ACTA AGRONOMICA SINICA
Volume 36, Issue 3, March 2010
Online English edition of the Chinese language journal

Available online at www.sciencedirect.com
ScienceDirect

Cite this article as: Acta Agron Sin, 2010, 36(3): 391–400.

RESEARCH PAPER

Variation in Photosynthetic Traits and Antioxidant Enzyme Activities in Wheat Seedlings Transferred from Low to High Light Growth Condition

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Abstract: The photooxidation induced by high light (HL) during the grain-filling period usually causes great yield loss of wheat (*Triticum aestivum* L.) in northern China. To understand the mechanism of wheat plant in response to HL, the photosynthetic characteristics of Xiaoyan 54, a winter wheat cultivar with high tolerance to HL, were investigated under HL condition. At third-leaf stage, seedlings of Xiaoyan 54 were transferred from low light to HL in a growth chamber and treated for 0, 1, 3, 8, 24, and 48 h. The net photosynthetic rate (P_n), chlorophyll content, and fluorescence parameters were measured with the second leaf. The activities of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR), were also determined. In addition, the expression patterns of genes encoding pigment-binding proteins were also evaluated. The results showed that the P_n increased gradually during the photosynthetic induction phase within 8 h of HL treatment, but decreased continuously during the photoinhibition phase longer than 8 h of HL treatment. The maximum P_n value of 18 μmol CO_2 m^{-2} s^{-1} was observed at the 8 h timepoint. The G_s, C_i, and T_r varied similarly to the P_n and reached their peaks at the 8 h timepoint. The contents of total chlorophyll and chlorophyll a were changed slightly during 48 h of HL treatment. In contrast, chlorophyll b reduced significantly at the 24 and 48 h timepoints, but the ratio of chlorophyll a to b continuously increased from the 8 h timepoint. One hour of HL treatment significantly reduced the maximum quantum efficiency of PSII (F_v/F_m), the maximum fluorescence (F_m), and the variable fluorescence (F_v), but enhanced the heat dissipation process. The activities of SOD, CAT, APX, and GR were induced by HL stress and reached the highest values at 48 h timepoint. At the photoinhibition stage, the RNA transcripts of *Talhcb* genes that encode LHCII subunits declined. *TaELIP1* and *TaELIP3* encoding early light-induced protein were induced by short-term HL (within 3 h) and repressed by long-term HL. As the key enzymes in xanthophyll cycle, the transcripts of *TaVDE* and *TaZEP* responded differently to the HL stress. The transcripts of *TaVDE* decreased remarkably after 8 h timepoint and remained low level. However, the transcripts of *TaZEP* showed an increase trend from 3 to 24 h of HL, and decreased at 48 h timpoints. In conclusion, when wheat seedlings were exposed to continuous HL for 48 h, photooxidative stress occurred, which resulted in a series of variations in physiology and biochemistry, such as reductions of P_n, F_v/F_m, and Chl b, the down-regulated expressions of pigment-binding protein genes, and the activation of antioxidant enzymes.

Keywords: wheat; high light; photooxidation; gene expression

Photooxidative stress induced by high light (HL) is a significant constraint for crop productivity. For example, photoinhibition causes photosynthetic loss to 10%, even under natural condition without other stresses [1]. For winter wheat (*Triticum aestivum* L.) grown in northern China, photooxidative stress during the late grain-filling period generally causes great yield loss. Even at seedling stage in cold winter days with bright light, chilling-dependent photoinhibition can lead to wheat leaf "photobleaching". Therefore, HL stress has been considered as a restraint for cereal crop production [2]. However, plants have evolutionally developed many mechanisms to cope with it. Firstly, when plants are transferred from low light (LL) to HL growth condition, the photosynthetic activity including photosynthetic rate [3], Rubisco activity [4], electric

Received: 25 September 2009; Accepted: 9 January 2010.
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DOI: 10.1016/S1875-2780(09)60039-0

transfer rate, and ATP synthesis increased shortly [5]. Secondly, plants reduce light absorbance through reduction of LHCII complex under HL condition [6, 7] and dissipate excessive light energy as heat via xanthophyll cycle [8]. Additionally, the activations of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) play photoprotective role for they can remove reactive oxygen species (ROS) produced in photosynthetic electron transport process [9]. Some important genes, such as *VDE* [10], *PsbS* [11], and *ELIP* [12], have been identified to be involved in nonphotochemical quenching (NPQ) process through which excess light energy is dissipated as heat. For example, *npq1* (for *VDE*) and *npq4* (for *PsbS*) mutants showed significantly reduction of NPQ and were susceptible to HL stress in *Arabidopsis thaliana* [10, 11]; early light induced protein (ELIP) mutants in *Arabidopsis* were also sensitive to photooxidation, suggesting its possible role in photoprotection [12]. Moreover, a large number of genes have been found to be response to HL via microarray technique [5].

