Impact of short-term exposure to elevated temperatures on physiology of Thai rice (cv. Riceberry)

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Abstract. High temperature is an important limiting factor for the growth and development of rice cultivation worldwide. The current research aimed to investigate the efficiency of PSII system, photosynthetic changes in photosynthetic pigments and membrane stability in Thai seedlings of rice cv. Riceberry after exposure to different temperatures viz., 25, 30, 35, 40, 45, 50 and 55°C for 30 min. The experiment was designed in CRD with four replications. The results showed that the highest $F_v/F_m$ was realized when seedlings were exposed to 30°C and 35°C and decreased when exposed to 40°C for 30 min. The content of photosynthetic pigments such as total chlorophyll (TC) and chlorophyll a (Chl a) were the highest after short-term exposure to a temperature of 25°C. However, the contents of all photosynthetic pigment such as TC, Chl a, Chl b and carotenoids were the lowest when exposed to 55°C for 30 min. The membrane stability of Riceberry seedlings affected on short-term exposure to temperatures of 50°C and 55°C. Thus, $F_v/F_m$ and Hill reaction were the first mechanisms to be significantly affected by the short-term exposure to a temperature of 40°C followed by membrane stability and contents of photosynthetic pigments which were affected by short-term exposure to temperature of 50°C-55°C and 55°C, respectively.

1. Introduction
Rice (Oryza sativa L.) is an essential food for a large part of the world’s population. It can adapt and grow well in many climatic zones. However, its production is severely affected by various factors such as pests, weeds, drought, high and low temperature, salinity and environmental pollution [1]. In recent years, the global warming and climate change have seriously affected the cultivation of rice and other crops. The Intergovernmental Panel on Climate Change (IPCC) [2] has predicted that the air temperature will increase by more than 4.0°C in the 21st century. High temperature affects the photosynthesis system especially the PSII efficiency because the PSII is the most heat-sensitive among the two photosynthetic systems [3]. Mathur et al [4] reported that, in wheat exposure to temperatures of 25°C, 35°C, 40°C and 45°C for 15 min showed a decreased in PSII efficiency with increasing temperature. High temperature decreased chlorophyll synthesis by inhibiting the chloroplast function [5] and also reduction in the Hill reaction rate [6]. Cui et al [7] reported that in tall fescue (Festuca arundinacea S.) seedlings after treatment with 35°C/30°C (day/night) for 20 days showed a reduction in chlorophyll a+b and chlorophyll/carotenoid ratio. Heat stress also damaged the cell membrane by...
2. Material and methods
Thai rice cv. Riceberry was used in this study. The seeds were sterilized and germinated on wet tissue papers until the root length was approximately 3 cm. Then, the germinated seeds were transplanted to the pots containing paddy soil in open air greenhouse at the Agronomy Field Station, Department of Agronomy, Khon Kaen University, Khon Kaen, Thailand. Further, 21-day-old seedlings were subjected to different temperatures in the temperature control cabinet (VRV. Corp. Ltd, Thailand). The initial temperature was 25°C which was further increased to 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, and maintained for 30 min. Relative humidity and light intensity were 70% and 400 μmol (photons) m⁻² s⁻¹, respectively. For the temperature was first increased to the target temperature, maintained for 30 min and then cooled down to 25°C [10] before measuring the PSII efficiency, Hill reaction, photosynthetic pigments and membrane stability. The PSII efficiency, as indicated by Fv/Fm is the maximal quantum yield of PSII efficiency in dark-adapted leaves, as described by Schreiber [11]. The Hill reaction was determined according to Giebel [12]. Fresh leaves (1 g) were extracted in sucrose-phosphate buffer pH 7.0 using a homogenizer (Vinayak Industries Thane, Maharashtra, India). Chloroplast (1 ml) was extracted in sucrose-phosphate buffer pH 7.0 (1.5 ml) and 0.28 mM 2,6-Dichlorophenolindophenol (DPCI) (0.5 ml). The Hill reaction was indicated by DCPIP reduction at 620 nm. It was expressed in μM.mg.Chl⁻¹.hr⁻¹. Arnon’s [13] procedure was used to determine photosynthetic pigment contents such as total chlorophyll (TChl), chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoid (Car). Fresh leaves (0.1 g) were collected, cut in small pieces and put in a glass test tube and then 80% acetone was added (10 ml) and kept in the dark for 72 h. For the total extraction volume (V) was recorded and the optical density of solution was measured and recorded at 440, 645 and 663 nm using by UV-VIS spectrophotometer (Model i3, Hanon, China). The contents of TChl, Chl a, Chl b and Car were calculated as described by Arnon [13] and the photosynthetic parameters were expressed as mg.gFW⁻¹. Membrane stability was indicated by electrolyte leakage (EL) as described by Bajji et al [14]. Fresh leaves (0.1 g) were soaked in deionized water (10 ml) for 24 h. The electrical conductivity (EC) of the suspension was measured. The suspension was incubated at 100°C for 15 min and cooled down a temperature to 25°C on ice and then the final electrical conductivity (ECf) was measured. For EL was calculated as follows: \( EL(%) = (ECf/ECi) \times 100 \). 

