Title: Growth light environment changes the sensitivity of photosystem I photoinhibition by depending on common wheat cultivar

Running title: Cultivar differences in PSI photoinhibition

Authors

Daisuke Takagi^{1,2}, Hiroaki Ihara¹, Shigeo Takumi¹, Chikahiro Miyake^{1,2*}

Author's address

¹Department of Biological and Environmental Science, Graduate School of Agricultural Science, Kobe University, 1-1 Rokkodai, Nada, Kobe, 657-8501 Japan ²Core Research for Environmental Science and Technology, Japan Science and Technology Agency, 7 Gobancho, Chiyoda-ku, Tokyo 102-0076, Japan

*For correspondence

Corresponding author: Chikahiro Miyake (fax; +81-78-803-5851, <u>cmiyake@hawk.kobe.u-ac.jp</u>)

Keywords

Photosystem I, Photoinhibition, Reactive oxygen species, Light acclimation, Wheat (*Triticum aestivum* L.)



The study of the cultivar differences in Y(II), Y(I), Y(ND) and Y(NA) among wheat plants grown under LL- and HL-conditions respectively. The data were same as shown in Figures 2-5. Data are expressed as mean \pm SD of three to four independent experiments. To examine the significant differences among the wheat cultivars, analysis of variance (ANOVA) and Tukey-Kramer honestly significant difference (HSD) test were conducted at the maximum light intensity. Red squares indicate the results of CS, orange circles indicate the results of Bob, dark yellow triangles indicate the results of Haru, blue inverted-triangles indicate the results of M808, green diamonds indicate the results of N61, and purple stars indicate the results of Aka. Open symbols indicate the results of LL-grown wheat plants, and closed symbols indicate the result of HL-grown wheat plants.



Analysis of the rapid change in Y(I), Y(ND) and Y(NA) at the beginning of rSP illumination in CS and M808 grown under both LL- (open symbols) and HL-conditions (closed symbols). Before rSP illumination, the wheat plants were adapted in the dark for at least 1 h. In these experiments, the wheat plants are illuminated with a short pulse (300 ms, 20,000 μ mol photons m⁻² s⁻¹) with the different illuminating intervals. From the time 0 min to 1 min, the short pulse was illuminated every 10 s. From 1 min to 4 min, the short pulse was illuminated every 1 min. From 4 min to 8 min, the short pulse was illuminated every 2 min. Data are expressed as mean \pm SD of three independent experiments. Black squares indicate Y(I), red circles indicate Y(NA) and blue triangles indicate Y(NA).



The susceptibility of PSII photoinhibition to rSP illumination in wheat cultivars grown under LLand HL-conditions. (A) showed the time-course analysis of the relative change in Y(II) during rSP illumination under atmospheric conditions (21 kPa O₂, 40 Pa CO₂) at 25°C. Before rSP illumination, wheat plants were adapted in the dark for at least 1 h. Wheat leaves were illuminated every 10 s with a saturating pulse (300 ms, 20,000 μ E m⁻² s⁻¹) for 1 h in the dark. Data were expressed as mean ± SD of three to five independent experiments. Black line indicates the mean result of LL-grown wheat cultivars and gray shadow indicates the SD of the results of the LLgrown wheat cultivars. Red line indicates the mean results of the LL-grown wheat cultivars and pink shadow indicates the SD of the results of the HL-grown wheat cultivars.



The P700⁺ kinetics in wheat cultivars under the determination of Pm before and after rSP illumination. Before the determination of Pm, wheat plants were adapted in the dark for 30 min. To determine Pm value, Far-red (FR) light was illuminated to wheat leaves, and steady-state P700⁺ value was obtained. Subsequently, pulse illumination (300 ms, 20,000 μ mol photons m⁻² s⁻¹) was applied and determined maximum P700⁺ signals by using extrapolation method (see Materials and Methods). After pulse illumination, stable zero level of P700⁺ was determined at the steady state. Data were expressed as mean \pm SD of three to five independent experiments. P700⁺ signal was normalized by the Pm before rSP illumination in LL- and HL-grown wheat plants respectively. Blue lines with gray shadows indicate the results of LL-grown wheat plants after rSP illumination. Dark blue with pink shadows indicate the results of LL-grown wheat plants before rSP illumination. Dark red lines with pink shadows indicate the results of LL-grown wheat plants after rSP illumination. Dark red lines with pink shadows indicate the results of LL-grown wheat plants after rSP illumination. The red bars indicate the FR-illumination, yellow bars indicate the pulse illumination, and dark gray bars indicate the dark respectively.





