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Physiological and proteome studies of responses to heat stress during grain filling in contrasting wheat cultivars☆

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ABSTRACT

Experiments to explore physiological and biochemical differences of the effects of heat stress in ten wheat (*Triticum aestivum* L.) cultivars have been performed. Based on the response of photosynthesis rates, cell membrane lipid peroxide concentrations and grain yield to heat, six cultivars were clustered as heat-tolerant (cv. '579′, cv. '810′, cv. '1110′, cv. Terice, cv. Taifun and cv. Vinjett) and four as heat-sensitive (cv. '490′, cv. '633′, cv. '1039′ and cv. '1159′). Higher rates of photosynthetic carbon- and light-use were accompanied by lower damage to cell membranes in leaves of tolerant compared to sensitive cultivars under heat stress. The tolerant cv. '810′ and the sensitive cv. '1039′ were selected for further proteome analysis of leaves. Proteins related to photosynthesis, glycolysis, stress defence, heat shock and ATP production were differently expressed in leaves of the tolerant and sensitive cultivar under heat stress in relation to the corresponding control. The abundance of proteins related to signal transduction, heat shock, photosynthesis, and antioxidants increased, while the abundance of proteins related to nitrogen metabolism decreased in the tolerant cv. '810′ under heat stress as compared to the control. Collectively, the results indicate that primarily changes in both the amount and activities of enzymes involved in photosynthesis and antioxidant activities in leaves contributed to higher heat tolerance in the cv. '810′ compared to the heat sensitive cv. '1039′.

1. Introduction

Increased frequency and duration of high temperature episodes during the last decade is becoming a limiting factor for plant growth [1,2]. In cereals, high temperature events especially occurring during the reproductive growth stage can significantly decrease both crop yield and quality [3,4]. Wheat, one of the most important crops, is sensitive to heat stress, which is considered to be one of the major limiting factors for wheat production in Europe [5]. The optimum temperature for wheat during grain filling is around 21 °C [6,7], and higher temperatures have been shown to significantly decrease grain yield [8]. Thus, improvement of crop tolerance under more frequent heat stress conditions will become essential for food supply.

The effects of heat stress on wheat production are quite complex [9]. Heat stress causes adverse effects for plant growth, development, crop yield and quality [10]. Photosynthesis as one of the most heat-sensitive physiological processes is significantly inhibited by high temperatures [11,12]. It has been reported that

Abbreviations: A/C_i, curve, carbon assimilation versus intercellular CO_2 concentration curve; APX, ascorbate peroxidase; A_{sat}, saturated net photosynthetic rate; CAT, catalase; CPN60, chaperonin 60 proteins; DHAR, dehydroascorbate reductase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GR, glutathione reductase; g_s, stomatal conductance; HSPs, heat shock proteins; J_{max}, maximum photosynthetic electron transport rate; MDA, malondialdehyde; NPQ, non-photochemical quenching of chlorophyll fluorescence; Pn, photosynthetic rates; PSII, photosystem II; ΦPSII, actual PSII photochemical efficiency; RCA, Rubisco activase; RLS, Rubisco large subunit; RSS, Rubisco small subunit; Rubisco, Ribulose-1,5-bisphosphate carboxylase/oxygenase; V_{max}, maximum carboxylation rate of Rubisco; SAMS, S-adenosylmethionine synthase; SOD, superoxide dismutase; TR, transpiration rate; TPU, triose phosphate utilization.

☆ This paper is dedicated to the late Susanne Jacobsen.

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heat stress inhibits photosynthesis by decreasing the Rubisco activation state *via* inhibition of Rubisco activase [13,14]. The oxygen evolution complex in PSII is also considered to be vulnerable to heat stress [15]. Higher photochemical light-use efficiency and heat dissipation correlate with heat tolerance [16–18]. The disturbance of photosynthesis and other metabolic processes will result in the generation of reactive oxygen species (ROS), leading to cell membrane peroxidation, protein oxidation and DNA damage [19]. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and dehydroascorbate reductase (DHAR) play important roles in detoxifying ROS [20]. Both enhanced activity and synthesis of antioxidant enzymes correlate with heat tolerance in crops [12,18,21,22].

Proteome analysis is as a useful tool to investigate the mechanisms of plant response to abiotic stresses [23,24]. Previous studies found that proteins related to the electron transport chain, redox homeostasis, heat shock proteins (HSPs) and glycolysis may play critical roles in protecting leaves against heat stress [25]. Increased abundance of sucrose synthase, glutathione S-transferase, SOD and of HSPs could be important in protecting plants from heat stress [26]. Few studies have combined physiological and proteome analyses to elucidate changes in the abundance of and/or activity of relevant enzymes in response to heat stress.

The hypothesis of this study has been that responses of physiological and proteomic parameters in tolerant and sensitive wheat cultivars will help to indicate possible thermo-tolerance mechanisms. The objective of this study was therefore to quantify the effects of a heat stress event during grain filling in ten different wheat cultivars followed by a detailed proteome analysis of differences to a heat stress event in the tolerant and the sensitive cultivar.

