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# Plant Temperature for Sterile Alteration of Rice

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## 1. Introduction

Temperature affects not only the growth rate, but also reproductive development of rice. When temperature was higher than a critical point value during 5-15 days before heading, rice thermo-sensitive genic male sterile (TGMS) line showed pollen sterility. Otherwise, it would be fertile (Lu *et al* 2001). The relationship between temperature and plant growth or reproductive development is usually studied by using a thermometer screen at a weather station placed at height of 150 cm ($T_A$), although a few studies were also performed to describe more direct and accurate effects of micro-climate temperature at rice canopy on plant growth and reproductive development.

Plant temperature ($T_p$) is regulated by various factors including solar radiation, cloud cover, wind speed, soil heat flux, and transpiration of plant. Besides, temperature and flow velocity of irrigated water have significant effects on rice $T_p$. Many methods and simulations on $T_p$ have been reported (Cellier 1993, Cui *et al* 1989, Ferchinger 1998, Hasegawa 1978, Leuning 1988a, 1988b, Lu *et al* 1998, Tetsuya *et al* 1982, Van *et al* 1989, Wei *et al* 1981, Zhao *et al* 1996). $T_p$ was used as the index of water supply regime for wheat or corn (Cheng *et al* 2000, Huang *et al* 1998, Shi *et al* 1997, Yuan *et al* 2000), freezing injury or grain filling rate of wheat (Feng *et al* 2000, Li *et al* 1999, Liu *et al* 1992, Xiang *et al* 1998, Xu *et al* 2000). In recent studies, it was found that the fertility alteration of rice TGMS line was sensitive to $T_p$ (Lu *et al* 2007, Xu *et al* 1996). Lu *et al* (2004, 2007) found that the sterility of TGMS was simulated more accurately by temperature at stem part of 20 cm height or air temperature ($T_a$) around the part when compared with $T_A$ at a weather station (Lu *et al* 2004, 2007, Zou *et al* 2005). So far, little is known about how $T_p$ is regulated by microclimate or irrigated water (Hu *et al* 2006).

## 2. Plant temperature and its simulation model of thermo-sensitive male sterile rice

The present chapter was performed to investigate the temporal and spatial distribution of $T_p$ and its relationships with microclimate of canopy and irrigated water by using a TGMS line under irrigated and non-irrigated conditions. Two models were established to understand how $T_p$ is regulated by environments.

A TGMS rice line, Peiai64S, was used as plant material. Flowing irrigated water depth of 10-15 cm was treated, and no irrigated (keeping humid) was treated as control.

$T_p$ and microclimatic factors were determined as below:

$T_p$: PTWD-2A sensors were inserted in stem sheaths at heights of 10 cm, 20 cm, 30 cm and 40 cm, respectively, to measure $T_p$.

Air, water and soil temperatures: PTWD-2A sensors were placed at 10 cm and 5 cm under the ground, and 5 cm, 20 cm, 40 cm, 60 cm, 100 cm, 150 cm above the ground, respectively, to measure temperatures of soil, water and air.

Wind speed: EC-9S sensors were used to measure wind speed at height of 150 cm.

All above data were obtained using TRM-ZS1, automatically collecting every 10 seconds and storing every 10 minutes.

When 50% of flag leaves appeared perfectly, five plants were sampled and dissected at height of each 10 cm to determine layered LAI. Layered LAI was determined with traditional method, i.e., calculated according to the dry matter of harvested layer and the specific leaf area of each layer from sampled leaves (SLA).

### 2.1 Change of rice $T_p$

#### 2.1.1 Daily change of $T_p$

Fig. 1 showed the daily changes of air temperature at height of 150 cm ($T_A$) and $T_p$ at heights of 20 cm and 40 cm ($T_{p20}$ and $T_{p40}$) under non-irrigated condition. The data were collected from random 30 days, during which 11 d were sunshine, 9 d were cloudy, and 10 d were overcast. $T_p$ showed different value from $T_A$, although they had a similar trend in daily change. $T_A$ and $T_p$ could be simulated by using same parameters and a similar equation.

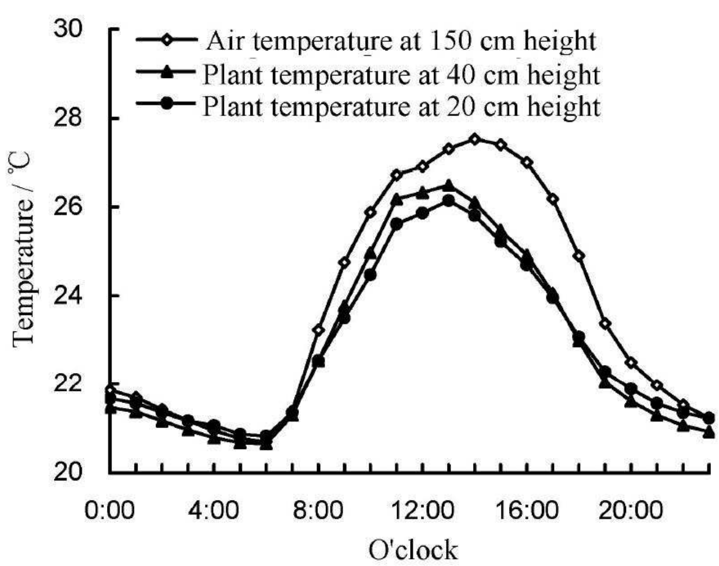



Fig. 1. Daily change of air temperature at 150 cm and plant temperature at 20 cm, 40 cm heights.

Calculated the data of Fig. 1, result showed that, during 08:00-20:00, $T_p$ was significantly lower than $T_A$. $T_{p20}$ was lower than $T_A$ by an average of 1.44°C and the largest margin of 2.3°C, while $T_{p40}$ was lower than $T_A$ by an average of 1.25°C and the largest margin of 2.1°C.

During 21:00-07:00, $T_{p20}$ and $T_{p40}$ were lower than $T_A$ only by 0.27°C and 0.06°C, respectively.

Fig. 1 and its simulation equations (1), (2), (3) showed that, during daytime (06:00-18:00), the variation of both $T_p$ and $T_A$ fit the sinusoid curve, but the coefficient showed $T_A > T_{p40} > T_{p20}$. During night time (18:00-06:00), the variation of both $T_p$ and $T_A$ fit the exponent curve, but the coefficient showed $T_A > T_{p40} \approx T_{p20}$. The simulation equations and their effects were described as follows：

$$T_{p20} = \begin{cases} 20.8 + 5.3\sin\left[\pi(t-5.78)/14.44\right] & 06:00 \le t \le 18:00 \\ \left\{19.54 + 2.3\exp\left[-(t+6)/4\right]\right\}/0.94 & t < 06:00 \\ \left\{19.54 + 2.3\exp\left[-(t-18)/4\right]\right\}/0.94 & t > 18:00 \end{cases} \quad R^2 = 0.956^{**} \tag{1}$$

$$T_{p40} = \begin{cases} 20.7 + 5.8\sin\left[\pi(t-5.78)/14.44\right] & 06:00 \le t \le 18:00 \\ \left\{19.38 + 2.3\exp\left[-(t+6)/4\right]\right\}/0.94 & t < 06:00 \\ \left\{19.38 + 2.3\exp\left[-(t-18)/4\right]\right\}/0.94 & t > 18:00 \end{cases} \quad R^2 = 0.957^{**} \tag{2}$$

$$T_{A} = \begin{cases} 20.7 + 6.8\sin\left[\pi(t-5.78)/16.44\right] & 06:00 \le t \le 18:00 \\ \left\{19.32 + 4.2\exp\left[-(t+6)/4\right]\right\}/0.94 & t < 06:00 \\ \left\{19.32 + 4.2\exp\left[-(t-18)/4\right]\right\}/0.94 & t > 18:00 \end{cases} \quad R^2 = 0.972^{**} \tag{3}$$

Differences (visual temperature difference from thermometer, >0.2°C) were detected in value and time between the maximum $T_p$ and the maximum $T_A$. The daily maximum value was 26.1°C for $T_{p20}$, and 26.5°C for $T_{p40}$, whereas the maximum value was 27.5°C for $T_A$. The maximum value of $T_p$ occurred at 13:00, 1 h earlier than that of $T_A$. Their minimum values, however, both appeared at 06:00. The fluctuation in daily change also showed significant difference: $T_A$ (6.8°C) > $T_{p40}$ (5.8°C) > $T_{p20}$ (5.3°C).

