Responses of Thai recommended rice cultivars to high temperature in \( PSII \) efficiency at panicle initiation stage

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Abstract. Photosynthesis is a major process for determining plant growth and development. The temperature plays important roles in plant photosynthesis. Thus, this research aimed to study the temperature response of \( PSII \) efficiency in different Thai recommended rice cultivars at panicle initiation (\( PI \)) stage. Youngest fully expanded leaves of fifteen rice cultivars namely, N22 (heat tolerance), IR64 (heat sensitive), CN1, Dular, PSL2, PT1, Riceberry, RD29, RD31, RD41, RD49, RD57, RD61, RD63 and SPT1 at \( PI \) stage were exposed to temperature ranged from 25-55°C. During the exposure experiment to the temperature, coinciding measurements on the effective quantum yield of \( PSII \) efficiency (\( \Delta F/F'_{m} \)) and maximum quantum yield of \( PSII \) efficiency (\( F'/F'_{m} \)) were carried out. The experiment was designed in CRD with 4 replications. Temperature response to \( \Delta F/F'_{m} \) and \( F'/F'_{m} \) showed a parabolic curve in all rice cultivars but \( F'/F_{m} \) showed a wider parabolic curve than \( \Delta F/F'_{m} \). Optimum temperature of \( \Delta F/F'_{m} \) and \( F'/F'_{m} \) was 31.83-35.64°C and 31.82-34.10°C, respectively. The ranges of supraoptimal temperature of \( \Delta F/F'_{m} \) and \( F'/F'_{m} \) were 47.15-50.60°C and 46.93-48.65°C, respectively. On the other hand, the high temperature stress of \( \Delta F/F'_{m} \) and \( F'/F'_{m} \) was 50.80-54.70°C and 50.93-52.50°C, respectively. Optimum temperature of \( \Delta F/F'_{m} \) showed high correlation with supraoptimal temperature especially in \( cv. \) PTT1. Broad sense heritability of \( \Delta F/F'_{m} \) under high temperature stress was the highest which could be used as selection criteria for heat tolerance in rice breeding programme.

1. Introduction

Photosynthesis is an important process for plant growth and it is sensitive to temperature. Thus temperature has been considered as a major factor for photosynthesis and high temperature can result in a decline in plant leaf photosynthesis [1]. The higher than normal temperature causes plant heat injury and photosynthetic damage [2]. Haldimann and Feller [3] reported that when temperature was increased rapidly or gradually photosynthesis decreased with increasing leaf temperature. This kind of effect reduced photosynthesis more than 90% at 45°C compared to 25°C. The photosynthetic fluorescence parameters such as \( F'/F_{m} \) also decreased with increasing temperature [4]. The \( F'/F_{m} \) declined markedly at 45°C within 30 min after the beginning of treatment. It is an important parameter for assessing \( PSII \) efficiency whereby, decreasing \( F'/F_{m} \) indicated loss of \( PSII \) efficiency. Therefore, changes in chlorophyll fluorescence can be used as plant screening method for heat tolerance. \( PSII \) efficiency was inhibited by high temperature and plant response to high temperature depended on the extent of increasing temperature, temperature duration and plant type [5]. When light falls on the
leaves resulted in increasing in leaf temperature. Therefore, leaf temperatures can be used as indicators of the optimum temperature for plant photosynthesis under different conditions [6]. Measurements of $\Delta F/F_m'$ and $F/F_m$ in response to temperature determined the whole thermal adaptation of photosynthesis [7] which used parabolic function of $\Delta F/F_m'$ for indicating optimum temperature, supraoptimal temperature and high temperature stress in tropical plant species. The purpose of the present research was to study the temperature response on photosynthetic efficiency of PSII in Thai recommended rice cultivars at panicle initiation stage and to indicate optimum temperature, optimum temperature range, supraoptimal temperature and high temperature stress by PSII efficiency response to temperature.

