This indicates the rate of photosynthesis remains high even in conditions of increasing environmental temperature. According to Yan et al. (2008) there is a $3.61 \pm 0.54^\circ\text{C}$ temperature differences between the internal temperature of the plants and the environment. Therefore the crop can still maintain their photosynthetic rate at high temperatures.

The rate of photosynthesis is also determined by the ability of the leaves to maintain the exchange of CO_2 in the elevated environmental temperature. The increase in elevated temperature in this study tends to reduce stomatal conductance. Stomatal conductance of the rice crops at 29.5°C decreased by 26.6 and 24.8% when compared with 27.6°C and 28.1°C . The results in this study were in line with Chakrabarti et al. (2013), showing that stomatal conductance in leaves decreased at higher temperature conditions. The decrease in stomatal conductance has a close relationship with the rate of transpiration in this study. Based on the correlation test carried out between stomatal conductance and transpiration rate has a strong correlation with the correlation coefficient ($r = 0.7^{**}$), which means a decrease in stomatal conductance may lead to a decrease in the transpiration rate. The results of this study supports the findings by Greer and Weedon (2012) who reported that at high temperature stomatal conductance and CO_2 concentrations in cells tend to decrease and affect the water status in cells. Higher temperature can cause the stomata to close to maintain water balance in the plant cells.

The rate of transpiration tends to decrease due at high environmental temperatures. The transpiration rate at 29.5°C decreased by 17.0 % compared to 27.6°C , and by 22.6% when compared to 28.1°C (Table 1). The lower transpiration rate at 29.5°C was likely due to the stomatal closure to avoid excessive water loss at high temperatures, which is one of the mechanisms of plant adaptation to reduce transpiration rate and maintaining water levels in the plant cells, and to stabilize metabolic processes under stressful conditions.

Conclusion

Environmental temperature of 29.5°C increased rice tiller number by 21.3 to 29.9% and shoot dry weight by 15.1 to 24.8 % compared to 27.6°C . Stomatal conductance and transpiration rate decreased at 29.5°C , but had no significant effects on photosynthesis rates of rice “Ciherang”, “Ciasem” and “IR64”.

Acknowledgment

The authors thanked LPDP (Indonesia Endowment Fund for Education) for funding provided for this research.