### 2.1.2 Difference of $T_p$ at vertical height

Fig. 2 showed $T_p$ and $T_a$ at different heights observed at 06:00 and 13:00 under non-irrigated condition (30 d, same as Fig.1). Although the differences between $T_p$ and $T_a$ were not significant, $T_p$ at 13:00 at heights of 10 cm, 20 cm, 30 cm and 40 cm were all higher than $T_a$ at corresponding heights without exception. $T_{p40}$ and $T_{p20}$ were higher by 0.24°C and 0.60°C, respectively. The decreased rate of $T_p$ (0.1°C /10 cm) was lower than that of $T_a$ (0.41°C /10 cm). At 06:00, $T_p$ at 30-40 cm was higher than $T_a$, whereas it showed an opposite difference at 10-20 cm.

### 2.2 Effects of environmental factors on $T_p$

Solar radiation and irrigated water were two main heat sources affecting rice $T_p$. Cloud cover, wind speed and LAI of canopy also affect $T_p$ by regulating heat transmission or radiation intensity. Temperature and flowing speed of irrigated water were two main factors regulating $T_p$.

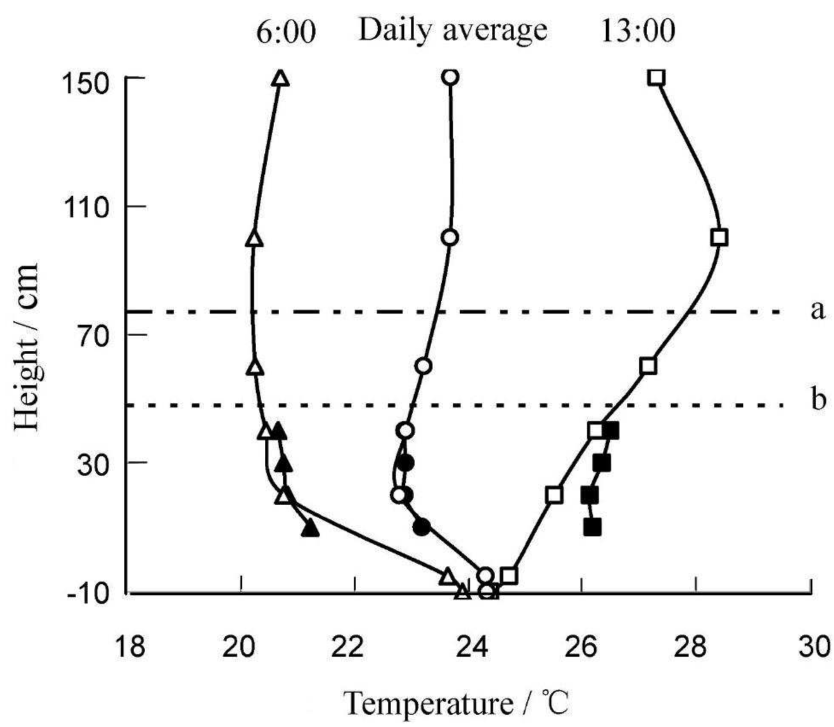
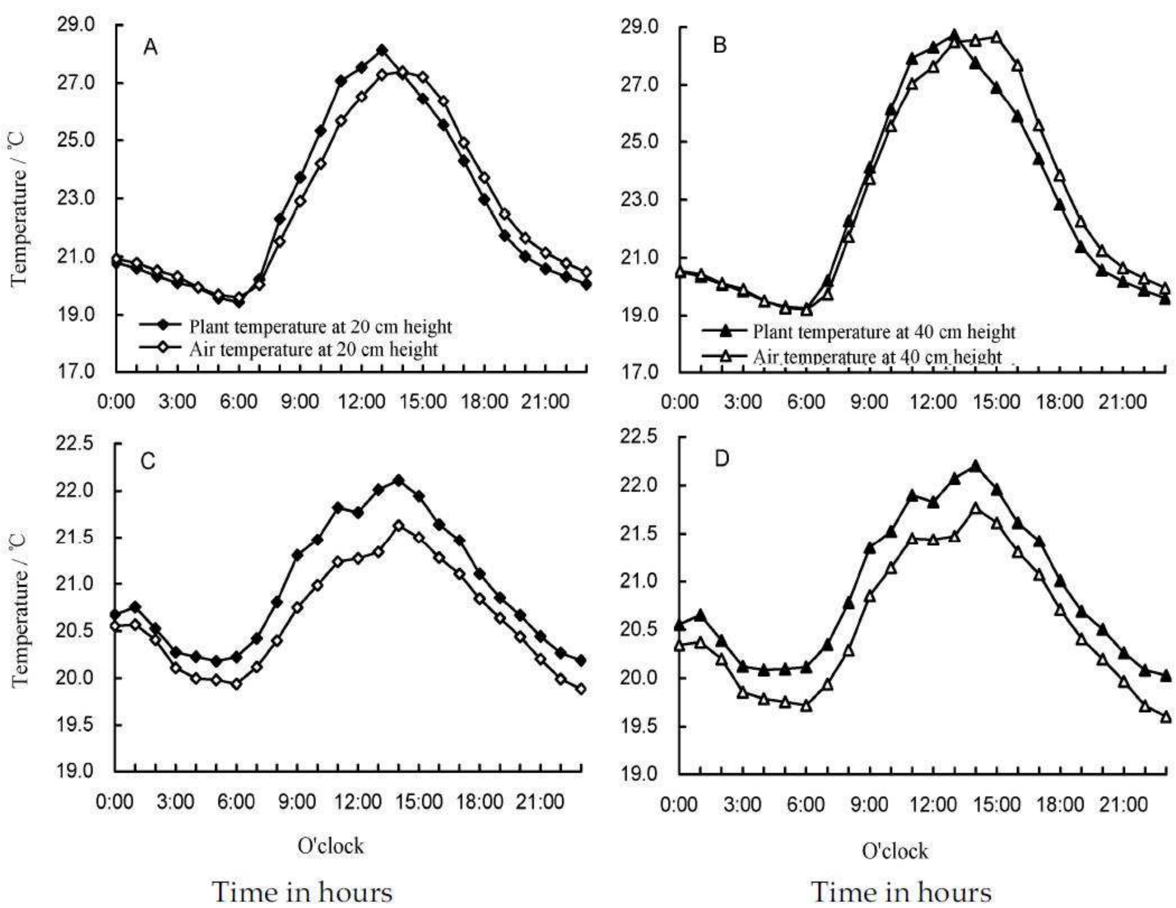



Fig. 2. Plant (▲●■) and air (△○□) temperatures at different heights. a and b lines denotes plant height and layer of maximum leaf density, respectively.

### 2.3 Effects of $T_a$ on $T_p$

#### 2.3.1 Change of $T_a$ and its effects on $T_p$

Fig. 3 showed daily changes of $T_p$ and $T_a$ at heights of 20 cm and 40 cm under sunshine (11 d) and cloudy (10 d) days. During 06:00-13:00 in sunshine days, $T_p$ was increased earlier than $T_a$ by 1 h, and $T_p$ was maximized at 13:00 (28.1°C for $T_{p20}$, and 28.7°C for $T_{p40}$). In contrast, $T_a$ was increased later than $T_p$ by 1 h, and $T_a$ was maximized at 14:00 (27.4°C for $T_{a20}$, and 28.5°C for $T_{a40}$). Besides, the raising intensity was $T_p$ stronger than $T_a$ by 0.7°C (20 cm) and 0.2°C (40 cm). $T_p$ showed close to or even little lower than $T_a$ during night time (18:00-06:00). The significant difference between $T_a$ and $T_p$ during daytime might be caused by the larger absorption of solar radiation by plant than air. When the heat was absorbed, the plant released its energy in long wave, which resulted in an increase of $T_a$ around the plant. After 13:00, along with the diminishing of solar radiation, $T_p$ began to decrease but $T_a$ was reacted dully (decreased one or two hours later under sunny days). Under cloudy days, $T_p$ was higher than $T_a$ all the day. At heights of 20 cm and 40 cm, $T_p$ was 0.4°C higher on average, and the maximum one by 0.5°C for $T_{p20}$ and 0.4°C for $T_{p40}$, and the minimum one by 0.3°C for $T_{p20}$ and 0.4°C for $T_{p40}$, respectively. This suggested that there was a weak exchange between plant and air on cloudy days.




A and B denote sunny day with sunshine time >8 h; C and D denote overcast day with no sunshine time.

Fig. 3. Daily change of air and plant temperature at 20 cm and 40 cm heights.