2. Materials and methods

Plant materials: Rice seeds of fifteen recommended rice cultivars such as N22 (heat tolerance), IR64 (heat sensitive), CN1, Dular, PSL2, PTT1, Riceberry, RD29, RD31, RD41, RD49, RD57, RD61, RD63 and SPT1 were used as experimental plant materials. Seeds were sterilized first by soaking in 70% alcohol for 1 minute followed by rinsing with tap water for 3 times. After that, the seeds were soaked in 10% Clorox for 15 minutes and rinsed further with tap water for 3 times. The seeds were soaked in tap water for 24 hours, and placed on wet paper until seeds germinated. Germinated seeds were transferred to pots of 20 cm in diameter containing paddy. Rice seedling was grown until panicle initiation (PI) stage in an open air greenhouse at the Agronomy Field Station, Faculty of Agriculture, Khon Kaen University, Thailand. At the PI stage photosynthetic efficiency of PSII in response to temperature were measured according to the method of Hacker and Neuner [8] and Dongsansuk [7]. Determination of PSII efficiency was carried out on youngest fully expanded leaf by using a chlorophyll fluorometer (Mini-PAM; Walz, Effeltrich, Germany). For preparing leaf sample, each leaf was placed on wet paper for keeping the leaf humidity under normal condition. The samples were then put in a temperature controlled cabinet (VRV. Corp. Ltd, Thailand) set with a relative humidity of 70%. All the samples were exposed to a temperature ranged from 25°C reached to 55°C coincided recorded fluorescence parameters and generated fluorescence response curve. The following fluorescence parameters were measured: effective quantum yield of PSII efficiency; $\Delta F/F_m'$ under light intensity at 61.80 $\mu$mol m$^{-2}$ s$^{-1}$ and maximum quantum yield of PSII efficiency; $F/F_m$ under dark 30 min condition according to Schreiber [9]. $\Delta F/F_m'$ and $F/F_m$ response curve was calculated for optimum temperature ($T_{opt}$), optimum temperature range, supraoptimal temperature and high temperature stress. Broad sense heritability ($h^2_b$) was calculated as following: $h^2_b = \delta^2_{G} / \delta^2_{P}$, according to Holland et al [10] ($\delta^2_{G}$; genotypic variance, $\delta^2_{P}$; phenotypic variance, $\delta^2_{P} = \delta^2_{G} + \delta^2_{E}$; error variance).

This experiment was designed in Completely Randomized Design (CRD) with 4 replications. Data were analyzed by one-way ANOVA and Duncan Multiple Range Test at $p \leq 0.01$ by software SPSS for windows version 16 to compare the significant differences among different rice cultivars.

3. Results and discussion

Temperature response of effective quantum yield of PSII efficiency; $\Delta F/F_m'$ and maximum quantum yield of PSII efficiency; $F/F_m$ exposed to temperature range of 25-55°C in each leaf of fifteen rice cultivars (N22, IR64, CN1, Dular, PSL2, PTT1, Riceberry, RD29, RD31, RD41, RD49, RD57, RD61, RD63 and SPT1) at PI stage are shown in figure 1. Temperature response curves of $\Delta F/F_m'$ and $F/F_m$ for fifteen rice cultivars fitted in a parabolic curve (figure 1). The result is in consistent with that obtained by Dongsansuk and Neuner [11] which showed parabolic response curve (from -5 to +60°C) of $\Delta F/F_m'$ and $F/F_m$ in tropical and temperate plants. The shape of parabolic function of $\Delta F/F_m'$ in fifteen rice cultivars showed unchanged value by the temperature between 12 and 21K (figure 1A) but curves of $F/F_m$ maintained value by temperatures in the range of 18-21K (from+25°C) (figure 1B) (data not shown). The curve range of $F/F_m$ was wider than the $\Delta F/F_m'$. The reduction of $F/F_m$ indicates damages of PSII function [12]. With increasing leaf temperature $F/F_m$ was declining, this indicated plants were under stress.
From the temperature response curves of $\Delta F/F_m'$ and $F_v/F_m$ in 15 rice cultivars at PI stage, optimum temperature, optimum temperature range, supraoptimal temperature and high temperature stress could be inferred as shown in table 1. The optimum temperature of $\Delta F/F_m'$ and $F_v/F_m$ was defined from parabolic curve according to Battaglia et al [13]. The optimum temperature of $\Delta F/F_m'$ and $F_v/F_m$ in 15 rice cultivars was found in the range of 31.83-35.64°C and 31.82-34.10°C, respectively. The highest optimum temperature of $\Delta F/F_m'$ and $F_v/F_m$ were found in cvs. CN1 and RD61, respectively (table 1). Whereas, the lowest optimum temperature of $\Delta F/F_m'$ and $F_v/F_m$ were found in cvs. SPT1 and RD57, respectively. The optimum temperature in 15 rice cultivars was related to the highest of $\Delta F/F_m'$ and $F_v/F_m$ as defined by Battaglia et al [13]. As a result, temperature increased up to optimum temperature resulted in an increasing photosynthesis and then photosynthesis tended to decline with increasing upper optimum level [14].