The study of the cultivar differences in Y(II), Y(I), Y(ND) and Y(NA) among wheat plants grown under LL- and HL-conditions after rSP illumination respectively. The data were same as shown in Figures 7-10. Data are expressed as mean \pm SD of three to four independent experiments. To examine the significant differences among the wheat cultivars, analysis of variance (ANOVA) and Tukey-Kramer honestly significant difference (HSD) test were conducted at the maximum light intensity. Different letters adjacent to the symbols indicate a significant difference between those wheat cultivars (Tukey-Kramer HSD test, p < 0.05). Red squares indicate the results of CS, orange circles indicate the results of Bob, dark yellow triangles indicate the results of Haru, blue inverted-triangles indicate the results of M808, green diamonds indicate the results of N61, and purple stars indicate the results of Aka. Open symbols indicate the results of LL-grown wheat plants, and closed symbols indicate the result of HL-grown wheat plants.



Supplemental Figure S6

The P700⁺ kinetics in wheat cultivars under the determination of Y(I), Y(ND) and Y(NA) before and after rSP illumination. To determine the Y(I), Y(ND) and Y(NA), AL (2439 μ mol photons m⁻² s⁻¹) was illuminated. Subsequently, at the steady-state conditions, pulse illumination (300 ms, 20,000 μ mol photons m⁻² s⁻¹) was applied and determined maximum P700⁺ signals by using extrapolation method (see Materials and Methods). After pulse illumination, stable zero level of P700⁺ was determined at the steady state. Data were expressed as mean ± SD of three to five independent experiments. P700⁺ signal was normalized by the Pm before rSP illumination in LLand HL-grown wheat plants respectively. Black lines with gray shadows indicate the results of LL-grown wheat plants after rSP illumination. Blue with gray shadows indicates the results of LL-grown wheat plants before rSP illumination. Dark red lines with pink shadows indicate the results of HL-grown wheat plants before rSP illumination. Dark red lines with pink shadows indicate the results of HL-grown wheat plants after rSP illumination. Dark red lines with pink shadows indicate the results of HL-grown wheat plants after rSP illumination. Dark red lines with pink shadows indicate the results of HL-grown wheat plants after rSP illumination. Dark red lines with pink shadows indicate the results of HL-grown wheat plants after rSP illumination. The purple bars indicate the ALillumination, yellow bars indicate the pulse illumination, and dark gray bars indicate the dark respectively.



The response of NPQ to light intensity in six wheat cultivars grown under LL (open symbol) and HL (closed symbol) conditions. Figure (A) displays the difference in NPQ between wheat plants grown under LL- and HL-conditions in respective wheat cultivars. Figure (B) displays the cultivar differences grown under LL- and HL-conditions respectively. The data of (A) and (B) is same. The measurement was conducted under atmospheric conditions (21 kPa O_2 , 40 Pa CO_2) at 25°C. Data were expressed as mean \pm SD of three to five independent experiments. Red squares indicate the results of CS, orange circles indicate the results of Bob, dark yellow triangles indicate the results of Haru, blue inverted-triangles indicate the results of M808, green diamonds indicate the results of N61, and purple stars indicate the results of Aka



The change in the decrease of Fm (NPQ) during rSP illumination in wheat cultivars grown under LL- and HL-conditions. (A) showed the time-course analysis of the decrease of Fm (NPQ) during rSP illumination under atmospheric conditions (21 kPa O₂, 40 Pa CO₂) at 25°C. Before rSP illumination, wheat plants were adapted in the dark for at least 1 h. Wheat leaves were illuminated every 10 s with a saturating pulse (300 ms, 20,000 μ E m⁻² s⁻¹) for 1 h in the dark. Data were expressed as mean ± SD of three to five independent experiments. Black line indicates the mean result of LL-grown wheat cultivars and gray shadow indicates the SD of the results of the LL-grown wheat cultivars. Red line indicates the mean results of the LL-grown wheat cultivars and pink shadow indicates the SD of the results of the HL-grown wheat cultivars are shown in (B). Data were expressed as mean ± SD of three to five independent the results of Bob, dark yellow bars indicate the results of Haru, blue bars indicate the results of M808, green bars indicate the results of N61, and purple bars indicate the results of Aka.