### 2.3.2 Statistical relation of $T_p$ to $T_A$

In the present chapter, $T_p$ at 20 cm and 40 cm, and $T_A$ at 150 cm observed at 06:00 and 13:00 were used to establish linear regression equations. Results showed that the regression coefficient of $T_{p40}$ was lower than that of $T_{p20}$, confirming that solar radiation was the key resource of heat in plant.

06:00 $T_p$: 20 cm: $T_{p20}$ =0.9949 $T_A$ + 0.1741 $R^2$ = 0.992**, $n$ = 30

40 cm: $T_{p40}$ =0.9670 $T_A$ + 0.8885 $R^2$ = 0.995**, $n$ = 30

13:00 $T_p$: 20 cm: $T_{p20}$ =0.9338 $T_A$ + 2.3121 $R^2$ = 0.973**, $n$ = 30

40 cm: $T_{p40}$ =0.9199 $T_A$ + 2.3416 $R^2$ = 0.982**, $n$ = 30

Daily average: 20 cm: $T_{p20}$ =0.9338 $T_A$ + 1.6011 $R^2$ = 0.996**, $n$ = 30

40 cm: $T_{p40}$ =0.9205 $T_A$ + 1.7991 $R^2$ = 0.997**, $n$ = 30

### 2.4 Effect of wind speed

Fig. 4 showed the relationship between $T_{p20}$ and wind speed at 150 cm (V). The equation was: $T_{p20} = -14.411V + 32.622$ ($R^2$=0.334*, $n$=39). The result indicated that wind speed on the top of canopy had a significant effect on $T_p$.

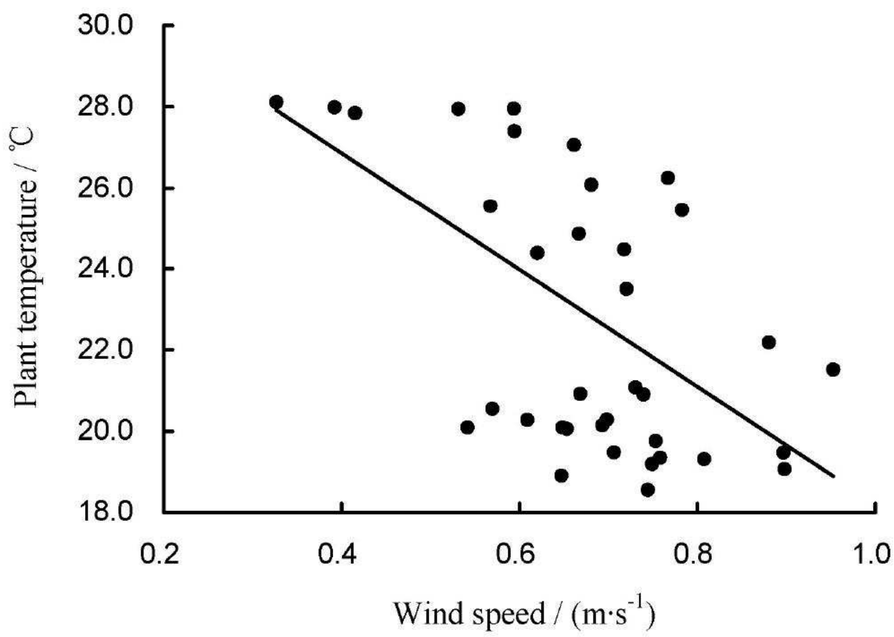



Fig. 4. Relationship between plant temperature at 20 cm and wind speed at 150 cm.

### 2.5 Effects of irrigated water

Irrigated water was another important effect regulating rice $T_p$. Water temperature ($T_w$) and $T_a$ were varying in different manners, which resulted in a remarkable difference in their effects on $T_p$.

#### 2.5.1 Temporal change of effects of irrigated water on $T_p$

Fig. 5 showed daily varying curves of $T_p$ (30 d) at 20 cm and 40 cm of plants grown with and without irrigated water. The average temperature of inflow water was 26.9°C (21.5-34.0°C), and $T_A$ was 23.7°C (13.5-36.5°C). Irrigated water increased $T_p$ at 20 cm and 40 cm by 0.7°C and 0.5°C, respectively, or by 0.35°C and 0.25°C, respectively, for per 1°C of water-air temperature margin. The effect of increased $T_p$ by irrigated water was higher at night than at daytime. $T_{p20}$ was increased by 1.01°C during 19:00-05:00, and by 0.37°C during 06:00-18:00, but decreased by 0.19°C during 11:00-14:00. At 40 cm, $T_{p40}$ was increased by 0.77°C during 19:00-05:00, and by 0.20°C during 06:00-18:00, but decreased by 0.25°C during 11:00-13:00. The results showed that irrigated water had more significant effects on $T_p$ during night than daytime. In daytime, solar radiation partly withstood the effects of water irrigated. At the noon when solar radiation was the maximum, irrigated water decreased $T_p$.

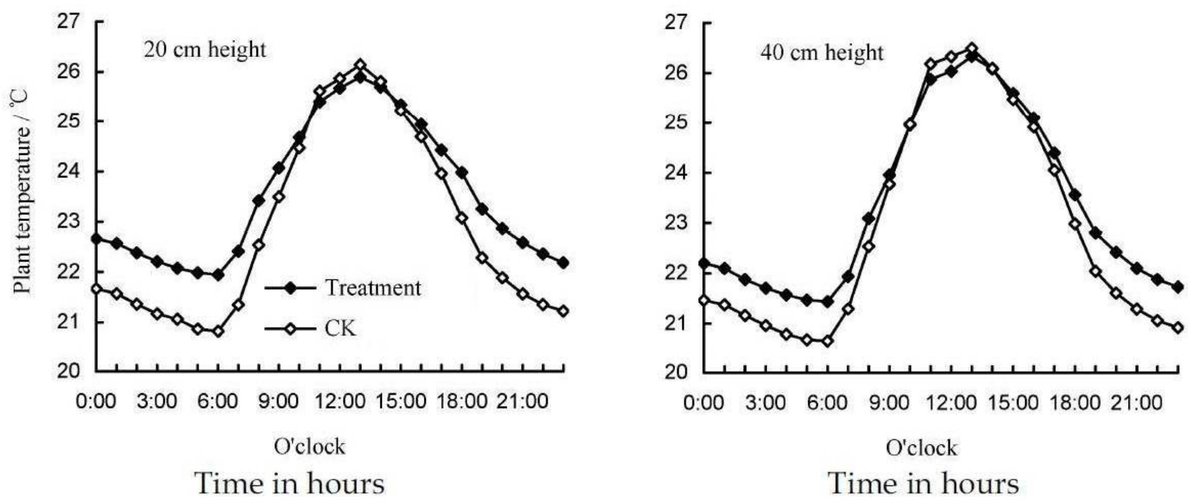



Fig. 5. Daily change of plant temperature at 20 cm and 40 cm under irrigated treatment and non-irrigated conditions.

### 2.5.2 Effects of irrigated water on $T_p$ at vertical height

$T_p$ (30 d, same as Fig. 1) at four heights at 06:00 and 13:00 was compared between irrigated and non-irrigated conditions (Table 1). The effect of irrigated water on $T_p$ was decreased lower along with the increase of height. It was a difference by 2.5°C at 10 cm, 0.6°C at 20 cm, 0.5°C at 30 cm, and 0.4°C at 40 cm. At 06:00, irrigated water could increase $T_p$ (increasing rate of 3.7°C at 10 cm), but the increasing rate was lowered along with the increasing of plant height. Around noon, $T_p$ showed 0.7°C increase at 10 cm but a decrease at 20-40 cm. The effect of irrigated water on $T_p$ was stronger at the lower site than at the higher site in plants.

| Item | | 06:00 | 13:00 | Daily average |
|---|---|---|---|---|
| | Irrigated 10 cm | 24.7±9.4 | 26.9±7.6 | 25.7±8.3 |
| $T_{p10}$ | Non-irrigated | 21.2±8.5 | 26.2±8.0 | 23.2±7.6 |
| | Difference | 3.5±3.9 | 0.7±3.5 | 2.6±2.2 |
| | Irrigated 10 cm | 21.9±8.7 | 25.9±7.9 | 23.5±8.0 |
| $T_{p20}$ | Non-irrigated | 20.8±8.4 | 26.1±8.1 | 22.9±7.5 |
| | Difference | 1.1±1.5 | -0.2±1.9 | 0.7±1.0 |
| | Irrigated 10 cm | 21.8±8.4 | 26.0±7.4 | 23.4±7.6 |
| $T_{p30}$ | Non-irrigated | 20.8±8.3 | 26.4±8.6 | 22.9±7.6 |
| | Difference | 1.0±2.3 | -0.3±2.0 | 0.5±1.0 |
| | Irrigated 10 cm | 21.4±8.3 | 26.3±8.3 | 23.3±7.7 |
| $T_{p40}$ | Non-irrigated | 20.7±8.4 | 26.5±8.6 | 22.9±7.6 |
| | Difference | 0.8±1.0 | -0.2±1.0 | 0.5±0.5 |

Note: Difference: difference of plant temperature between irrigated water of 10 cm depth and non-irrigated.