In the present research, the optimum temperature range of $\Delta F/F_m'$ in 15 rice cultivars at PI stage were in the range between 25°C and 45°C (table 1). Relating this, Dongsansuk et al [15] suggested that the optimal temperature of healthy structure was between 30°C and 45°C and for inactive structure was higher than 60°C. For their study, the optimum temperature range of $F_v/F_m$ was found only maximum value (approximately 42°C) but the minimum of optimum temperature range of $F_v/F_m$ could not be defined from $F_v/F_m$ curve. For other plant species, Dongsansuk et al [15] suggested that optimum temperature range of $\Delta F/F_m'$ in Musa sp. and A. ursinum exposed to a wide range of temperature (-15 and 60°C) showed a range of 27-36°C and 18-27°C, respectively. In the present investigation, the supraoptimal temperature of $\Delta F/F_m'$ and $F_v/F_m$ ranged 47.15-50.60°C and 46.93-48.65°C, respectively (table 1). The highest supraoptimal temperature of $\Delta F/F_m'$ and $F_v/F_m$ was found in cvs. PTT1 and RD49, respectively. On the other hand, the lowest supraoptimal temperature of $\Delta F/F_m'$ and $F_v/F_m$ was found in cvs. SPT1 and N22, respectively. High temperature stress of $\Delta F/F_m'$ and $F_v/F_m$ in 15 rice cultivars at PI stage was in the range of 50.80-54.70°C and 50.93-52.50°C, respectively. The highest and the lowest high temperature stress of $\Delta F/F_m'$ were found in cvs. PTT1 and SPT1, respectively. For $F_v/F_m$, the highest and the lowest high temperature stress were found in PSL2 and RD57, respectively (table 1). As a result, each species showed their critical and lethal temperature at which it could not survive and caused damage in cell structure resulting cell death [14].

