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Imaging of fast chlorophyll fluorescence induction curve (OJIP) parameters, applied in a screening study with wild barley (*Hordeum spontaneum*) genotypes under heat stress

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ABSTRACT

We quantified the influence of heat stress (HS) on PSII by imaging of parameters of the fast chlorophyll fluorescence (CF) induction (OJIP) kinetic of 20 genotypes of wild barley (*Hordeum spontaneum*) covering a broad geographical spectrum. We developed a standardised screening procedure, allowing a repetitive fluorescence measurement of leaf segments. The impact of HS was quantified by calculating a Heat Resistance Index (HRI), derived from the decrease of the Performance Index (PI) caused by HS treatment and following recovery. For the genotype showing the lowest HRI, reduced maximum quantum yield (φP_0) and increased relative variable fluorescence of the O-J phase (K-Peak) were detected after HS, whereas the basal fluorescence (F_0) remained stable. An additional feature was a lowered fraction of active (Q_A-reducing) reaction centres (RCs). The disturbances disappeared after one day of recovery. Spatial heterogeneities of fluorescence parameters were detected, as the negative effect of HS was stronger in the leaf areas close to the leaf tip. The results of this study prove that chlorophyll fluorescence imaging (CFI) is suitable for the detection of HS symptoms and that imaging of JIP-Test parameters should be considered in future screening and phenotyping studies aiming for the characterisation of plant genotypes.

1. Introduction

Heat is one major abiotic factor affecting plant productivity. HS disturbs plant development, and it consequently acts as a limiting factor for biomass production and crop yield [1]. It is predicted that Climate Change, a major threat for future agriculture and food security, will increase the frequency of weather extremes like heat waves and drought periods, *e.g.* for the Eastern Mediterranean [2,3]. Thus, understanding the physiological and molecular impact of HS on plants and using the inherent potential of natural genetic diversity to withstand extreme temperatures should be an essential component of scientific research.

HS affects the photosynthetic process in multiple ways. The carbon assimilation in the Calvin–Benson Cycle is already diminished under moderate HS (at approx. 35–40 °C) by deactivation of Rubisco [4]. This effect is thought to be caused mainly by the heat sensitivity of Rubisco activase [5]. There is a general consensus that PSII is the main target for heat mediated disturbances of the photosynthetic electron transport [6], and in particular the functionality of the oxygen evolving complex (OEC) is affected [7].

The wild relatives of cultivated crops are sources for genetic adaptations to unfavourable environmental conditions. Cultivated barley's wild progenitor (*Hordeum spontaneum*) is distributed from the Eastern Mediterranean region, North Africa over the "Fertile Crescent" and the Middle East to Central Asia [8], (Fig. 1). Wild barley penetrates into mountainous and arid areas [9], and genotypes growing in those regions are believed to carry adaptations to extreme abiotic conditions. These adaptations may help to improve the resistance of cultivated barley to biotic and abiotic stresses [10,11].

Abbreviations: CCD, charge-coupled device; CF, chlorophyll fluorescence; CFI, chlorophyll fluorescence imaging; DF, driving force; F_V/F_M, maximum quantum efficiency of PSII; HRI, Heat Resistance Index; HS, heat stress; NPQ, nonphotochemical quenching; OEC, oxygen evolving complex; PI, Performance Index; RC, reaction centre; ROS, reactive oxygen species; RT, room temperature; T_c, critical temperature; φP_0, maximum quantum efficiency of PSII in the context of JIP-Test analysis; ΦPSII, actual photochemical efficiency of PSII.

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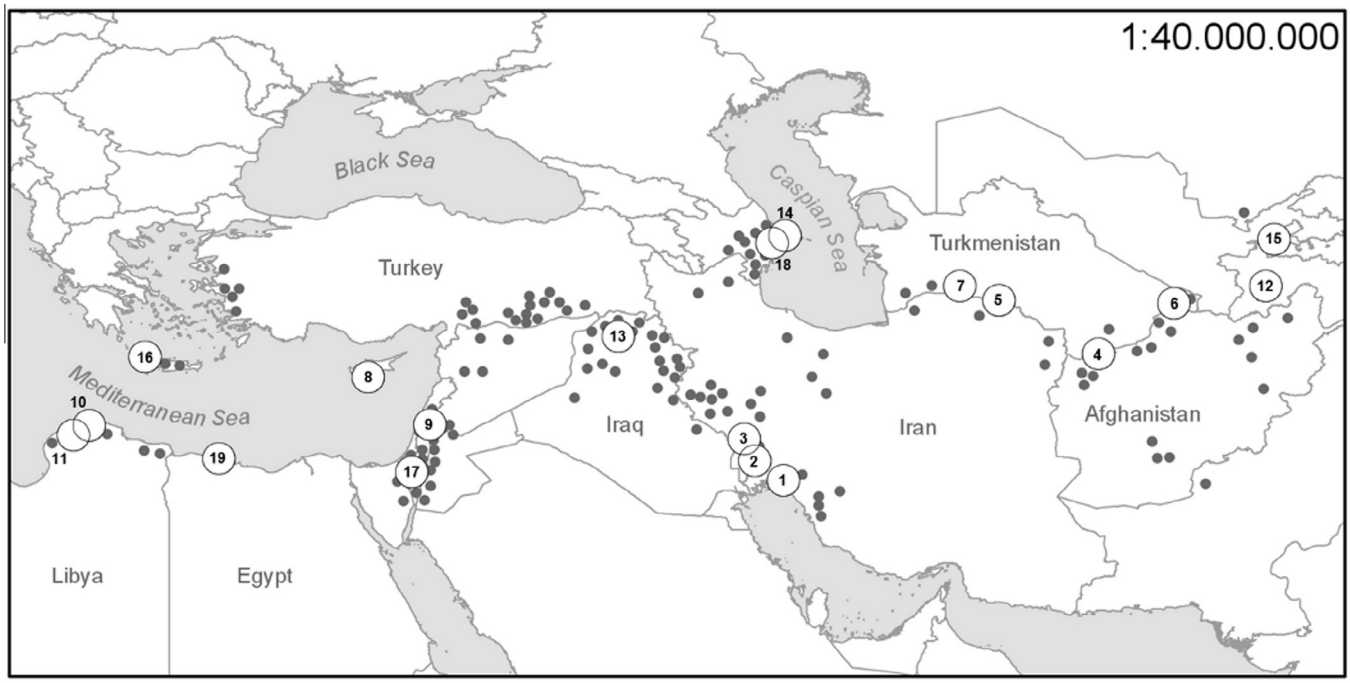