Table 1. Comparison of plant temperature observed at four heights at 06:00 and 13:00 between irrigated and non-irrigated conditions

### 2.5.3 Effects of irrigated water temperature on $T_p$

Irrigated water temperature also regulated $T_p$, due to the difference between water and air temperature. The simulated equation between water-air temperature difference ($T_{w-A}$) and average $T_A$ of 117 days was： $T_{w-A} = \pm 25.8\left[1-(\frac{T_A-10.6}{19.0})^{0.048}\right]^{0.5}$ ($R^2$=0.422**, $n$=117), based on the weather record ($T_A$ from 17.4-30.5°C with the average of 23.7±8.6°C, $T_w$ from 22.2-31.9°C with the average of 26.9±6.7°C). It implied that, when $T_A$=29.6°C, then $T_{w-A}$=0°C；when $T_A$＞29.6°C, $T_{w-A}$ was lower than $T_A$, irrigated water decreased $T_p$. Conversely, when $T_A$＜29.6°C, irrigated water increased $T_p$. When $T_A$ decreased from 29.6°C, $T_{w-A}$ would be decreased in power. When $T_A$ decreased from 27.4°C to 22.0°C (decreased by 5.4°C), $T_{w-A}$ was enlarged from 2°C to 4°C. When $T_A$ decreased from 16.5°C to 12.9°C (decreased only by 3.6°C), $T_{w-A}$ was enlarged from 6°C to 8°C. Obviously, the regulatory effect would be enlarged under a lower $T_A$.

Fig. 6 showed the relationship in $T_p$ difference (at 20 cm and 40 cm) between irrigated and non-irrigated ($\Delta T_p$) and the difference between water-air temperature ($T_{w-A}$, average value of 720 samples) (equation 4 and 5). The results showed that, in a range of (-5.45°C)–(+10.32°C) for $T_{w-A}$, $\Delta T_p$ and $T_{w-A}$ showed a significant conic relationship. When $T_{w-A}$= -5°C, $\Delta T_p$ at 20 cm and 40 cm was -1.10°C and -0.71°C, respectively. When $T_{w-A}$=5°C , $\Delta T_p$ at 20 cm and 40 cm was 0.84°C and 0.66°C, respectively. When $T_{w-A}$=10°C, $\Delta T_p$ at 20 cm and 40 cm was 1.42°C and 1.26°C, respectively.

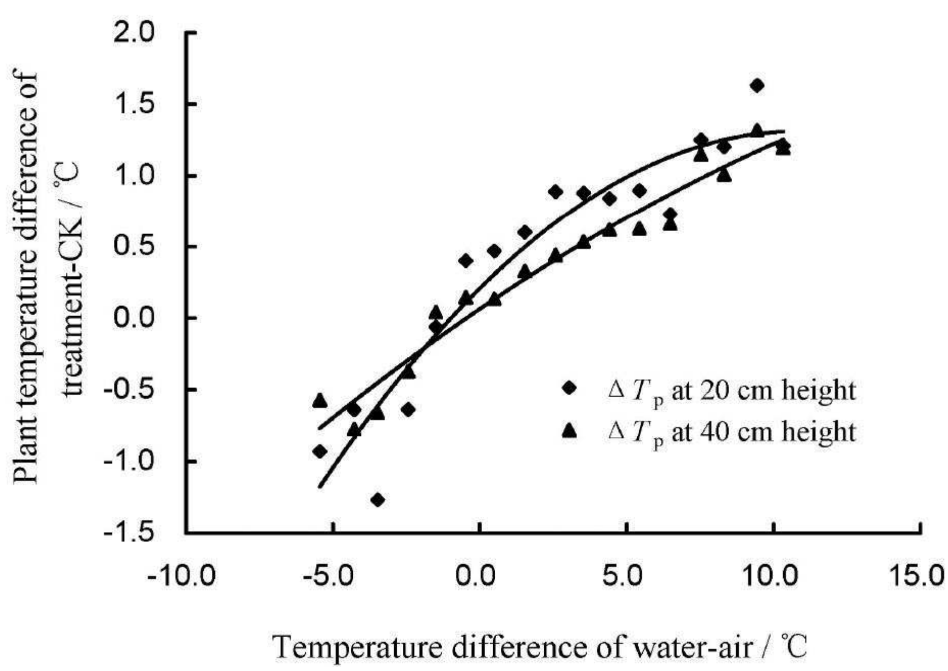



Fig. 6. Relationship between plant temperature difference of irrigated-non-irrigated ($\Delta T_p$) and temperature difference of water-air ($T_{w-A}$).

$$\text{20 cm：} \Delta T_{p20} = -0.0051\, T_{w-A}^2 + 0.1934\, T_{w-A} \quad R^2 = 0.870^{**}, n = 17 \tag{4}$$

$$\text{40 cm：} \Delta T_{p40} = -0.0011\, T_{w-A}^2 + 0.1368\, T_{w-A} \quad R^2 = 0.950^{**}, n = 17 \tag{5}$$

### 2.5.4 Effects of flowing speed of irrigated water on $T_p$

Flowing speed of irrigated water was also an important factor affecting $T_p$. The margin of $T_w$ between inflow and outflow (50 m between them) denotes the flowing speed of irrigated water. The equation of $T_p$ established by margin of $T_w$ between inflow and outflow of 13 days (with $T_A$=17.4-22.5°C) showed that $T_p$ was affected significantly by flowing speed of irrigated water (Fig.7).

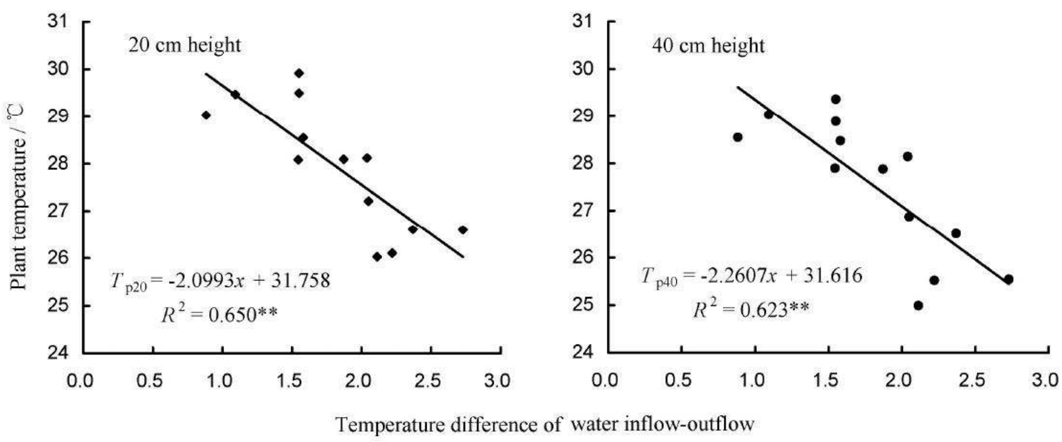



Fig. 7. Relationship between plant temperature and temperature difference of water inflow-outflow.

### 2.6 Effects of LAI of canopy on $T_p$

Rice layered LAI was decreased along with the increase of plant height. The relationship between $T_p$ at 06:00 and 13:00 and their corresponding LAI at 5 heights was shown in Fig. 8. At 13:00, $T_p$ was increased (actual value was increased from 26.9°C to 28.4°C) along with the decrease of the accumulated LAI. At 06:00, $T_p$ was decreased (actual value was decreased from 20.6°C to 19.7°C) along with the decrease of the accumulated LAI. It indicated that the canopy absorbed solar radiation at daytime and released the heat energy during night, which regulated $T_p$.

