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Physiological and transcriptional analyses of induced post-anthesis thermo-tolerance by heat-shock pretreatment on germinating seeds of winter wheat

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ABSTRACT

Heat stress occurring during grain filling is a worldwide environmental constraint limiting wheat production. To evaluate the alleviation effects of heat-shock pretreatment during germination on post-anthesis heat stress, germinating seeds were firstly heat-shocked at 40 °C for four hours. The plants were later given a five-day high temperature stress from ten days after anthesis. The post-anthesis heat stress resulted in significant grain yield reduction. However, the heat-shock pretreatment caused less yield loss. Physiological analyses revealed that capacities of leaf photosynthesis and antioxidation were improved to benefit the less yield loss in the pretreated plants. This was consistent with the differentially regulated expressions of the involving genes as revealed by the transcriptomic analysis. In addition, the transcriptome profiling indicated that the stress signaling processes were triggered, and expressions of stress related genes such as heat-shock proteins and osmotins were up-regulated in the pretreated plants. Thus, the up-regulated physiological processes of photosynthesis, antioxidation and HSPs accumulation because of the modified expressions of the related genes could contribute to the enhanced thermo-tolerance induced by heat-shock pretreatment.

1. Introduction

Wheat is one of the most important staple crops in the world by virtue of its key contribution in food security (Ainsworth and Ort, 2010). Heat stresses, especially those occurred during grain filling, caused severe grain yield losses (Hays et al., 2007; Tan et al., 2008; Wang et al., 2011). The yield losses were ascribed to the reduction in photosynthesis, the imbalance in carbon and nitrogen metabolism, the accumulation of toxic compounds and reactive oxygen species (ROS), and the shortened period of grain filling (Hays et al., 2007; Lim et al., 2007; Dias et al., 2010). Importantly, heat stresses are predicted to become more frequent and severe due to the future climate change (Hays et al., 2007; Wang et al., 2011). Thus, it is of key importance to improve heat-stress tolerance to reduce yield loss of wheat under global warming scenarios.

Induction of thermo-tolerance has been demonstrated to be an effective measure to alleviate the negative effects of high-temperature stress. Thermo-tolerance can be achieved not only by breeding approaches, but also via plant training, such as high-temperature preconditioning or priming, or via applications of plant growth regulators or antioxidants (Kotak et al., 2007; Wahid et al., 2007; Hayat et al., 2010; Wang et al., 2011). Recently, high-temperature priming was found to be very effective in alleviating the negative effects of recurrent severe heat stresses on plant growth and yield (Wang et al., 2011; Mittler et al., 2012). The enhanced thermo-tolerance was related to the improvement of photosynthesis, better maintenance of reactive oxygen species (ROS) homeostasis and membrane thermo-stability at the physiological level (Dias et al., 2010; Cossani and Reynolds, 2012), and to the regulated expressions of a large number of genes associated with stress and signal transduction systems at the molecular level (Wahid et al., 2007; Ahuja et al., 2010).

One of the best characterized aspects of acquired thermo-tolerance is the production of heat-shock proteins (HSPs)

Abbreviations: CAT, catalase; Ci, intercellular CO_2 concentration; Fv/Fm, maximum photochemical efficiency after full dark adaptation; gs, stomatal conductance; H_2O_2, hydrogen peroxide; MDA, malonildialdehyde; NPQ, non-photochemical quenching; $O_2^{\cdot-}$, superoxide anions radical; Pn, net photosynthetic rate; POD, peroxidase; ΦPSII, actual photosynthetic efficiency; qP, photochemical quenching; ROS, reactive oxygen species; SOD, superoxide dismutase; Tr, transpiration rate.

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(Xue et al., 2014). HSPs play important physiological roles in response to many kinds of abiotic stresses, such as heat, chilling, salinity and oxidative stresses (Wahid et al., 2007; Kosova et al., 2011). In addition, HSPs are also involved in numerous regulatory pathways and act as molecular chaperones by interacting with other proteins (Wang et al., 2004). Previous studies have shown that HSP synthesis can be induced by a mild heat acclimation or priming, and also by heat shock (Helm and Abernethy 1990; Shah et al., 2011; Mittler et al., 2012). However, the effects of HSPs induced by heat priming on alleviating the reoccurring high-temperature stress, and the underlying mechanisms, remain largely unclear.

Seed germination is the original and critical stage over the whole plant growth period. During germination, a large number of genes are activated to encode numerous germination-related proteins, which further trigger a series of biochemical and physiological processes (Toole et al., 1956; Yang et al., 2007). Thus, we supposed that heat-shock pretreated plants during this period might induce more robust responses to abiotic stress and enable them to better cope with the recurring stresses than those applied at other growth stages.

Thereafter, we pretreated germinating wheat seeds with a heat shock, and then exposed the plants to a five-day high-temperature stress after anthesis. One objective of this study is to test whether the plants from the heat-shock pretreated germinating seeds could acquire tolerance to the post-anthesis heat stress. Leaf gas exchange, chlorophyll fluorescence, antioxidant enzymes activities, and transcriptional profiling were measured to reveal the underlying physiological mechanisms. The results could provide new knowledge of crop plants in response to heat stress, and help explore new approaches to alleviate the post-anthesis heat stress in wheat production for future climates.

