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Genotypic response of detached leaves versus intact plants for chlorophyll fluorescence parameters under high temperature stress in wheat

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ABSTRACT

The genotypic response of wheat cultivars as affected by two methods of heat stress treatment (treatment of intact plants in growth chambers versus treatment of detached leaves in test tubes) in a temperature controlled water bath were compared to investigate how such different methods of heat treatment affect chlorophyll fluorescence parameters. A set of 41 spring wheat cultivars differing in their maximum photochemical efficiency of photosystem (PS) II (F_v/F_m) under heat stress conditions was used. These cultivars were previously evaluated based on the heat treatment of intact plants. The responses of the same cultivars to heat stress were compared between the two methods of heat treatment. The results showed that in detached leaves, all of the fluorescence parameters remained almost unaffected in control (20 °C at all durations tested), indicating that the detachment itself did not affect the fluorescence parameters. In contrast, heat induced reduction in the maximum photochemical efficiency of PSII of detached leaves occurred within 2 h at 40 °C and within 30 min at 45 °C, and the response was more pronounced than when intact plants were heat stressed for three days at 40 °C. The proportion of total variation that can be ascribed to the genetic differences among cultivars for a trait was estimated as genetic determination. During heat treatment, the genetic determination of most of the fluorescence parameters was lower in detached leaves than in intact plants. In addition, the correlation of the cultivar response in intact plants versus detached leaves was low ($r = 0.13$ (with expt.1) and 0.02 with expt.2). The most important difference between the two methods was the pronounced difference in time scale of reaction, which may indicate the involvement of different physiological mechanisms in response to high temperatures. Further, the results suggest that genetic factors associated with cultivar differences are different for the two methods of heat treatment.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important food crops, cultivated on more than 216 million hectares of farmland worldwide with overall production of 651 million tons (FAO, 2012). Heat stress as a result of climate change may negatively affect wheat grain yields (Ortiz et al., 2008). The effect of heat stress on photosynthetic performance was foreseen by the discovery of thermal instability of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activase (Feller et al., 1998; Sayed, 2003) and inhibition of electron transport in photosystem II (PSII) (Haldimann and Feller, 2005; Mathur et al., 2011).

In addition to agronomic and other management strategies, improvement of heat tolerance in wheat through breeding and

Abbreviations: $(1 - V_j)/V_j$, variable fluorescence at J step in the fast fluorescence transient curve (OJIP curve); Area, relative area above the OJIP curve from F_m; F_m, maximum fluorescence; F_o, minimum fluorescence; F_v, variable fluorescence; F_v/F_m, maximum photochemical efficiency of PSII; F_v/F_o, maximum primary yield of photochemistry of PSII; PI, performance index; PPFD, photosynthetic photon flux density; PSII, photosystem II; RC/ABS, active reaction centers per absorbance; TF_m, time to reach F_m.

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molecular tools is in progress (Farooq et al., 2011). However, the large diversity of wheat cultivars combined with polyploidy and gene redundancy makes it difficult to identify desirable genotypes (Dong et al., 2009; Farooq et al., 2011). Therefore, physiological and biochemical screening techniques are desirable to complement phenotypic measurements in order to increase the selection efficiency (Ibrahim and Quick, 2001; Reynolds et al., 1994). Traits previously studied for screening of wheat for heat tolerance include cell membrane thermo stability (Farooq et al., 2011; Ibrahim and Quick, 2001), cellular respiration (Farooq et al., 2011), canopy temperature depression (Ali et al., 2010; Reynolds et al., 1994) and individual kernel weight (Sharma et al., 2008). Most of these methods require long measurement time or involve complex laboratory techniques. In addition, evaluation of heat tolerance under field conditions based on yield parameters is a slow process, influenced by many factors. The multiple environmental factors affecting field trials complicate phenotyping of material adapted to different growing conditions, because the response to a specific stress confounds the effects of plant adaptation.

In stress physiology, chlorophyll fluorescence is an important non-invasive technique in assessing and quantifying damage to the leaf photosynthetic apparatus, particularly PSII activity in response to environmental stresses (Baker and Rosenqvist, 2004; Maxwell and Johnson, 2000). However, this method also has some common pitfalls associated with the measurements and analysis (Logan et al., 2007). Thus, due care should be taken to create uniform conditions including the use of uniform illumination of sampling leaves during the heat treatment, plants of equal developmental stage, etc., all factors that may influence the fluorescence measurements, when different genotypes of diverse origin are being treated and compared (Sharma et al., 2012).