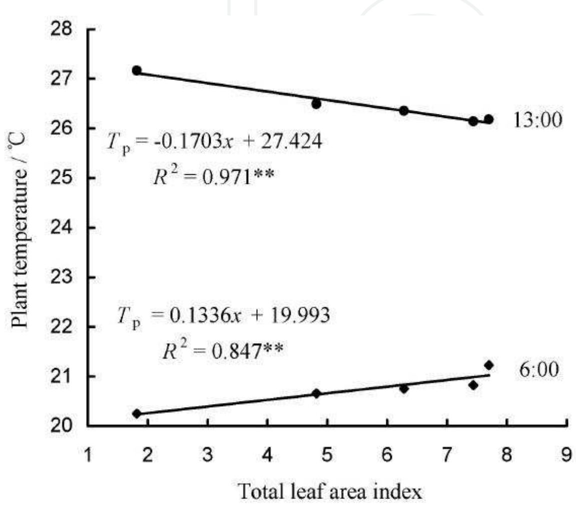



Fig. 8. Relationship between plant temperature ($T_p$) and LAI ($x$) at 06:00 and 13:00.

### 2.7 Simulation model of rice $T_p$ by environmental factors

#### 2.7.1 Analysis on correlations between $T_p$ and environmental factors

Table 2 listed the correlations between $T_p$ and weather or field environmental factors. It implied that $T_p$ was regulated by various factors of canopy.

| Item | $T_{p20}$ | $T_{p40}$ | $T_{a20}$ | $T_{a40}$ | 150 cm Wind speed (V) | Inflow tempe-rature ($T_{in}$) | Outflow tempe-rature ($T_{out}$) | $T_A$ | Sunshine hours (S) |
|---|---|---|---|---|---|---|---|---|---|
| $T_{p20}$ | 1 | 0.997** | 0.985** | 0.990** | -0.360** | 0.944** | 0.973** | 0.981** | 0.260* |
| $T_{p40}$ | | 1 | 0.982** | 0.995** | -0.366** | 0.944** | 0.971** | 0.988** | 0.270* |
| $T_{a20}$ | | | 1 | 0.973** | -0.392** | 0.941** | 0.971** | 0.971** | 0.320* |
| $T_{a40}$ | | | | 1 | -0.359** | 0.950** | 0.975** | 0.994** | 0.296* |
| 150 cm Wind speed (V) | | | | | 1 | -0.330* | -0.372** | -0.390** | -0.200 |
| Inflow water temperature ($T_{in}$) | | | | | | 1 | 0.977** | 0.943** | 0.362** |
| Outflow water temperature ($T_{out}$) | | | | | | | 1 | 0.967** | 0.316* |
| $T_A$ | | | | | | | | 1 | 0.269* |
| Sunshine hours (S) | | | | | | | | | 1 |

* Sample no. 60, $\alpha_{0.05}$=0.259, $\alpha_{0.01}$=0.335.

Table 2. Correlations between plant temperature and weather or field environmental factors

#### 2.7.2 Models of $T_p$ at heights of 20 cm and 40 cm

Four indices were chosen to establish models for $T_p$ at heights of 20 cm and 40 cm in the present study: average temperature of inflow and outflow water [$(T_{in}+T_{out})/2$], temperature margin between inflow water and air at 150 cm ($T_{in}$-$T_A$), wind speed at 150 cm (V), and sunshine hours (S). Results showed that both of two equations showed significant high $R^2$ (equations 6 and 7 and Fig. 9). Among the five parameters, $T_{in}$ and $T_{out}$ can be obtained by an actual measure, and $T_A$, V and S can be obtained from local weather station.

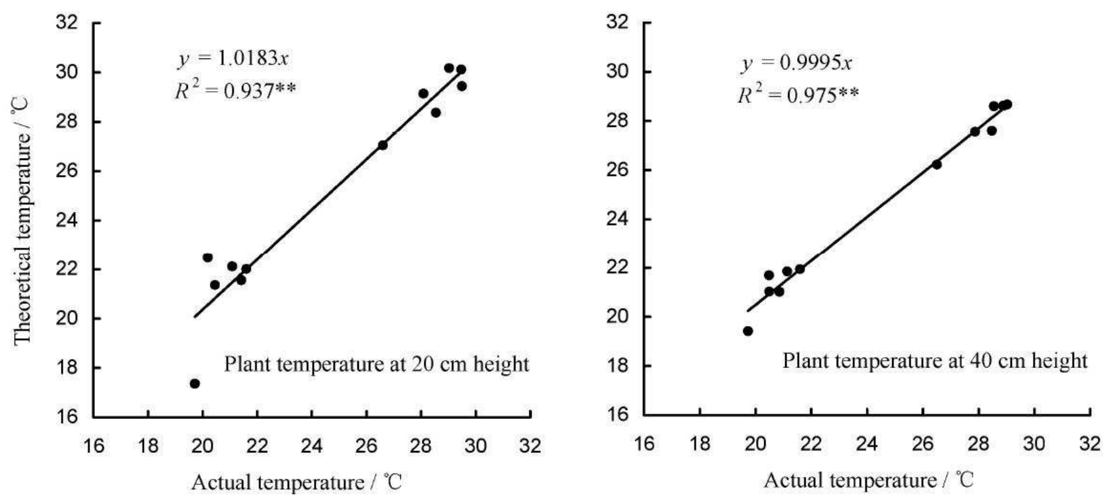



Fig. 9. Simulation effect of daily plant temperature at 20 cm and 40 cm heights.

Validation test showed that, the theoretical and practical values of $T_{p20}$ and $T_{p40}$ had significant correlations, and the slopes of the linear equation were 1.0183 and 0.9995, close to 1 (Fig. 9). The relative error [(∑ | practical−theoretical | /practical)/*n*] of $T_{p20}$ and $T_{p40}$ were 3.93% and 2.95%, respectively.

$T_p$ of 20 cm:

$$Tp20 = 0.964(T_{in} + T_{out}) / 2 - 0.803(T_{in} - T_A)V + 0.085(12 - S) \quad R^2 = 0.993^{**}, n = 61 \qquad (6)$$

$T_p$ of 40 cm:

$$Tp40 = 0.937(T_{in} + T_{out}) / 2 - 0.595(T_{in} - T_A)V + 0.063(12 - S) \quad R^2 = 0.998^{**}, n = 15 \qquad (7)$$

### 2.8 Function for rice plant temperature

It could be traced root as early as 1960's when field microclimate regulated by irrigated water was researched. The detailed procedure, however, was not described until two-line hybrid rice was applied (Xiao *et al* 2000, Zhou *et al* 1993). The sterility of rice TGMS line was controlled by temperature. A credible alteration point in temperature was important not only for selection and identification of TGMS lines, but for monitoring sterility alteration, determining effective methods to keep sterility of such TGMS, and increasing seed production of two-line hybrid rice (Lu *et al* 2004, 2007). In the practice of past two decades, techniques commonly used temperatures from thermometer-screen which was located in a 25×25 $m^2$ green plot and 150 cm height in local weather station ($T_A$). The sensitive part of rice plant to temperature, however, was in its canopy (Lu *et al* 2004, 2007, Zou *et al* 2005). We have noticed that the sterility of rice TGMS lines was affected directly by $T_p$ rather than $T_A$. Therefore, it would be more accurate to monitor sterility of TGMS by using $T_p$ in sensitive organs (Lu *et al* 2007). $T_p$ was the final consequence of various environmental factors including air, water, soil, and the heat exchange among them. When attacked by low temperature during sensitive stage, how does the plant response, and how are agronomical methods used for safeguarding the sterility of rice TGMS? Such issues should be addressed by further studies. It will be too late to guarantee seed production of TGMS rice if only traditional methods are used. Simulation models of rice $T_P$ established in this chapter and its regulation by environmental factors such as by inflow and outflow temperature of irrigated water, and $T_A$, wind speed, sunshine hours from local weather station have provided a more effective method for seed production of two-line hybrid rice.

