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Research article

Photosystem II thermostability *in situ*: Environmentally induced acclimation and genotype-specific reactions in *Triticum aestivum* L

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ABSTRACT

Photosystem II (PSII) thermostability and acclimation effects on PSII photochemical efficiency were analyzed in thirty field grown winter wheat (*Triticum aestivum* L.) genotypes using prompt chlorophyll *a* fluorescence kinetics before and after dark heat treatment. A gradual increase in temperature caused the appearance of K-bands at 300 μs on the chlorophyll fluorescence induction curve, indicating the impairment of the PSII donor side (even by heat treatment at 38 °C). An increase in basal fluorescence, commonly used as a criterion of PSII thermostability, was observed beyond a temperature threshold of 44 °C. Moreover, an acclimation shift (increase of critical temperature) was observed at the 3.5 °C identified for K-band appearance, but only by 1.1 °C for a steep increase in F_0. The single temperature approach with regular weekly observations completed within two months using dark heat treatment at 40 °C demonstrated that the acclimation effect is not gradual, but occurs immediately and is associated with an increase of daily temperature maxima over 30 °C. The acclimated heat treated samples had less effect on the donor side of PSII, the higher fraction of active $Q_{\bar{A}}$ reducing reaction centers and causing a much lower decrease of connectivity among PSII units compared to non-acclimated samples. In the non-treated plants the reduction of antennae size, increase of PSII connectivity and changes in the acceptor side occurred as a result of heat acclimation. The enhancement of PSII thermostability persisted over several weeks regardless of weather conditions. The genotype comparison identified three groups that differed either in initial PSII thermostability or in acclimation capacity; these groupings were clearly associated with the origin of the genotypes.

1. Introduction

Photosynthesis is a very heat-sensitive process that can be partially or even completely inhibited by high temperature before other symptoms of the stress are detected [1]. Exposure of plants to heat stress typically induces inactivation of photosynthesis regardless of whether the stress is applied to whole plants, intact tissues or isolated organelles [2]. The primary targets of high temperature effects in plants are the photosystem II (PSII), carbon fixation by Rubisco and the ATP-generating system [3–5]. Moderately high temperature inhibits the repair of PSII damaged by photoinhibition and activity of reactive oxygen species [5]. However, the specific effects of high temperatures on photosynthesis depends strongly on plant species [2], the age of leaf or plant tissue [6], physiological status of plant as well as on the level of acclimation [7–9]. Furthermore, the magnitude of the effect depends not only on the temperature level, but also on the time spent at the higher temperature [10].

Photosynthetic acclimation to high temperatures represents an adjustment in photosynthetic processes and structures at each level, leading to a shift in the optimum temperature toward the new conditions [11]. This process is usually accompanied by an increase in the upper limit of the maximum physiologically tolerable temperature. Improvement of thermotolerance through

Abbreviations: PSII, photosystem II; PSI, photosystem I; OEC, oxygen evolving complex; RC, reaction center; Chl., chlorophyll; ABS, absorption flux; TR, electron trapping flux; Q_A, primary PSII quinone acceptor; QB, secondary PSII quinone acceptor; PQ, plastoquinone; cytb6/f, cytochrome b6/f; O–J–I–P, transient, fluorescence induction transient defined by the names of its intermediate steps; O-level, fluorescence level at 50 μs; J-level, fluorescence plateau at ~2 ms; I-level, fluorescence plateau at ~30 ms; P-level, the maximum fluorescence.

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acclimation has been observed in many plant species [12,13]. Different stress factors can also induce an increase of heat tolerance. For example, an increase of high temperature tolerance of PSII was observed as a result of drought stress [14,15] and salinity [16,17]. There are various published explanations for such an increase including the accumulation of glycine betaine [18–20], zeaxanthin [21,22], synthesis of isoprene [23,24], or heat shock proteins [25]. Moreover, an important role has been attributed to a wide range of other stress-related proteins and the complex antioxidative system has also been implicated in this process [26].

The assessment of heat stress effects on photosynthetic apparatus, particularly on PSII status and linear electron transport rate, has been frequently performed through chlorophyll (Chl) *a* fluorescence measurements [27]. Specifically, analysis based on high time-resolution measurements of the Chl *a* fluorescence transient represents a particularly quick method for gaining detailed information about PSII photochemical activity, electron transport events and the different regulatory processes [28]. Fast Chl *a* fluorescence kinetics data are derived from the time dependent increase in fluorescence intensity achieved upon application of continuous bright light to a previously dark adapted sample; the result is called the Kautsky curve or Chl *a* fluorescence transient [29]. The group of fluorescence parameters called JIP-test that quantify the stepwise flow of energy through PSII, using input data from the fluorescence transient, was formulated with a simplified model of the energy fluxes incorporating the parameters that define each type of flux [29,30]. The energy fluxes consist of an absorbed flux (ABS), trapping flux (TR), electron transport flux (ET) and the flux defining the dissipation of non-trapped energy as heat (DI) [29] - a flux quantifying the reduction of PSI end acceptor (RE) was later introduced [31]. The method is widely used for screening the stress effects in plants, especially the impacts of high temperature [32,33], low temperature [34,35], drought [36,37], salinity [38–40], and submergence [41].