We have used F_v/F_m as the selection criterion for heat stress tolerance (Sharma et al., 2012). As a measure of the maximum photochemical efficiency of PSII (F_v/F_m) (Baker and Rosenqvist, 2004), usually any decrease in F_v/F_m results in a corresponding decrease in the maximum apparent quantum yield of the photosynthetic light response curve measured by gas exchange at ambient CO_2 concentration (Ögren and Sjöström, 1990) as well as when measured as oxygen evolution at saturating CO_2 (Demmig and Björkman, 1987). Changes in F_v/F_m thus may have a direct effect on the carbon gain of the plant, especially when light is a limiting factor in the photosynthesis. In a heat pulse treatment (50 °C for 20–40 s), the reduction in F_v/F_m has been reported to represent the loss of PSII capacity rather than loss of maximum quantum yield of PSII (Tóth et al., 2005), and the destruction of the manganese cluster by a heat pulse affected the oxygen evolution but had a small effect on the quantum yield of PSII (Tóth et al., 2007). The quantitative meaning of F_v/F_m is based on the Q_A model (Duysens and Sweers, 1963), with the assumption that change in fluorescence reflects the changes in the redox state of Q_A in PSII, and this parameter has been used in numerous stress physiological studies to relate the maximum quantum yield of PSII photochemistry. Recently, the Q_A plus light-induced conformational change model have been proposed to better understand the quantitative meaning of F_v/F_m (Schansker et al., 2013). Some fluorometers also calculate other parameters from the fast phase of the fluorescence induction curve, also called the OJIP curve, used for the JIP-test. They are *e.g.* the maximum primary yield of photochemistry of PSII (F_v/F_o), active reaction centers per absorbance (RC/ABS) and the performance index (PI), while time to reach F_m (*T*Fm) is a descriptive parameter estimating the time to reach maximum fluorescence, $(1 - V_j)/V_j$ a fluorescence transient curve-derived parameter related to the probability with which a PSII trapped electron is transferred from Q_A to Q_B and Area is the relative area above the OJIP curve (Stirbet and Govindjee, 2011; Strasser et al., 2004). Many of the JIP-test parameters are interdependent (Strasser et al., 2004) and it is not clear yet how the overall effect of heat stress on carbon gain in plants is reflected by these parameters.

Under field conditions, the effects of heat stress on plants are often confounded with other factors such as drought and light. Apart from the high cost of conducting yield trials under various stress conditions, the difficulty in separating such effects of different stresses and stress periods complicates the screening protocol for single stress factors. Treating intact plants in controlled environments is a viable option, but limitations in capacity and resources restrict the number of genotypes to be evaluated. Therefore, cheaper, faster and easier screening methods to measure heat tolerance capacity in large numbers of genotypes would be valuable for the selection of wheat cultivars.

In general, many plant physiological responses to elevated temperatures have been investigated by *in vitro* studies with the use of detached leaf segments or isolated organelles. Some studies have been performed using a water bath to heat stress detached leaves in tubes, demonstrating the influence of elevated temperature on chlorophyll fluorescence parameters (Havaux, 1993; Mathur et al., 2011). This detached leaf method not only allows an excellent isolation of heat stress from the influence of other abiotic factors such as light, water and nutrient supply, but it also provides an opportunity for fast evaluation of a large number of plant samples. However, it is still not known if a detached leaf system can also be used to select plants for their heat tolerance. The main aim of the present study was to test the genotypic response of wheat cultivars to the effect of heat treatment on the detached leaves versus intact plants using the chlorophyll fluorescence technique of stress detection.

Materials and methods

Plant material

The plant material consisted of 41 spring wheat cultivars, previously shown, using intact plants, to differ in their heat tolerance, evaluated as their maximum photochemical efficiency of photosystem II (PSII) (F_v/F_m) by chlorophyll *a* fluorescence (Sharma et al., 2012). These cultivars belong to different wheat growing regions of the world (Sharma et al., 2012).

Growing conditions

Single seeds were sown in pots (11 cm diameter; 0.59 L) filled with peat (Pindstrup 2, Pindstrup Mosebrug A/S, Ryomgaard, Denmark). The plants were grown in a greenhouse under long day conditions (16/8 h day/night) with air temperature of 15 ± 3 °C during the day and 12 ± 3 °C during the night, 50–70% relative humidity and ambient CO_2 concentration. Supplementary light of 60 μmol photons m^{-2} s^{-1} was provided from high-pressure sodium lamps (SON-T Agro, 600W, Phillips, Eindhoven, The Netherlands) in the evening time, so as to maintain the photoperiod of 16 h. Plants were fertilized with a nutrient solution consisting of: N (185 mg L^{-1}), P (27 mg L^{-1}), K (171 mg L^{-1}), Mg (20 mg L^{-1}) and full micronutrients. The plants were grown under these conditions until the phenotypic stage ranged from late stem elongation to the inflorescence emergence stage based on BBCH identification keys of wheat (Lancashire et al., 1991).

Experimental set up

Two experiments (separated in time) were conducted with detached leaf treatment in a water bath. Each experiment had three complete randomized blocks and the blocks were separated in time by one week in experiment 1 and two weeks in experiment 2. In experiment 1, four plants of each cultivar were used in each block (3 blocks × 4 plants = 12 plants per cultivar). In experiment 2,