## 3. Plant temperature for sterile alteration of a thermo-sensitive genic male sterile rice, Peiai64S

Rice thermo-sensitive genic male sterile (TGMS) line showed their sterile alteration along with the temperature change. An exact parameter to indicate sterile alteration was useful not only for breeding and identification of such TGMS, but was also helpful to determine and estimate the sterility security of TGMS, and select suitable methods for safeguarding the sterility of TGMS in two line hybrid rice seed production. Up to date, the forecast of TGMS sterile alteration was only based on the daily average temperature determined by local weather station (screen temperature at a height of 150 cm in a 25 m × 25 m green plot), and used three days average temperature as the alteration point temperature (from sterility transformed to partial fertility, seed setting rate from zero increased to 0.5%) (Lu *et al* 2001, Yao *et al* 1995). For instance, a TGMS, Peiai64S, set its alteration point temperature to be 23.5-24°C for average temperature with three days duration. When the three days average temperature was higher than the point, Peiai64S will exhibit sterile, otherwise partial fertility (Liao *et al* 2000, Lu *et al* 1999, Zou *et al* 2003). However, rice production practices showed that such parameter revealed its shortages in the following points: I: Temperature forecasted by a weather station was the same value within a county (or a city), and it could not express the difference of microclimate of individual field. Furthermore, the ground of screen in the weather station was different from rice field. It would cause the difference of temperature (Xie *et al* 2001). II: The sensitive part of rice to the environment condition was lower than the height of screen (Xu *et al* 1996), which would cause the difference of temperature as well. III: It was confirmed that when TGMS was attacked by lower temperature weather, it was useful of water irrigated to increase field or plant temperature for maintaining its sterility (Lu *et al* 2004, Xiao *et al* 1997, 2000, Zou *et al* 2005). However, it is difficult to estimate the increased degree of temperature by such forecasted screen temperature. IV: Seed production practices of two-line hybrid rice showed that under the same lower temperature weather, individual field exhibited diversified seed purity for the differences of microclimate. It is inaccurate to estimate fertility by screen temperature. V: The alteration point temperature for fertility used only the upper point temperature (seed setting rate from zero to 0.5%) and no scale for lower and optimum points. Also, there is no research report for such item so far. The author consider that the fertility of TGMS was affected by plant temperature, which was caused by all environmental conditions including air, water, soil, wind and so on. During the fertility sensitive period, for rice TGMS of each seed production field, the damaged degree, and the effect of measures against lower temperature must be estimated immediately. If these are estimated only by the pollen fertility even seed set, it will be late for taking measures to avoid such damage of lower temperature. Thus, it is important to establish an effective estimating and adjusting method for safeguarding seed production of two-line hybrid rice.

The temperature indices of sterile alteration must include two aspects as parameter of type and scale. The parameter type denoted the type of temperature (screen temperature of average or maximum or minimum one, otherwise as plant temperature of stem or leaf). The temperature scale will include the upper point (seed setting rate from zero increased to 0.5%), the optimum (show highest seed setting rate), and the lower point (seed setting rate returned to zero again) of temperature, which were considered as the three basic temperature points. The present chapter was aimed to establish such three basic temperature points.

A lower thermo-sensitive genic male sterile line, Peiai64S, which was widely used in two-line hybrid rice breeding, was chosen as plant material. During the fertility-sensitive period of all treatments, the microclimate and plant temperature were regulated by irrigated water. Three irrigated depths of 5 cm, 10 cm, and 15 cm, each of these with flowing and staying-water were used. Flowing irrigated water of 15 cm depth was also treated. During the fertility sensitive stage, the stem temperature at plant height of 20 cm was measured continually per 10 s with an improved needle thermocouple sensor under various types of weather. At the fertility sensitive stage, the plant and panicle height were determined, and furthermore the distance between the last two leaves. For all the sowing date treatments, their self-fertilized seed setting rate was measured by 30 paper bag insulated panicles.

### 3.1 Temperature differences between rice field and screen of weather station

Table 3 shows the temperature differences between the screen of weather station and rice field at heights of 150 cm, 100 cm, 60 cm, 40 cm, and 20 cm. The four periods in Table 3 are: I: from 20 August to 29 September in 2004 and from 9 August to 30 September in 2005. II: the earliest lower temperature days, 3 to 5 September in 2004. III: the earliest lower temperature days, 18 to 20 August in 2005. IV: the highest temperature day of 16 August, 2005. Table 3 shows that under various weather conditions, the temperatures of rice field at each height were different from the screen one of the weather station.

| Tempe-rature | I | | II | | III | | IV | |
|---|---|---|---|---|---|---|---|---|
| Location | Tempe-rature | Difference # | Tempe-rature | Difference | Tempe-rature | Difference | Tempe-rature | Difference |
| Screen | 24.6±5.82 | | 23.0 | | 22.7 | | 32.0 | |
| 150cm | 23.91±5.82 | 0.69** | 22.89 | 0.11 | 22.32 | 0.38 | 31.05 | 0.95 |
| 100cm | 23.50±6.80 | 1.10** | 22.79 | 0.21 | 22.21 | 0.49 | 30.79 | 1.21 |
| 60cm | 23.77±5.94 | 0.83** | 22.86 | 0.14 | 22.33 | 0.37 | 30.68 | 1.32 |
| 40cm | 23.42±5.88 | 1.18** | 22.69 | 0.31 | 22.18 | 0.52 | 30.21 | 1.79 |
| 20cm | 22.97±5.29 | 1.63** | 22.46 | 0.54 | 22.25 | 0.45 | 29.44 | 2.56 |

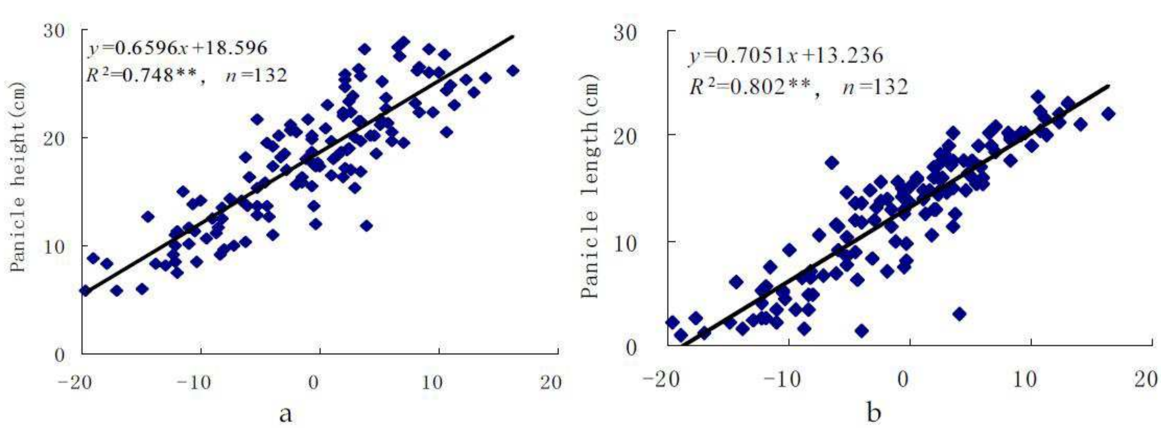
# Temperature difference between screen and various heights in rice field. ** Different at $p<0.01$.

Table 3. Temperatures at different locations in rice field and their difference when compared to that in weather station

Owing to the difference in the underlay surface and growing plant, the temperature at each canopy height was lower than that of the screen of weather station in the four periods. For the period Ⅰ, which represented the various weather conditions, the air temperature at height of 150 cm showed 0.69°C lower than the screen temperature of the weather station, which was at the same height. At heights of 100 cm, 60 cm, 40 cm, and 20 cm, owing to the energy absorbing and reflecting, temperature difference was enlarged at the four heights. The average temperature at heights of 100 cm, 60 cm, 40 cm, and 20 cm of period Ⅰ was significantly lower than screen one by 1.10°C, 0.83°C, 1.18°C, and 1.63°C, respectively. A tendency of larger difference along with the height increase was seen, and the largest one was seen at 20 cm.

### 3.2 Fertility sensitive position and its height

It was reported that developing panicle was the fertility sensitive part of rice (Xu *et al* 1996). Thus, panicle height was important for determining the fertility sensitive position and the water depth for irrigation to regulate the temperature of the fertility sensitive part. The preceding studies proved that the sensitive stage was around the stage of meiosis of pollen mother cells (panicle developing stage IV – VI), and the visible morphological trait was at ±5 cm distance between the last upper two leaves (DL) (Lu *et al* 2001). Fig. 10 shows the correlation between the panicle height, length, and the DL. Result showed that during the stage, panicle height from the base was 15.3-21.9 cm with an average value of 18.5 cm, and meanwhile, the panicle length was 9.7-16.8 cm with an average value of 13.2 cm when DL was ±5 cm. It indicated that height of 20 cm was the suitable location for fertility sensitivity.




** Significant at $p$<0.01.

Fig. 10. Correlation of distance between the uppermost two leaves and panicle height (A) or length (B) of Peiai64S.