Table 1. Optimum temperature, optimum temperature range, supraoptimal temperature and high temperature stress of $\Delta F/F_m'$ and $F_v/F_m$ between 25°C and 55°C in fifteen rice cultivars at PI stage. The values were mean ± SE (n=3-4). The different small letters indicated significantly different by Duncan’s New Multiple Range Test at $p<0.01$. * indicated calculation by fitting a parabolic function. ** is significant different at $p<0.01$. N22 is heat tolerance rice cultivar and IR64 is heat sensitive rice cultivar.
| Rice cultivars | ΔF/F₀<sup>opt</sup> | T<sub>we</sub> range (°C) | T<sub>we</sub> span | Supraoptimal temperature (°C±SE)<sup>opt</sup> | High temperature stress (°C±SE)<sup>opt</sup> | F/F₀<sup>opt</sup> | T<sub>we</sub> range (°C) | Supraoptimal temperature (°C±SE)<sup>opt</sup> | High temperature stress (°C±SE)<sup>opt</sup> |
|---------------|----------------|---------------------|----------------|---------------------|---------------------|----------------|---------------------|---------------------|---------------------|
| N22           | 34.26±0.42<sup>abcd</sup> | 29                  | 42              | 13                  | 47.90±0.10<sup>abcd</sup> | 51.51±0.04<sup>abcd</sup> | 32.64±0.08<sup>abcd</sup> | 32                  | 47.22±0.10<sup>abcd</sup> | 51.24±0.04<sup>abcd</sup> |
| IR64          | 33.44±0.20<sup>cd</sup> | 26                  | 41              | 15                  | 48.92±0.12<sup>cd</sup> | 53.02±0.14<sup>cd</sup> | 33.26±0.17<sup>cd</sup> | 38                  | 47.95±0.07<sup>cd</sup> | 51.84±0.09<sup>cd</sup> |
| CN1           | 35.64±0.41<sup>c</sup> | 27                  | 45              | 18                  | 49.42±0.67<sup>ce</sup> | 52.67±0.67<sup>ce</sup> | 33.26±0.30<sup>ce</sup> | 39                  | 48.34±0.10<sup>c</sup> | 52.03±0.33<sup>c</sup> |
| Dular         | 33.89±0.87<sup>ce</sup> | 26                  | 43              | 17                  | 48.23±0.37<sup>ce</sup> | 52.30±0.19<sup>ce</sup> | 32.76±0.11<sup>ce</sup> | 31                  | 48.06±0.06<sup>ce</sup> | 52.12±0.09<sup>ce</sup> |
| PSL2          | 32.93±0.63<sup>ef</sup> | 26                  | 42              | 16                  | 47.92±0.48<sup>ef</sup> | 51.89±0.45<sup>ef</sup> | 33.68±0.27<sup>ef</sup> | 40                  | 48.52±0.27<sup>ef</sup> | 52.45±0.28<sup>ef</sup> |
| PTT1          | 35.11±0.22<sup>ace</sup> | 28                  | 41              | 13                  | 50.60±0.20<sup>ace</sup> | 54.70±0.30<sup>ace</sup> | 32.55±0.26<sup>ace</sup> | 40                  | 47.67±0.13<sup>ace</sup> | 51.68±0.10<sup>ace</sup> |
| Riceberry     | 35.21±0.25<sup>ab</sup> | 27                  | 41              | 14                  | 50.32±0.34<sup>ab</sup> | 54.33±0.38<sup>ab</sup> | 32.64±0.26<sup>ab</sup> | 42                  | 47.72±0.48<sup>ab</sup> | 51.88±0.45<sup>ab</sup> |
| RD29          | 34.61±0.05<sup>ad</sup> | 27                  | 44              | 17                  | 50.11±0.25<sup>ad</sup> | 54.36±0.21<sup>ad</sup> | 32.84±0.25<sup>ad</sup> | 39                  | 47.73±0.17<sup>ad</sup> | 51.67±0.16<sup>ad</sup> |
| RD31          | 35.17±0.17<sup>ab</sup> | 28                  | 41              | 13                  | 50.17±0.25<sup>ab</sup> | 52.14±0.28<sup>ab</sup> | 33.58±0.23<sup>ab</sup> | 37                  | 47.98±0.34<sup>ab</sup> | 51.96±0.26<sup>ab</sup> |
| RD41          | 33.34±0.42<sup>cd</sup> | 26                  | 42              | 16                  | 48.06±0.38<sup>cd</sup> | 52.08±0.39<sup>cd</sup> | 33.38±0.36<sup>cd</sup> | 36                  | 48.27±0.10<sup>cd</sup> | 52.22±0.10<sup>cd</sup> |
| RD49          | 33.44±0.89<sup>cd</sup> | 28                  | 41              | 13                  | 47.46±0.08<sup>cd</sup> | 51.17±0.16<sup>cd</sup> | 34.20±0.38<sup>cd</sup> | 40                  | 48.64±0.03<sup>cd</sup> | 52.13±0.35<sup>cd</sup> |
| RD57          | 32.63±0.18<sup>cd</sup> | 28                  | 37              | 9                   | 48.36±0.10<sup>cd</sup> | 52.53±0.08<sup>cd</sup> | 31.82±0.18<sup>cd</sup> | 39                  | 46.93±0.16<sup>cd</sup> | 50.93±0.16<sup>cd</sup> |
| RD61          | 33.95±0.51<sup>cd</sup> | 25                  | 45              | 20                  | 48.81±0.38<sup>cd</sup> | 53.38±0.49<sup>cd</sup> | 34.10±0.18<sup>cd</sup> | 41                  | 48.65±0.22<sup>cd</sup> | 52.50±0.30<sup>cd</sup> |
| RD63          | 34.53±0.30<sup>cd</sup> | 26                  | 42              | 16                  | 49.84±0.20<sup>cd</sup> | 53.90±0.20<sup>cd</sup> | 33.16±0.21<sup>cd</sup> | 37                  | 47.83±0.17<sup>cd</sup> | 51.71±0.16<sup>cd</sup> |
| SPT1          | 31.83±1.09<sup>cd</sup> | 26                  | 41              | 15                  | 47.15±0.80<sup>cd</sup> | 50.80±0.33<sup>cd</sup> | 32.92±0.30<sup>cd</sup> | 40                  | 48.05±0.41<sup>cd</sup> | 52.26±0.34<sup>cd</sup> |

Mean            | 34.00                  | 26.87                | 41.87            | 15.00                | 48.88                  | 52.85                | 33.12                  | 38.07                | 47.97                  | 51.91                  |