2. Material and methods

2.1. Plant material and experimental setup

An outdoor pot experiment was done at the Research Centre Flakkebjerg, Aarhus University, Denmark in 2011. Ten kg soil (16:9:4 v/v/v mixture of peat moss, loamy soil and sand) was filled into each pot (18 cm height and 23 cm in diameter). Ten wheat (*Triticum aestivum* L.) cultivars were used in this study (Table 1) and for each cultivar 4 pots were used for each treatment. The plants were thinned to 4 seedlings per pot at the three-leaf stage. The phenology of each cultivar was carefully recorded according to Zadoks et al. [27]. The heat stress treatment started at 15 days after anthesis (determined individually for each cultivar as shown in Table 1) and lasted for 5 days. One half of the plants were moved into a climate chamber (PGV36; Conviron, Montreal, QC, Canada) with halogen lamps (HRI.BT 400W/D Pro daylight E40 (Radium Lampenwerk GmbH, Wipperfürth, Germany)) and with temperatures set to 35 °C/26 °C (day/night) and with a day length of 14 h. The photosynthetically active radiation was set to 400 μmol m^{-2} s^{-1}. The control plants were moved into a climate chamber with temperatures set to 20 °C/12 °C (day/night). The relative humidity was controlled around 60% in the control and 70% in the heat treatment chamber. After the heat stress treatment, all plants were moved outdoors again until maturity.

2.2. Gas exchange parameters and chlorophyll content

After 5 days of heat stress treatment, flag leaves were used for the gas exchange measurements according to Wang et al. [12]. The LI-6400 system (LI-COR Biosciences, Lincoln, NE, USA) was used to measure photosynthesis rates from 8:30 a.m. to 11:30 a.m. with the light level set at 1200 μmol m^{-2} s^{-1}. The response curves of net carbon assimilation rate versus intercellular CO_2 concentration (A/C_i) were determined in three biological replicates (flag leaves) using the LI-COR "A/C_i curve" program, with CO_2 concentrations set to 400, 300, 200, 150, 100, 50, 400, 800 and 1500 ppm. The maximum carboxylation rate of Rubisco (V_{max}), maximum electron transport rate (J_{max}), triose phosphate utilization (TPU) and saturated net photosynthesis rate (A_{sat}) were determined according to Long and Bernacchi [28]. Chlorophyll content was measured on six biological replicates (leaves) with a chlorophyll meter SPAD502 (Soil Plant Analysis Development; Minolta, Japan).

2.3. Chlorophyll a fluorescence

After 5 days of heat stress treatment, the plants were dark adapted for at least 20 min before measuring leaf chlorophyll fluorescence in the flag leaves (PAM chlorophyll fluorometer, M-series, Heinz Walz, Effeltrich, Germany). The actual PSII photochemical efficiency (ΦPSII) and non-photochemical quenching of chlorophyll fluorescence (NPQ) were recorded. Three biological replicates (leaves from different plants in different pots) were done.

2.4. Cell membrane peroxidation and antioxidant enzyme activities

The extract for determining the membrane lipid peroxidation was prepared according to Dhindsa et al. [29]. The extent of membrane lipid oxidation in flag leaves was determined by analyzing the malondialdehyde (MDA) content according to Heath and Packer [30]. Ascorbate peroxidase (APX) was assayed according to Nakano and Asada [31], glutathione reductase (GR) activity was measured according to Foyer and Halliwell [32], and dehydroascorbate reductase (DHAR) was determined according to Mishra et al. [33]. Soluble protein content was estimated by the method of Bradford [34]. The above measurements were done after 5 days of heat stress in three biological replicates (leaves).

2.5. Leaf proteomic analysis

2.5.1. Protein extraction and quantification

After heat stress treatment, the flag leaves of cv. '810′ and cv. '1039′ were harvested for proteome analysis. Protein extraction was performed according to Rinalducci et al. [35]. Briefly, flag leaves were ground in liquid nitrogen, and then aliquots of 0.5 g were extracted with 5 mL 10% (w/v) trichloroacetic acid in acetone containing 0.07% (w/v) DTT and 1 tablet protease inhibitor cocktail (Roche, Basel, Switzerland) per 50 mL extraction solution, vortexed and incubated at −20 °C overnight. The extraction was centrifuged at 35,000 × *g* for 1 h (4 °C), and the pellet was washed three times with cold acetone containing 0.07% (w/v) DTT, incubated for 2 h and centrifuged at 20,000 × *g* for 30 min (4 °C). The pellet was dried and then solubilised in lysis buffer containing 9 M urea, 4% (w/v) CHAPS, 1% (w/v) DTT, 1% (v/v) ampholytes pH 4–7 (GE Healthcare, Freiburg, Germany) and 35 mM Tris (Sigma). The mixture was centrifuged at 12,000 × *g* for 20 min (room temperature) and the supernatant was used for two-dimensional gel electrophoresis. The protein concentration was analysed according to Ramagli [36]. Three biological replicates were done.

2.5.2. Two-dimensional gel electrophoresis

The IPG strips (linear pH 4–7, 18 cm, GE Healthcare) were rehydrated in 350 μL of solubilisation solution containing 1% ampholyte pH 4–7 (GE Healthcare), Orange G and 400 μg protein. After isoelectric focusing, the strips were subsequently equilibrated in 5 mL equilibration buffer (50 mM Tris–HCl pH 8.8, 6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS, 0.01% (w/v) bromophenol blue) with 1%, w/v DTT for 15 min and then incubated in 5 mL equilibration buffer with