Table 4 shows the correlation coefficients between the self-fertilized seed setting rate and air temperature at various plant heights or screen one with a liner, conic, and present model (See the part '3.3 Model of fertility-temperature'). The result showed that the coefficients between the self-fertilized seed setting rate and stem or air temperature at 20 cm height were the highest and showed significant correlation.

It indicated that height of 20 cm was the location expressing the highest correlation coefficient. The result happened to meet the height of panicle at that time. Thus, 20 cm was taken as the suitable location for expressing the fertility of TGMS.

| Item | 20 cm Stem tempe-rature | 20 cm Air tempe-rature | 40 cm Air tempe-rature | 60 cm Air tempe-rature | 100 cm Air tempe-rature | 150 cm Air tempe-rature | Screen tempe-rature |
|---|---|---|---|---|---|---|---|
| Line model | -0.316** | -0.410** | -0.275* | -0.225* | -0.224* | -0.200 | -0.180 |
| Conic model | 0.318** | 0.424** | 0.275* | 0.240* | 0.238* | 0.227* | 0.208* |
| Present model | 0.853** | 0.778** | 0.503** | 0.314** | 0.362** | 0.369** | 0.265* |
| Sample No. | 66 | 85 | 85 | 85 | 85 | 85 | 89 |

*, ** Significant at $p<0.05$ and $p<0.01$, respectively.

Table 4. Correlation of the seed setting rate of Peiai64S and temperature at different heights in field

### 3.3 Model of fertility-temperature

#### 3.3.1 Fertility sensitive stage

Determining fertility sensitive stage was the base to establish a model of fertility-temperature. The fertility sensitive stage can be determined by distance between the last upper two leaves or can be checked by the length of panicle. However, a method by growth days was popular for its advantage in quantitative analysis (Lu *et al* 2001). The method was given a range of growing days and it will cause difficulty for calculating temperature accumulation (Lu *et al* 2001), since it does not consider the growing difference owing to temperature. The chapter determined the initiative date (total days as well) by effective accumulated temperature >24. The method was set as：

| >24°C°C effective accumulated temperature (Total sensitive days) | Initiative days from heading |
|---|---|
| <10°C.d | 15 |
| 10-20°C.d | 12 |
| >20°C.d | 10 |

#### 3.3.2 Fertility-temperature model

By analyzing the relationship between the fertility and temperature, it was found that the fertility showed a rule of recovery-increase-decrease along with temperature decreasing. Thus, the chapter set the base of the model as: when the temperature decreased to an upper limit (self-fertilized seed setting rate of 0.5%), the TGMS was regarded as fertility, and at the optimum temperature, it showed the highest fertility. When the temperature decreased furthermore to a lower limit, the self-fertilized seed setting rate returned to zero.

Thus, equation (8) was established as:

$$P = P_0\left(\frac{T - T_L}{T_0 - T_L}\right)^A \left(\frac{T_H - T}{T_H - T_0}\right)^B \tag{8}$$

Here, P indicated self-fertilized seed setting rate, while $P_0$ indicated its maximum value. T denoted the averaged temperature of the sensitive stage, and $T_H$, $T_L$ indicated the upper and lower limit temperature, respectively, when fertility was zero. $T_0$ denoted the optimum temperature when the TGMS showed maximum fertility. A and B were undetermined parameters and were both > 0. Equation (8) shows the following parameters:

The derivative equation:

$$P' = P_0 \frac{A(T - T_L)^{A-1}(T_H - T)^B}{(T_0 - T_L)^A (T_H - T_0)^B} - P_0 \frac{B(T - T_L)^A (T_H - T)^{B-1}}{(T_0 - T_L)^A (T_H - T_0)^B}$$

i. When P' =0, the optimum temperature $T_0$ was calculated, and the highest self-fertilized seed setting rate, $P_{max} = P_0$
ii. When $T \geq T_H$ and $T \leq T_L$, then $P_{min} = 0$

### 3.3.3 Analysis of temperature indices for sterile alteration by the model

Table 5 shows the simulation of stem or air temperatures with self-fertilized seed setting rate by three parameters as average, maximum, and minimum temperatures, with three durations of 1, 3, 5 days, and by 0.1°C of temperature step length for the simulation. Result showed that there were significant correlations between self-fertilized seed setting rate and the temperatures of 20 cm stem, air temperature or 150 cm air temperature with the three temperatures and three durations. It also implied that the effect of average temperature was better than that of the maximum or minimum temperature, and three-day duration was better than one-day or five-day durations. The result also showed that the stem and air temperatures at 20 cm were better than 150 cm air temperature, and the stem temperature at 20 cm showed the best effect of simulation. Thus, stem temperature at 20 cm was selected as the best simulating parameter for the model.

Equations (9), (10), and (11) are the statistic models of the self-fertilized seed setting rate simulated with the actual self-fertilized seed setting rate and temperatures of 20 cm stem, air, and 150 cm air. With the self-fertilized seed setting rate of 0.5% as initial (upper limit) and that of zero as the lower limit, and with stem temperature at 20 cm for three days, using equation (9), upper limit temperature and lower limit temperature was determined to be 22.8°C and 21.7°C, respectively. When compared with air temperature at 150 cm, there was 1.2°C and 1.1°C lower, respectively. With air temperature at 20 cm for three days, using equation (10), upper limit temperature and lower limit temperature was determined to be 23.2°C and 21.5°C, respectively. When compared with air temperature at 150 cm, there was 0.8°C and 1.3°C lower, respectively.

| Location | | | A | B | $T_L$ | $T_0$ | $T_H$ | $P_0$ | R | n |
|---|---|---|---|---|---|---|---|---|---|---|
| 20 cm Stem tempe-rature ($T_{p20}$) | One day | Average | 1 | 0.24 | 21.6 | 22.3 | 22.5 | 10.82 | 0.755** | 68 |
| | | Maximum | 1 | 0.55 | 27.9 | 28.1 | 28.2 | 10.82 | 0.371** | 68 |
| | | Minimum | 1 | 1.12 | 17.0 | 17.7 | 18.5 | 10.82 | 0.964** | 34 |
| | Three days | Average | 1 | 0.37 | 21.7 | 22.5 | 22.8 | 10.82 | 0.853** | 66 |
| | | Maximum | 1 | 0.55 | 28.5 | 28.7 | 28.8 | 10.82 | 0.470** | 66 |
| | | Minimum | 1 | 1.33 | 17.0 | 17.5 | 18.1 | 10.82 | 0.550** | 66 |
| | Five days | Average | 1 | 19.35 | 22.2 | 22.4 | 26.0 | 10.82 | 0.586** | 64 |
| | | Maximum | 1 | 0.62 | 28.0 | 28.2 | 28.3 | 10.82 | 0.344** | 64 |
| | | Minimum | 1 | 0.63 | 18.7 | 18.9 | 19.0 | 10.82 | 0.610** | 64 |
| 20 cm Air Tempe-rature ($T_{a20}$) | One day | Average | 1 | 1.17 | 21.9 | 22.1 | 22.3 | 10.82 | 0.715** | 83 |
| | | Maximum | 1 | 1.50 | 26.6 | 26.8 | 27.1 | 10.82 | 0.360** | 83 |
| | | Minimum | 1 | 27.27 | 17.8 | 18.0 | 23.0 | 10.82 | 0.659** | 76 |
| | Three days | Average | 1 | 12.14 | 21.5 | 21.8 | 26.0 | 10.82 | 0.778** | 85 |
| | | Maximum | 1 | 0.60 | 26.8 | 27.0 | 27.1 | 10.82 | 0.356** | 85 |
| | | Minimum | 1 | 5.02 | 17.7 | 17.9 | 19.1 | 10.82 | 0.684** | 83 |
| | Five days | Average | 1 | 0.88 | 22.0 | 22.3 | 22.6 | 10.82 | 0.491** | 86 |
| | | Maximum | 1 | 0.97 | 26.7 | 26.8 | 26.9 | 10.82 | 0.408** | 86 |
| | | Minimum | 1 | 0.56 | 19.1 | 19.3 | 19.4 | 10.82 | 0.361** | 85 |
| 150 cm (ck) Air Tempe-rature ($T_A$) | One day | Average | 1 | 0.22 | 22.9 | 23.4 | 23.5 | 10.82 | 0.559** | 41 |
| | | Maximum | 1 | 0.67 | 29.5 | 29.6 | 29.7 | 10.82 | 0.280 | 41 |
| | | Minimum | 1 | 0.55 | 17.7 | 17.9 | 18.0 | 10.82 | 0.426** | 37 |
| | Three days | Average | 1 | 12.61 | 22.8 | 23.0 | 26.0 | 10.82 | 0.594** | 42 |
| | | Maximum | 1 | 0.34 | 28.5 | 28.8 | 28.9 | 10.82 | 0.548** | 42 |
| | | Minimum | 1 | 15.56 | 17.5 | 17.8 | 22.9 | 10.82 | 0.686** | 41 |
| | Five days | Average | 1 | 9.15 | 22.9 | 23.2 | 26.0 | 10.82 | 0.504** | 43 |
| | | Maximum | 1 | 0.08 | 27.1 | 28.3 | 28.4 | 10.82 | 0.223 | 43 |
| | | Minimum | 1 | 0.31 | 18.7 | 19.0 | 19.1 | 10.82 | 0.623** | 42 |