3. Results and discussion

3.1. Effect of short-term exposure to elevated temperature on PSII efficiency
In rice (cv. Riceberry), the efficiency of PSII was influenced by short-term exposure to elevated temperatures, as indicated by decreased Fv/Fm values as shown in figure 1. The highest Fv/Fm was observed after short-term exposure to temperatures of 30°C and 35°C. A significant reduction in Fv/Fm was found when the temperature increased from 40°C to 55°C (figure 1). These results suggested that Fv/Fm in rice seedling was significantly affected by heat exposure at 55°C for 30 min (table 1), which was in agreement with the effect on rice cv. Pathumthani 60 at heading stage in which Fv/Fm decreased after exposure to 45°C for 30 min [10]. Moreover, Tang et al [15] also showed a decrease in Fv/Fm with increasing temperature from 45°C to 50°C in spinach seedlings (Spinacia oleracea) and also in rice cv. 9311 exposed to 35°C for 1 h [16].
Figure 1. Effect of short-term treatment at different temperatures on \( F_v/F_m \) in seedlings of rice cv. Riceberry. Data were expressed as means ± SE \((n=3-4)\). The different letters showed significant differences among different temperatures by DMRT at \( P \leq 0.05 \).

3.2. Effect of short-term exposure to elevated temperature on Hill reaction and photosynthetic pigments

The effect of short-term exposure to elevated temperatures on Hill reaction was indicated by a reduction of DCPIP in rice cv. Riceberry as shown in figure 2A. The highest rate of Hill reaction was observed after short-term exposure to temperature of 35°C (approximately 62.18±3.07µ M.mg.Chl \(^{-1}.\)hr\(^{-1}\)) and the decreasing trend of Hill reaction rates was found on increasing temperature from 40°C to 55°C (figure 2A). According to Misr and Meena [17] reported that Hill reaction dropped in rice (\textit{Oryza sativa} L. cv. Mousouri) when 12-day-old seedlings were exposed to high temperature (40°C) [17]. The photosynthetic pigment contents, namely \( TC \) and \( Chl \ a \), were the highest after short-term exposure to a temperature of 25°C (approximately 1.853±0.129 and 1.391±0.096 mg.gFW\(^{-1}\), respectively); the contents decreased when exposed to temperatures of 30°C to 55°C (figures 2B and 2C). However, \( Chl \ b \) and \( Car \) contents were the highest after short-term exposure to a temperature of 45°C, which then significantly decreased at 55°C (for \( Chl \ b \)) and 50°C -55°C (for \( Car \)) (figures 2D and 2E). The \( Chl \ b \) and \( Car \) contents were the lowest after treatment of 55°C for 30 min. This suggested that short-term exposure to elevated temperatures (50°C -55°C) affected the Hill reaction and the contents of \( TC \), \( Chl \ a \), \( Chl \ b \) and \( Car \) (table 1). Earlier studies showed a decrease in the contents of \( TC \), \( Chl \ a \) and \( Chl \ b \) in five rice cultivars (N22, Dular, IR64, KDML105 and PTT1) at dough grain stage when they were exposed to 42°C for 30 min [18].

Table 1. Analysis of variance of the effects of short-term exposure to different temperatures on \( F_v/F_m \), Hill reaction and contents of \( TC \), \( Chl \ a \), \( Chl \ b \) and \( Car \) in seedlings of rice cv. Riceberry \((n=3-4)\).

| Parameters | \( F_v/F_m \) | Hill reaction | Mean Square | \( TC \) | \( Chl \ a \) | \( Chl \ b \) | \( Car \) | EL |
|------------|---------------|---------------|-------------|---------|----------|----------|--------|----|
| Temperature | 0.013*        | 800.095*      | 0.110*      | 0.074*  | 0.004*   | 0.007*   | 880.9* |    |
| Error      | 9.54x10\(^{-5}\) | 13.844       | 0.024       | 0.013   | 0.001    | 0.001    | 46.435 |    |

* indicated significant differences at \( P \leq 0.05 \) by DMRT.
Figure 2. Effect of short-term exposure to different temperatures on Hill reaction (A) and contents of total chlorophyll (B), chlorophyll a (C), chlorophyll b (D) and carotenoid (E) in seedlings of rice cv. Riceberry. Data were expressed as means ± SE (n=3-4). The different letters showed significant differences among different temperatures by DMRT at P≤0.05.

3.3. Effect short-term exposure to elevated temperature on membrane stability

The effect of short-term exposure to elevated temperatures on electrolyte leakage (EL) in seedlings of rice cv. Riceberry is shown in figure 3. The highest EL value was observed after short-term exposure to temperatures of 50°C - 55°C (figure 3). This suggested that rice seedlings were affected by short-term exposure to elevated temperatures (table 1), resulting in increased cell membrane damage and high leaked solute to outside the cell which probably induced the death of rice cell [19]. The lowest of EL was found when rice was exposed to a temperature of 35°C (figure 3). This suggested that a temperature of 35°C was the optimum temperature for rice [20]. Ali et al [21] showed the highest EL in rice seedlings on exposure to 42°C for 72 h. Other studies also reported that the membrane leakage significantly increased in wheat [22] and lentil (Lens culinaris Medik.) [23] after exposure to high temperatures.
Figure 3. Effect of short-term exposure to different temperatures on electrolyte leakage in seedlings of rice cv. Riceberry. Data were expressed as means ± SE (n=3-4). The different letters showed significant differences among different temperatures by DMRT at $P \leq 0.05$.

4. Conclusions

Based on the results of the effect of short-term temperature on the physiology of rice seedlings (cv. Riceberry) indicated that short-term exposure to temperatures of 40°C - 55°C affected the efficiency of PSII by decreasing $F_v/F_m$ values. Also the short-term heat stress (at 55°C) severely affected Hill reaction and the contents of photosynthetic pigments ($TC$, $Chl$ a, $Chl$ b and $Car$) leading to low membrane stability as indicated by increased membrane damage and high $EL$. Short-term temperature of 35°C could be the optimum temperature for growing seedling rice (cv. Riceberry).

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