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Research article

Cold priming drives the sub-cellular antioxidant systems to protect photosynthetic electron transport against subsequent low temperature stress in winter wheat

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ABSTRACT

Low temperature seriously depresses the growth of wheat through inhibition of photosynthesis, while earlier cold priming may enhance the tolerance of plants to subsequent low temperature stress. Here, winter wheat plants were firstly cold primed (5.2 °C lower temperature than the ambient temperature, viz., 10.0 °C) at the Zadoks growth stage 28 (i.e. re-greening stage, starting on 20th of March) for 7 d, and after 14 d of recovery the plants were subsequently subjected to a 5 d low temperature stress (8.4 °C lower than the ambient temperature, viz., 14.1 °C) at the Zadoks growth stage 31 (i.e. jointing stage, starting on 8th April). Compared to the non-primed plants, the cold-primed plants possessed more effective oxygen scavenging systems in chloroplasts and mitochondria as exemplified by the increased activities of SOD, APX and CAT, resulting in a better maintenance in homeostasis of ROS production. The trapped energy flux (TR_O/CS_O) and electron transport (ET_O/CS_O) in the photosynthetic apparatus were found functioning well in the cold-primed plants leading to higher photosynthetic rate during the subsequent low temperature stress. Collectively, the results indicate that cold priming activated the sub-cellular antioxidant systems, depressing the oxidative burst in photosynthetic apparatus, hereby enhanced the tolerance to subsequent low temperature stress in winter wheat plants.

1. Introduction

As one of the most critical abiotic stresses limiting growth and productivity of winter wheat (*Triticum aestivum* L.), low temperature seriously depresses plant growth and causes significant reduction in grain yield (Kosova et al., 2013). It induces a series of metabolic changes, including inactivation of many metabolic enzymes and disturbance of the metabolic regulations (Xu et al., 2012), accumulation of osmolytes (e.g. proline, glycinebetaine) (Thakur and Nayyar, 2013; Ruelland et al., 2009), modifications of the carbohydrate metabolism and photosynthetic properties (Ruelland et al., 2009; Crosatti et al., 2013).

Photosynthesis converts light energy into ATP and redox equivalents (NADPH), which is the primary metabolic sink for plant growth (Ruelland et al., 2009). Low temperature stress affects many aspects of photosynthesis. For instance, it can inhibit thylakoid electron transport by increasing membrane viscosity and restricting the diffusion of plastoquinone. Light energy is trapped by the antenna of PSI and PSII to drive the charge separation in the reaction centres (RCs). This process can be disturbed by low temperatures, since the chlorophyll antenna complexes are able to trap more energy than the capacity of biochemical procession in the photosynthetic RCs, resulting in over-energized status in the thylakoid membranes (Ensminger et al., 2006). One of the consequences of this over-energized state is photodamage due to overproduction of reactive oxygen species (ROS) (Ruelland et al., 2009; Ashraf and Harris, 2013). It has been documented that the activities of the scavenging enzymes are depressed by low temperature stress (Thakur and Nayyar, 2013; Ruelland et al., 2009; Li et al.,

Abbreviations: APX, ascorbate peroxidase (EC 1.11.1.11); BSA, bovine serum albumin; CAT, catalase (EC 1.11.1.6); CS, cross-section; DTT, dithiothreitol; EGTA, ethylene glycol tetra acetic acid; FNR, ferredoxin-$NADP^+$ reductase; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MOPS, 3-(N-morpholino) propanesulfonic acid; PMSF, phenylmethylsulphonyl fluoride; PVP, polyvinylpyrrolidone; RC, reaction centre; ROS, reactive oxygen species; SLA, specific leaf area; SOD, superoxide dismutase (EC 1.15.1.1).

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2013), leading to inefficiency in counterbalancing the ROS production in the mitochondrial and chloroplastic electron transfer reactions (Ruelland et al., 2009). To survive under low temperature stress, plants have developed some defense mechanisms to enhance their tolerance to the stress (Thakur and Nayyar, 2013). Priming, defined as a temporally limited experience of an environmental stimulus which prepares the plant to cope more successful with a future environmental stimulus, has been find in wheat plants under varied abiotic stresses, including heat (Wang et al., 2014) and waterlogging (Li et al., 2011). However, the priming induced responses to cold stress remain largely elusive.

Higher plants possess active oxygen scavenging systems including several antioxidant enzymes, such as superoxide dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase (APX, EC 1.11.1.11) and catalase (CAT, EC 1.11.1.6), and the non-enzymatic antioxidants (Thakur and Nayyar, 2013). It is well known that the oxygen scavenging systems activated by cold hardening play a key role in enhancing cold tolerance (Thakur and Nayyar, 2013), especially those in chloroplasts and mitochondria, the major sites of ROS production in plant cells (Crosatti et al., 2013; Jacoby et al., 2012). However, little is known about the responses of the oxygen scavenging systems in chloroplasts and mitochondria to cold priming treatment and their roles in improving low temperature tolerance in wheat.