Table 5. Simulation of stem or air temperature with three parameters and three durations

$$T_{p20} = 10.82 \times (\frac{T-21.7}{22.5-21.7})(\frac{22.8-T}{22.8-22.5})^{0.37} \quad (R=0.853^{**}; n=66) \tag{9}$$

$$T_{a20} = 10.82 \times (\frac{T-21.5}{21.8-21.5})(\frac{26.0-T}{26.0-21.8})^{12.14} \quad (R=0.778^{**}; n=85) \tag{10}$$

$$T_{A} = 10.82 \times (\frac{T-22.8}{23.0-22.8})(\frac{26.0-T}{26.0-23.0})^{12.61} \quad (R= 0.594^{**}; n=42) \tag{11}$$

### 3.4 Stem and air temperatures at 20 cm

Considering energy exchange and balance, stem and air temperatures in rice canopy were connected with two energy sources: solar radiant energy and water heat energy. To avoid trouble in actual operation, the chapter established effective statistic models as equations (12) and (13), which included the screen temperature ($T_A$), cloud cover (N, from 1 to 10), and the water temperature at inflow ($T_{in}$) and outflow ($T_{out}$). Only by determining $T_{in}$ and $T_{out}$, stem or air temperature at 20 cm could be calculated by screen temperature and cloud cover, which were both offered by the local weather station. It was helpful to estimate the damage of lower temperature weather and the effects of the adjusting measures in actual seed production.

$$T_{p20} = \frac{N}{15.2}\frac{(T_{in}+T_{out})}{2} + (1-\frac{N}{15.2})T_A \quad (R=0.812^{**}, n=46) \tag{12}$$

$$T_{a20} = (1-\frac{N}{34.9})\frac{(T_{in}+T_{out})}{2} + \frac{N}{34.9}T_A \quad (R=0.975^{**}, n=46) \tag{13}$$

### 3.5 Techniques by used plant temperature to safeguard sterile of Peiai64S

Peiai64S was a widely used TGMS in two-line hybrid rice breeding. Its fertility was controlled by temperature during its sensitive stage. Its sterility usually fluctuated owing to the frequent fluctuation caused by monsoon, which resulted in damage to the seed purity of seed production in China southern rice area (Lu *et al* 2001, Yao *et al* 1995). The first so-called super hybrid rice in China, Liangyoupeijiu, which was released by the authors' research group, was popularized over 7 million ha, and has been the major planted rice for its largest planting area of China from 2002. However, in lower reaches of Yangtze River, in the past five years, there was twice lower temperature weather (daily averaged temperature for three days lower than 24°C) in August, during which was the sterility sensitive period of TGMS, that caused damage to the seed production of Liangyoupeijiu and other two-line hybrid rice. It was a hidden problem for seed production of two-line hybrid rice. In the seed production practices of Liangyoupeijiu, it was found that when attacked by lower temperature weather, the seed purity exhibited a large difference even if the TGMS was in a same weather condition, owing to the individual differences at landform and water treatment and so on. Researches showed that the fertility of rice TGMS was affected directly by plant temperature, which was infected by the microclimate of the field (Hu *et al* 2006). When attacked by lower temperature weather, by irrigating warmer water from river or deep pool, plant temperature would be increased by 2°C, which was effective for safeguarding the sterility of TGMS (Lu *et al* 2004, Zou *et al* 2005). So far, the forecast of sterile alteration is only based on the temperature information from weather station; there is lack of any study on field microclimate or plant temperature, and especially no research has focused on the temperature scale of plant or air around it. In researches of wheat, some researchers used plant temperature as the parameter for freeze injury and grain growing speed (Feng *et al* 2000, Liu *et al* 1992).

The chapter put forward a method to conclude the fertility of TGMS by stem or air temperature at 20 cm height, which takes various factors including microclimate and location of field into account. It is more direct and exact than the traditional method. In rice production, we can use it to estimate any field or representative plot of a large field, and to monitor directly the result of regulation for safeguarding seed production in two-line hybrid rice. The technique is: when attacked by lower temperature weather with average temperature lower than 24°C during 5-15 days before TGMS heading, using infrared or thermosensor temperature indicator to determine plant stem temperature at 20 cm height or air temperature around it at 02:00, 08:00, 14:00, 20:00 (or only 08:00 and 20:00) every day. If the averaged value is lower than the line of 22.8°C for stem temperature or air temperature is lower than 23.2°C, it implies that the TGMS will transform its sterility to fertility. For safeguarding its sterility, it is necessary to irrigate by warmer water higher than 25°C, and by depth of 15 cm, until the temperature is higher than the above index.

The present chapter also established a statistic model for stem or air temperature at 20 cm height. By the inflow and outflow water temperatures of any field, and screen temperature and cloud cover from the local weather station, stem or air temperature at 20 cm height can be concluded. For an application example, if one day, the average temperature and cloud cover was 22°C and 9, respectively, and the actual water temperature of the field was 23°C, by (12) and (13), stem or air temperature at 20 cm height was calculated to be 22.55°C and 22.74°C, respectively, both of which were lower than the above temperature index. By irrigating warmer water from river, the inflow and outflow water temperature were measured as 26°C and 24°C, by (12) and (13), the stem and air temperature at 20 cm height was calculated to be 23.74°C and 24.23°C, respectively, since both are higher than the above temperature index, it will be effective for safeguarding the sterility of TGMS.

## 4. Abbreviation

Tp: plant temperature. $T_{p10}$, $T_{p20}$, $T_{p30}$, $T_{p40}$ denotes rice stem temperature at plant heights of 10cm, 20cm, 30cm and 40cm, respectively.
$\Delta T_p$: plant temperature difference between water irrigated and non-irrigated.
$T_{a20}$, $T_{a40}$ and $T_a$: air temperature at heights of 20cm, 40cm and 150cm, respectively.
$T_w$: water temperature.
$T_{in}$: temperature of inflow water.
$T_{out}$: temperature of outflow water.
$T_{w\text{-}A}$: temperature difference between water temperature and air temperature at height of 150cm.
LAI: leaf area index.
DL: distance between the last upper two leaves of rice plant.

## 5. Notes

The most data were published in the journals of <Agricultural Sciences in China>, 2007, 6(11):1283-1290, and <Rice Science>, 2008, 15(3):223-231.

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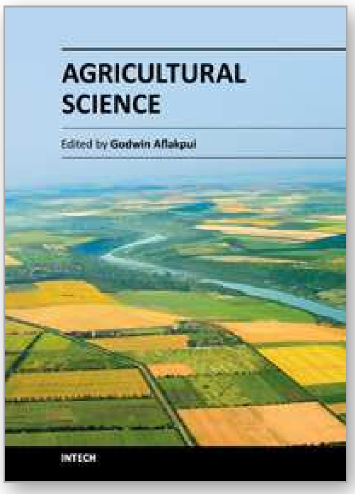


**Agricultural Science**
Edited by Dr. Godwin Aflakpui

ISBN 978-953-51-0567-1
Hard cover, 252 pages
**Publisher** InTech
**Published online** 27, April, 2012
**Published in print edition** April, 2012

This book covers key areas in agricultural science, namely crop improvement, production, response to water, nutrients, and temperature, crop protection, agriculture and human health, and animal nutrition. The contributions by the authors include manipulation of the variables and genetic resources of inheritance of quantitative genes, crop rotation, soil water and nitrogen, and effect of temperature on flowering. The rest are protecting crops against insect pests and diseases, linking agriculture landscape to recreation by humans, and small ruminant nutrition. This book is a valuable addition to the existing knowledge and is especially intended for university students and all professionals in the field of agriculture.