In this study we investigated the *in situ* acclimation of the photosynthetic apparatus of wheat (*Triticum aestivum* L.) leaves during the growth season, leading to enhanced PSII thermostability. An increase of PSII thermostability at the end of vegetation period is typically observed, but it is not clear whether this phenomenon is associated with the plant maturing or whether it is an environmentally induced effect. In order to distinguish between these hypotheses we conducted weekly measurements over two months to assess heat thermostability using Chl *a* fluorescence measurements. Wheat has the longest history of cultivation and breeding of any plant and therefore has an enormous diversity of germplasm worldwide – in most of cases well adapted to the local conditions. In this work, we use a relatively large collection of wheat genotypes of different origin in order to identify any genotype-specific reactions. Such a large number of measurements over a relatively short time period were only feasible because of the application of fast fluorescence kinetics. In addition to providing an overview of environmentally induced acclimation and heat effects at the level of PSII photochemistry in wheat plant, we also discuss the feasibility of the screening within the crop germplasm for improved heat tolerance as a potential practical use of the chlorophyll fluorescence method.

2. Material and methods

2.1. Plant material

Winter wheat plants (*T. aestivum* L.) were cultivated for two years in small-plot field trials within Genebank of Research Institute of Plant Production in Piestany, Slovak Republic (N 48° 38′, E 17° 49′); annual average temperature 9.2 °C, in vegetation period 15.5 °C; annual precipitation 593 mm, soil type the luvi-haplic chernozem, pH 6.5 to 7.2. The crop was sown in September and cultivated according to standard agronomic practices.

Within the experiment, 30 genotypes of different geographic origins were used: Astella, Malvina, Venistar, Torysa, Vanda, Kosutska, Viglasska Cervenoklasa, Samorinska, Vrakunska, Radosinska Norma (Slovakia); Komfort (Austria); Tamaro (Switzerland); Biscay (Germany); Griffen (G. Britain); GK Forras (Hungary); Mewa, Gedania (Poland); Echo (Russia); Mottin, Verna (Italy), Steklovidnaja-24 (Kazakhstan); Bbyo-17, Dagdas-94, Pehlivan (Turkey); Shaan 8007-7, Cha-bej (China); Hokushin, Nanbu Komugi (Japan); Piopio-4, Shark-4 (Mexico).

The measurements were performed on detached leaves taken from plants in April, May and June; young and mature, healthy, sun-exposed leaves from the main stems of plants inside the canopy were used. Collected leaves were protected from direct sunlight in the field, then kept in low light and fully hydrated under laboratory conditions.

2.2. Heat treatment

The heat treatment was performed in darkness. Tissue samples of approximately 50 mm length were cut from the middle part of the leaf and placed into glass tubes with a small amount of water to ensure leaf hydration. The tubes were closed and completely submersed in a water bath with precisely controlled water temperature for 30 min. The time of exposure was known to be sufficient on the basis of previous analyses using different exposure times. The temperature within some tubes was measured, demonstrating that the temperature at the level of leaf surface reached the water bath level within 1–2 min. After 30 min of exposure, the tube was removed from the bath (in the dark) and, after cooling, transferred to laboratory temperature (5–10 min) for further measurements.

In the experiments with graduated temperature levels, temperatures of 38, 40, 42, 44 and 46 °C were used; a new leaf sample was always used for each temperature. In single temperature experiments, a temperature level of 40 °C was used.

2.3. Chlorophyll fluorescence measurements

Chl *a* fluorescence measurements were performed using the Handy-PEA Continuous Excitation Plant Efficiency Analyser (Hansatech Instruments Ltd, UK). The leaf samples were illuminated with continuous red light (wavelength in peak 650 nm; spectral line half-width 22 nm). The light was provided by array of 3 light-emitting diodes. The light pulse intensity used was 3500 μmol m^{-2} s^{-1} and the duration of the light pulse was 1 s. The fluorescence signal was recorded with a maximum frequency of 10^5 points s^{-1} (each 10 μs) within 0–0.3 ms, after which the frequency of recording gradually decreased collecting a total of 118 points within 1 s.

Leaf segment measurements were performed in the middle part of a leaf blade, away from the main leaf vein, after 30 min of dark adaptation, using leaf clips.

Chl *a* fluorescence transient data were used to calculate basic parameters and the parameters needed for the JIP-test (Table 1) [30,42,43]. The F_0 level was measured as the fluorescence at 50 μs ($F_{50\mu s}$).

2.4. Statistical analysis

The majority of reported data represent the weighted mean ± standard error. Statistical analysis was performed using analysis