In the present study, wheat plants were firstly cold-primed for 7 d, and after two weeks recovery the plants were then exposed to a 5 d low temperature stress. The changes of oxygen scavenging systems in chloroplasts and mitochondria were investigated during the cold priming and the low temperature stress periods. It was hypothesized that modulations of the antioxidant systems in chloroplasts and mitochondria are involved in the acquirement of low temperature tolerance induced by the cold priming in winter wheat plants. The results will be helpful in understanding the roles of antioxidant systems in enhancing cold tolerance in winter crops.

2. Materials and methods

2.1. Plant materials and treatments

A semi-field experiment was conducted in the open-top chamber (OTC, Southeast Co. Ltd, Ningbo, China) at Lianyungang Experimental Station (119°32′ E, 34°30′ N) of Nanjing Agricultural University, Jiangsu Province, China during the wheat growing season in 2010–2011. Two winter wheat cultivars were used, i.e. a cold susceptible cultivar Funo and a cold tolerant cultivar HM18 (Data of preliminary experiment not published). The seeds of these two cultivars were sowed in the chamber on 16th October 2010. Before sowing, 120 kg N ha^{-1}, 60 kg P_2O_5 ha^{-1} and 120 kg K_2O ha^{-1} were applied as basal fertilizer and another 120 kg N ha^{-1} was top-dressed after jointing. The experimental design is shown in Fig. 1. In brief, a 7 d cold priming where 5.2 °C lower than the ambient temperature (10.0 °C) was conducted at the Zadoks growth stage 28 (Zadoks et al., 1974) (plants possessed one main shoot and eight tillers, starting on 20th of March), and after 14 d recovery a 5 d low temperature stress was imposed in which the temperature was 8.4 °C lower than the ambient temperature (14.1 °C) at the Zadoks growth stage 31 (Zadoks et al., 1974) (jointing stage, i.e. the 1st node was detectable, starting on 8th April). The detailed temperature data during the cold priming and subsequent low temperature stress was shown in Fig. 2. Low temperature treatments were applied with the OTC system. Air was cooled by a compressor (5.5 kW, CC-107, Ningbo Southeast Co., China), and then the pre-cooled air was driven by an air blower (350 W, AH9, Ningbo Southeast Co., China) through a major ducting tube connected with the sub-ducting tubes. In the sub-ducting tubes, small holes were drilled with uneven distance to make sure that very similar volume of pre-cooled air was released by each hole (the hole distance in the sub-conducting tube was longer near the major conducting tube while was shorter at the distal end). Six temperature and humidity sensors were installed to record the real-time data at a 10-min interval in the treatment and reference chambers, respectively. Finally, three treatments were conducted; non-cold priming + low temperature stress (NL), cold priming + low temperature stress (CL), non-cold priming + no low temperature stress (NN). The experiment was a split-plot design with temperature treatment as the main plot and wheat cultivar as subplot, and with three replicates for each treatment. Samples and measurements were conducted just before the onset and just after the ending of the cold priming and the low temperature stress, and after 5-day recovery of the low temperature stress.

2.2. Shoot biomass, concentrations of N and chlorophyll in the last fully expanded leaves

Five wheat plants were harvested for each replicate. The harvested shoot samples were oven-dried to get shoot biomass. Fresh leaf (0.1 g) was sliced and incubated in 50 ml of pigment extraction solution containing acetone and anhydrous ethanol (1: 1, v/v) in dark at 25 °C for 12 h. The supernatant was collected and the absorbance measured at 663 nm and 647 nm. Total chlorophyll concentration was then calculated according to Arnon (1949). Concentrations of total nitrogen in dry leaf samples were measured using the Kjeldahl method (Zhang et al., 2011).

2.3. Gas exchange, Chl a fluorescence transient and leaf area of the last fully expanded leaves

Photosynthetic rate (P_N) and stomatal conductance (g_s) of the latest fully expanded leaf were measured with a portable photosynthesis system (LI-6400, LI-Cor, NE, USA) at a CO_2 concentration of about 380 μmol mol^{-1}, at a photosynthetically active radiation of 1200 μmol m^{-2} s^{-1}. On each measurement occasion, five leaves

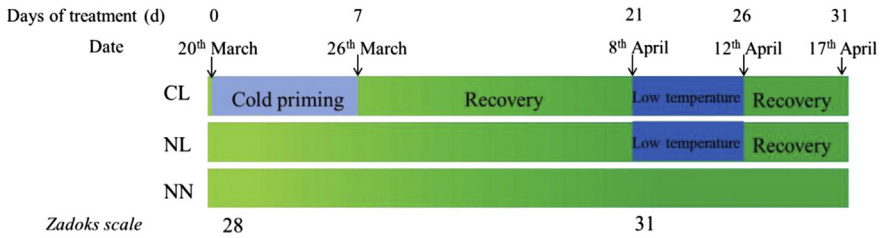

Fig. 1. Schematic representation of experimental design and treatments. The morphology date was indicated following the Zadoks scale: Stage 28 indicates plants possessing one main shoot and eight tillers; Stage 31 indicates the 1st detectable node emerges (Jointing stage). CL refers to cold priming + spring low temperature treatment without cold priming; NL refers to spring low temperature treatment; NN refers to the normal temperature control.