The mechanisms of plants in response to HL have been studied in model plants *Arabidopsis thaliana* [10–12] and rice (*Oryza sativa* L.) [5]. However, there are few in-depth studies on this mechanism in winter wheat, although it is often subjected to HL stress especially during grain-filling period. Much knowledge obtained from the model plants cannot be directly used in wheat because of their different growth conditions and their complexly genetic response mechanisms. Therefore, this study aimed at understanding the physiological and gene expression responses of wheat to HL stress.

Xiaoyan 54 was selected from a mutant line of Xiaoyan 6 that was obtained through artificial selection from the distant hybridization between common wheat and decaploid *Agropyron elongatum*. Xiaoyan 54 possesses not only disease resistance and good flour quality but also tolerance to photooxidation induced by HL. It is an ideal material to study the genetic and physiological mechanisms of HL tolerance in wheat. In our previous studies, Xiaoyan 54 showed significant tolerance to HL through photosynthetic measurements of attached flag leaf in the field or detached in laboratory [13–15]. As we know, uncontrolled temperature, humidity, light intensity, and soil water availability in the field often make the situations even more complex than HL itself. In addition, the results based on detached leaf do not reflect the HL response of attached leaf completely. To better understand HL response in Xiaoyan 54, we simplified the research model by adopting seedlings of Xiaoyan 54 as the material and artificially controlling HL condition at room temperature in this study. Time course of photosynthesis-related traits, antioxidant activities, and RNA transcript levels involved in LHCII and NPQ were determined when Xiaoyan 54 seedlings were transferred from LL to HL condition.

1 Materials and methods

1.1 Plant materials and HL treatment

Surface-sterilized seeds of Xiaoyan 54 were germinated in dark at 22°C for 2 d. The germinated seeds were then transferred into growth chamber under the conditions of light intensity 100 μmol m^{-2} s^{-1} (provided by fluorescence lamps), photoperiod 16 h, temperature 20°C, and relative humidity 40–60%. Until the first leaf fully expanded, similar plants were cultured with nutrient medium [16]. At three-leaf stage, the seedlings were pretreated continuously with LL of 100 μmol m^{-2} s^{-1} for 2 d at 26–28°C before they were subjected to HL intensity at 1200 μmol m^{-2} s^{-1}, which was provided by metal halide Lamp (Osram, Germany). The second leaves of seedlings were sampled for measurements at 0, 1, 3, 8, 24, and 48 h timepoints after HL treatment with 4 to 6 repeats at each timepoint.

1.2 Evaluation of photosynthesis-related parameters

Chlorophyll content was determined as described by Arnon [17]. Net photosynthetic rate (P_n), stomata conductivity (G_s), intercellular CO_2 concentration (C_i), and transparent rate (T_r) were simultaneously measured using the photosynthesis system LI-6400 (LI-COR, USA). When measuring the gas exchange parameters, environmental CO_2 concentration was 410 ± 16 μmol mol^{-1}. Internal light intensity provided by red and blue light source was set to 1200 μmol m^{-2} s^{-1}. Using Handy-PEA (Hansatech, UK), chlorophyll fluorescence parameters, including minimum fluorescence (F_o), maximum fluorescence (F_m), variable fluorescence (F_v), maximum photochemical quenching efficiency of PSII (F_v/F_m), trapped energy flux (TR_o/CS), electron transport (ET_o/CS), and dissipated energy per cross section (DI_o/CS), performance index, Q_A-reducing PSII reaction centers at minimum fluorescence (RC/CS_o), and Q_A-reducing PSII reaction centers at maximum fluorescence (RC/CS_m) [18, 19], were determined after dark adaptation for half an hour. The saturated flash light intensity was 3000 μmol m^{-2} s^{-1} with 1 s of duration.

1.3 Measurements of activities of antioxidant enzymes

Crude enzymes were extracted as described by Grace and Logan [20]. The activities of the antioxidant enzymes were determined according to Grace and Logan [20] with small modifications. The activities of APX, CAT, and GR were defined as absorbance variation per minute per gram of fresh weight. The SOD activity was evaluated using the method describe by Wang et al. [21], which was defined as the reduction of nitroblue tetrazolium (NBT).

1.4 Gene expression analysis by semiquantitative RT-PCT

Total RNA was extracted using Trizol reagent (Invitrogen, USA). DNase-pretreated RNA was used as template to