2. Materials and methods

2.1. Experimental design

2.1.1. Heat-shock pretreatment and plant growth

The experiment was conducted during wheat growing season of 2011–2012 at the Experimental Station of Nanjing Agricultural University, Nanjing, Jiangsu Province, P. R. China. Uniform seeds of winter wheat (*Triticum aestivum* L.) cv. Yangmai 16 were surface-sterilized with 30% H_2O_2 for 15 min and then quickly rinsed several times with distilled water. Thereafter, the seeds were soaked in distilled water in a RXZ-430D growth chamber (Dongnan Corporation, China) at 20 °C. The heat-shock pretreatment was performed by transferring the seeds into another growth chamber at 40 °C. The heat-shock pretreatment lasted for 4 h, the seeds were then moved back to the chamber at 20 °C for another 20 h. The pretreatment protocol was set according to the results of preliminary experiments (Table S1) and previous studies regarding effects of heat-shock on seed germination (Burke and Orzech Usda-Ars, 1988; Helm and Abernethy, 1990). Seeds which had been soaked with distilled water consistently at 20 °C for 24 h were set as a control.

Thereafter, the heat-shock pretreated seeds and the non-pretreated seeds were separately sown in plastic pots (22 cm in height and 25 cm in diameter). All pots were filled with 7.5 kg clay soil which contained 13.1 g kg^{-1} organic matter, 1.1 g kg^{-1} total N, 72.4 mg kg^{-1} available N, 41.9 mg kg^{-1} Olsen-P, 146.2 mg kg^{-1} available K. The soil was mixed with 0.9 g N, 0.36 g P_2O_5, and 0.9 g K_2O per pot before sowing, and another 0.3 g N per pot was applied at the jointing stage. Fifteen seeds were sown in each pot, and the seedlings were thinned to six at the three-leaf stage. The plants were grown in the semi-field facilities covered with bird net and equipped with transparent rainproof top (except during heat stress treatment). The plants were watered every two or three days to control the soil relative water content at around 70%.

2.1.2. Post-anthesis heat stress

Post-anthesis heat stress was performed from 10 days after anthesis by moving wheat plants into a growth chamber at a day/night temperature of 35/27 °C, since the high temperature stress events (over 32 °C) in Yangtze River Plain usually occur during this time (Liu et al., 2016). All plants from the heat-shock pretreated seeds and half of the plants from the non-pretreated seeds were exposed to the high-temperature stress, and were denoted as HS-H and CH, respectively. The remaining plants from the non-pretreated seeds were moved into another growth chamber at a day/night temperature of 28/20 °C as control (CC). The light intensity during the day time was set at 1000 μmol m^{-2} s^{-1}, while the humidity was controlled at around 70%. After a five-day period of heat stress, all plants were moved out of the chambers and grown under semi-field conditions until maturity. At the last day of the high temperature stress event, flag leaves of each treatment were harvested. One share of the samples was immediately frozen in liquid nitrogen, and then stored at −80 °C until analysis of enzyme activity, western blot and microarray. Another share was oven-dried at 80 °C to analyze the contents of total soluble sugars and free amino acids. At maturity, all plants in each pot were harvested to determine grain yield. Three pots of each treatment were harvested as three biological replicates. The conditions over the period of post-anthesis high temperature events and the climate data over the whole growth stage were shown in Supplementary files (Fig. S1 and Fig. S2).

2.2. Measurements

2.2.1. Gas exchange

Gas exchange of flag leaf was measured on the last day of the post-anthesis heat stress, using a LI-6400 system (Portable Photosynthesis System, Li-Cor, Lincoln, USA) equipped with a standard 2 × 3 cm chamber with light-emitting diode light sources. All the measurements were taken under a light intensity of 1000 μmol m^{-2} s^{-1} and at a CO_2 concentration of ca. 380 μmol mol^{-1} with a constant flow rate of 500 μmol s^{-1}, as reported previously (Wang et al., 2011).

2.2.2. Chlorophyll fluorescence

Chlorophyll fluorescence parameters were measured using the same leaves as for gas exchange measurements, using a portable pulse amplitude modulation fluorescence monitoring system (FMS2, Hansatech Instruments Ltd, King's Lynn, Norfolk, UK). After at least 20 min of dark adaption, the initial chlorophyll fluorescence yield (Fo) was determined at a low modulated measuring light. Thereafter, a pulse of saturating white light (>3000 μmolm^{-2} s^{-1}) was applied to obtain the maximum chlorophyll fluorescence yield (Fm). Following measurement of the steady state fluorescence (Fs) using actinic light, a pulse of saturating light was applied to obtain the maximum fluorescence under the light-adapted state (Fm'). Afterwards, a far-red light (20 μmolm^{-2} s^{-1}) was applied for determination of the minimal level of fluorescence (Fo'). The variable fluorescence (Fv = Fm − Fo), actual photosynthetic efficiency (ΦPSII = (Fm' − Fs)/Fm'), maximum photochemical efficiency of photosystem II under dark-adapted conditions (Fv/Fm), photochemical quenching (qP = (Fm' − Fs)/(Fm' − Fo'), and non-photochemical quenching (NPQ = (Fm − Fm')/Fm') were then calculated according to Kramer et al. (2004).

2.2.3. Contents of total soluble sugars, sucrose, and free amino acids

The contents of total soluble sugars (TSS) and sucrose were measured using the anthrone reagents method, and the content of