Fig. 1. Geographical distribution of wild barley (grey dots, original data taken from [8]) and collection sites of the *H. spontaneum* genotypes used in our study (numbered white dots). Detailed information about the genotypes is given in Table 1.

The fragility of PSII under high temperatures makes measurements of chlorophyll fluorescence (CF) induction curves an attractive, non-invasive method for the detection of HS symptoms. Chlorophyll fluorescence imaging (CFI) is a popular tool used in a large number of studies in plant stress research [12–14]. One main advantage of CFI is the detection of spatial heterogeneities of samples, which are likely missed by single point measurements of CF. Recently, there is a growing interest in the inclusion of CFI into automated high-throughput phenotyping platforms [15,16], using CF parameters under controlled environmental conditions in combination with other traits (*e.g.* leaf temperature *via* thermal imaging) for the characterisation of a large number of plants in a short time. In these studies, most frequently measurements of the maximum quantum yield of primary photochemistry of PSII (F_V/F_M) or of parameters based on Pulse-Amplitude-Modulated techniques [17], *e.g.* ϕPSII or NPQ, are favoured [18,19]. However, the imaging of fast CF induction curves (OJIP) – used successfully in numerous studies dealing with the impact of environmental stresses on PSII [20,21] – can as well be expected to provide valuable information about photosynthetic electron transport.

The aims of this study were the establishment of a reliable and standardised screening procedure for the measurement of OJIP parameters *via* CFI, the examination of differential reactions of *H. spontaneum* genotypes, covering a broad geographic spectrum to increased temperatures, and the detection of spatial heterogeneities of the fluorescence signal under HS.

Table 1
Detailed information about the *H. spontaneum* genotypes used in this study. The locations are numbered according to the labelling in Fig. 1; IPK = Leibniz Institute of Plant Genetics and Crop Plant Research, MPI = Max Planck Institute for Plant Breeding Research.

Genotype		Location		Source
1	HOR2680	①	Aghajari, Iran	IPK
2	HOR2686	②	Ahvaz, Iran	IPK
3	HOR2688	③	Shush, Iran	IPK
4	HOR2710	④	Kalai-Mor, Turkmenistan	IPK
5	HOR2826			
6	HOR4855	⑤	Manysh, Turkmenistan	IPK
7	HOR4859	⑥	Tashrabad, Turkmenistan	IPK
8	HOR4861	⑦	Koyno-Kumbez, Turkmenistan	IPK
9	HOR8266	⑧	Limassol, Cyprus	IPK
10	HOR9475	⑨	Tiberias, Israel	IPK
11	HOR9721	⑩	Bayda, Libya	IPK
12	HOR10164	⑪	Tacnis, Libya	IPK
13	HOR10404	⑫	Nurek, Tajikistan	IPK
14	HOR10478	⑬	Mosul, Iraq	IPK
15	HOR10710	⑭	Baku, Azerbaijan	IPK
16	HOR10977	⑮	Khujand, Tajikistan	IPK
17	HOR11017	⑯	Chania, Greece	IPK
18	HOR12818	⑰	Sede Boker, Israel	IPK
19	GOB	⑱	Gobustan, Azerbaijan	Own collection
20	WH		Wadi Habies, Egypt	MPI

2. Materials and methods

2.1. Plant material and growth conditions

In total, 20 genotypes of *H. spontaneum* Koch were used in this study (Fig. 1; Table 1). The seed material of the genotypes was provided by the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK, Gatersleben, Germany), the Max Planck Institute for Plant Breeding Research (MPI, Cologne, Germany) and from a collection of our working group. Seeds were germinated on Vermiculite for 48–72 h under the growing conditions described below. After germination, the seedlings were transferred into plastic pots containing one litre of commercial peat soil (*type C700*, *Stender Erden*, Schermbeck, Germany), using one individual per pot. Wild barley plants were cultivated in a growth chamber (*Fitotron SGC120 Plant Growth Chamber*, *Weiss Umwelttechnik*, Reiskirchen, Germany) at a day/night rhythm of 12/12 h at 25 °C (day)/20 °C (night), 50% relative humidity and a light intensity of 250 µmol m^{-2} s^{-1} under daily watering.