F-test          | **                     | **                    | **               | **                   | **                     | **                    | **                     | **                   | **                     | **                     |
For 15 rice cultivars at PI stage, significant positive relationships were found between optimum temperature and supraoptimal temperature ($\Delta F/F'_m$; $r=0.82$ and $F_v/F_m$; $r=0.89$), between optimum temperature and high temperature stress ($\Delta F/F'_m$; $r=0.71$ and $F_v/F_m$; $r=0.77$) and between supraoptimal temperature and high temperature stress ($\Delta F/F'_m$; $r=0.97$ and $F_v/F_m$; $r=0.93$) as shown in figures 2-4, respectively. The highest positive of all relationships of $\Delta F/F'_m$ was found in cv. PTT1 and the lowest positive of all correlation of $\Delta F/F'_m$ and $F_v/F_m$ was found in cvs. SPT1 and RD57, respectively. This positive relationship suggested that the increasing optimum temperature resulted in increasing trend in supraoptimal temperature and high temperature stress in 15 rice cultivars at PI stage.

**Figure 2.** Relationships between optimum and supraoptimal temperature of $\Delta F/F'_m$ (A) and $F_v/F_m$ (B) for fifteen rice cultivars at panicle initiation (PI) stage; N22, IR64, CN1, Dular, PSL2, PTT1, Riceberry, RD29, RD31, RD41, RD49, RD57, RD61, RD63 and SPT1.

**Figure 3.** Relationships between optimum temperature and high temperature stress of $\Delta F/F'_m$ (A) and $F_v/F_m$ (B) for fifteen rice cultivars at panicle initiation (PI) stage; N22, IR64, CN1, Dular, PSL2, PTT1, Riceberry, RD29, RD31, RD41, RD49, RD57, RD61, RD63 and SPT1.
Figure 4. Relationships between supraoptimal temperature and high temperature stress of $\Delta F/F_m'$ (A) and $F_v/F_m$ (B) for fifteen rice cultivars at panicle initiation (PI) stage; N22, IR64, CN1, Dular, PSL2, PTT1, Riceberry, RD29, RD31, RD41, RD49, RD57, RD61, RD63 and SPT1.

Broad sense heritability ($h^2_b$) of $\Delta F/F_m'$ under supraoptimal temperature and high temperature stress for 15-rice cultivars at PI stage showed higher than of $F_v/F_m$ (table 2). The highest $h^2_b$ of $\Delta F/F_m'$ was found in high temperature stress ($h^2_b=0.760$) followed by supraoptimal temperature and optimum temperature as 0.693 and 0.466, respectively. This result suggested that, for rice cultivars at PI stage, screening for high temperature stress tolerance by measuring $\Delta F/F_m'$ should be performed under supraoptimal temperature and high temperature stress.

Table 2. Broad sense heritability of optimum temperature, supraoptimal temperature and high temperature stress of $\Delta F/F_m'$ and $F_v/F_m$ in fifteen rice cultivars at PI stage.

| Parameter               | $\delta^2_G$ | $\delta^2_E$ | $h^2_G$ | $\delta^2_G$ | $\delta^2_E$ | $h^2_E$ |
|-------------------------|---------------|---------------|---------|---------------|---------------|---------|
| Optimum temperature     | 0.789         | 0.905         | 0.466   | 0.305         | 0.215         | 0.586   |
| Supraoptimal temperature| 1.134         | 0.502         | 0.693   | 0.173         | 0.215         | 0.445   |
| High temperature stress | 1.367         | 0.431         | 0.760   | 0.120         | 0.241         | 0.332   |

4. Conclusion
From the results it could be concluded that temperature response of $\Delta F/F_m'$ and $F_v/F_m$ showed a parabolic function in all 15 rice cultivars at PI stage. However, the $F_v/F_m$ showed a wider parabolic curve. Moreover, the PSII efficiency was limited by high temperature stress. High temperature significantly reduced the leaf PSII efficiency. Therefore, the temperature response of PSII efficiency could be used as indicators of the optimum temperature, supraoptimal temperature and high temperature stress in different rice cultivars and also indicated temperature tolerance in rice. There was the highest positive relationship of $\Delta F/F_m'$ between optimum temperature and supraoptimal temperature. The high temperature stress of $\Delta F/F_m'$ could be considered as a useful parameter to perform screening of heat tolerance rice in breeding programme.

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