References

- Bahuguna, R.N. and Jagadish, S.V.K. (2015). Temperature regulation of plant phenological development. *Environmental and Experimental Botany* **111**, 83–90.
- Barlow, K.M., Christy, B.P., O'Leary, G.J., Riffkin, P.A., and Nuttall, J.G. (2015). Simulating the impact of extreme heat and frost events on wheat crop production: a review. *Field Crops Research* **171**, 109–119.
- Barnabas B., Jager, K., and Feher, A. 2008. The effect of drought and heat stress on reproductive processes in cereals. *Plant Cell and Environment* **31**, 11–38.
- Chakrabarti, B., Singh, S.D., Kumar, V., Harit, R.C., and Misra, S. (2013). Growth and yield response of wheat and chickpea crops under high temperature. *Indian Journal of Plant Physiology* **18**, 7-14.
- Cheikh, N. and Jones, R.J. (1995). Heat stress effects on sink activity of developing maize kernels grown in vitro. *Physiologia Plantarum* **95**, 59–66.
- Endo, M., Tsuchiya, T., Hamada, K., Kawamura, S., Yano, K., Ohshima, M., Higashitani, A., Watanabe, M., and Kawagishi-Kobayashi, M. (2009). High temperatures cause male sterility in rice plants with transcriptional alterations during pollen development. *Plant and Cell Physiology* **50**, 1911–1922.
- Greer, D.H. and Weedon, M.M. (2012). Modeling photosynthetic responses to temperature of grapevine (*Vitis vinifera* cv. Semillon) leaves on vines grown in a hot climate. *Plant Cell and Environment* **35**, 1050-1064.
- Harsant, J., Pavlovic, L., Chiu, G., Sultmanis, S., and Sage, T.L. (2013). High temperature stress and its effect on pollen development and morphological components of harvest index in the C₃ model grass *Brachypodium distachyon*. *Experimental Botany* **64**, 2971-2983.
- Hatfield, J.L. and Prueger, J.H. 2015. Temperature extremes: effect on plant growth and development. *Weather Climate Extremes* **10**, 4-10.
- Hedhly, A., Hormaza, J.I., and Herrero, M. (2009). Global warming and sexual plant reproduction. *Trends in Plant Science* **14**, 30–36.
- Hedhly, A. (2011) Sensitivity of flowering plant gametophytes to temperature fluctuations. *Environmental and Experimental Botany* **74**, 9–16.
- IPCC (Intergovernmental Panel on Climate Change). 2014. Climate change: synthesis report summary for policymakers. <https://www.ipcc.ch>. [July 16, 2017].
- Jagadish, S.V.K., Craufurd, P.Q., and Wheeler, T.R. (2008). Phenotyping parents of mapping populations of rice (*Oryza sativa* L.) for heat tolerance during anthesis. *Crop Science* **48**, 1140–1146.
- Jumiatus, Junaedi, A., Lubis, I., Chozin, M.A., and Miyazaki, A. (2016). Morphological, physiological and yield responses of some rice varieties (*Oryza sativa* L.) as exposed under high temperature in Indonesia. *American Journal of Plant Physiology* **11**, 33-41.
- Kurniawan, D.Y. 2018. "Karakter Morfologi, Fisiologi, dan Fisikokimia Tiga Varietas Padi Sawah pada Kondisi Peningkatan Suhu Lingkungan" [Thesis]. Institut Pertanian Bogor.
- Makarim, K. and Suhartatik, E. (2009). "Morfologi dan Fisiologi Tanaman Padi". Balai Besar Tanaman Padi, Subang.
- Oh-e, I., Saitoh, K., and Kuroda, T. (2007). Effects of high temperature on growth, yield and dry-matter production of rice grown in the paddy field. *Plant Production Science* **10**, 412– 422.
- Prasad, P.V.V., Boote, K.J., and Allen, L.H. (2011). Longevity and temperature response of pollen as affected by elevated growth temperature and carbon dioxide in peanut and grain sorghum. *Environmental and Experimental Botany* **70**, 51–57.
- Ruan, Y.L., Ye, J., Yue, J.Y., Gou, J.L., and John, S.B. (2010). Sugar input, metabolism, and signaling mediated by invertase: role in development, yield potential, and response to drought and heat. *Molecular Plant* **3**, 942-955.
- Shaheen, M.R., Choudhary, M.A., Muhammad, A., and Ejaz, A.W. (2015). Morpho-physiological evaluation of tomato genotypes under high temperature stress conditions. *Journal of The Science of Food and Agriculture* **96**, 2698-

2704.

- Suwa, R., Hakata, H., Hara, H., El-Shemy, H.A., Adu-Gyamfi, J.J., Nguyen, N.T., Kanai, S., Lightfoot, D.A., Mohapatra, P.K., and Fujita, K. (2010). High temperature effects on photosynthate partitioning and sugar metabolism during ear expansion in maize (*Zea mays* L.) genotypes. *Plant Physiology and Biochemistry* **48**, 124–130.
- Teixeira, E.I., Fischer, G., Van-Velthuisen, H., Walter, C., and Ewert, F. (2013). Global hot spots of heat stress on agricultural crops due to climate change. *Agricultural and Forest Meteorology* **170**, 206–215.
- Wahid, A., Gelani, S., Ashraf, M., and Foolad, M.R. (2007). Heat tolerance in plants: an overview. *Environmental and Experimental Botany* **61**, 199–233.
- Wassmann, R., Jagadish, S.V.K., Sumfleth, K., Pathak, H., Howell, G., Ismail, A., Serraj, R., Redoña, E., Singh, R.K., and Heuer, S. (2009). Regional vulnerability of climate change impacts on Asian rice production and scope for adaptation. *Advances in Agronomy* **102**, 91–133.
- Xie, X.J., Shen, S.H.H., Li, X.Y., Zhao, X.Y., Li, B.B., and Xu, D.F. (2011). Effect of photosynthetic characteristic and dry matter accumulation of rice under high temperature at heading stage. *African Journal Agricultural Research* **6**, 1931–1940.
- Yan, C., Ding, Y.F., Liu, Zh., Wang, Q.S., Li, G.H., He, Y., and Wang, S.H. (2008). Temperature difference between the air and organs of rice plant and its relation to spikelet fertility. *Agricultural Sciences* **7**, 678–685.