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Differential activity and expression of antioxidant enzymes and alteration in osmolyte accumulation under high temperature stress in wheat seedlings

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ABSTRACT

The objective of this study was to investigate the effects of high temperature (HT) stress on activity and expression of antioxidant enzymes and osmotic adjustment substance content in seedlings of four wheat cultivars with contrasting levels of HT tolerance. The results showed that the differences in superoxide dismutase (SOD) activity, glutathione reductase (GR) activity, soluble protein content and proline content between HT-resistant cultivars and HT-sensitive cultivars were more obvious than those in the activities of peroxidase (POD) and catalase (CAT). Moreover, the transcript levels of *Fe-SOD* and *Mn-SOD* were consistent with the trend of variation in SOD activity, which suggested that *Fe-SOD* and *Mn-SOD* might play important roles in SOD activity under HT stress. Finally, the transcript levels of *CAT* and *GR* were more sensitive than the activities of CAT and GR in response to HT stress in wheat seedlings. Collectively, our results indicated that HT-resistant wheat cultivars had higher SOD activity, GR activity and proline content than HT-sensitive wheat cultivars under HT stress. These data indicated that HT-resistant wheat cultivars resisted the damage caused by HT stress more effectively, which could be applied in predicting HT-resistant wheat cultivars used for field production.

1. Introduction

High temperature (HT), as a major abiotic stress, affects plant growth, photosynthesis, metabolism and productivity worldwide. Plants must resist HT stress by developing a series of mechanisms and activating biological processes because they are sessile organisms which can not flee to more beneficial environments (Mittler et al., 2012).

HT stress can result in the excessive accumulation of reactive oxygen species (ROS, viz., $O_2^{\bullet-}$, H_2O_2, O_2, $HO_2^{\bullet-}$, OH•, ROO• and RO•) which can cause lipid peroxidation, damage nucleic acids, oxidize proteins and ultimately lead to cell death (Miller et al., 2010). In order to scavenge ROS and maintain redox homeostasis under HT stress, plant cells must elevate capacity to trigger the gene expression and enhance activity of antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and glutathione reductase (GR) (Farooq et al., 2008).

SOD constitutes the first line of a defense system which can resist oxidative damage in plants and catalyzes the dismutation of two superoxide radicals to hydrogen peroxide and oxygen (Xu et al., 2011). There are three groups of SOD categorized by the metal cofactor, namely Cu/Zn-SOD, Fe-SOD and Mn-SOD (Melchiorre et al., 2009). CAT is a tetrameric heme and mostly located in peroxisomes (Mhamdi et al., 2010). CAT has the highest conversion efficiency of all antioxidant enzymes and one molecule of CAT can remove about six million molecules of H_2O_2 per minute (Gill and Tuteja, 2010). POD can act as a scavenger which catalyzes the decomposition of H_2O_2 by oxidizing phenolic compounds (Wu et al., 2013). GR locates in chloroplasts, mitochondria and cytosol and catalyzes the reduction of glutathione, which plays an important role within the cell system including participation in the ascorbic acid-glutathione cycle (Asada, 1992; Ceylan et al., 2013). A great number of researches reported that the induction of the cellular antioxidant enzymes is important for protection against various stresses. Temperature tolerant wheat genotypes had higher activities of SOD and CAT under heat stress

Abbreviations: CAT, catalase; GR, glutathione reductase; H_2O_2, hydrogen peroxide; HT, high temperature; JMC, Jimai 20 control; JMH, Jimai 20 under HT stress; JNC, Jinan 17 control; JNH, Jinan 17 under HT stress; LXC, Liangxing 77 control; LXH, Liangxing 77 under HT stress; $O_2^{\bullet-}$, superoxide; qRT-PCR, quantitative real-time polymerase chain reaction; ROS, reactive oxygen species; SOD, superoxide dismutase; SNC, Shannong 23 control; SNH, Shannong 23 under HT stress.

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(Sairam et al., 2000). Almeselmani et al. (2006) found that the activities of SOD and CAT increased in HT tolerant wheat genotypes under HT stress, and GR maintained higher activity in HT tolerant wheat genotypes than in other genotypes. A drought-tolerant Kentucky bluegrass (*Poa pratensis*) cultivar had significantly higher activities of CAT, POD, and ascorbate peroxidase compared with a drought-sensitive cultivar under re-watering after drought stress (Xu et al., 2011). Up-regulation of antioxidant enzyme-related genes may be associated with HT tolerance. The transcript levels of the antioxidant enzyme-related genes *Cu/Zn-SOD, Mn-SOD* and *CAT* were higher in wheat plants which were acclimated to a heat treatment before a subsequent (post-anthesis) heat stress treatment than in non-acclimated plants subjected to a post-anthesis heat stress treatment (Wang et al., 2011). Heat-tolerant Kentucky bluegrass cultivars had significantly higher transcript levels of chloroplast *Cu/Zn-SOD, Fe-SOD, POD* and *CAT* than heat-sensitive cultivars under heat stress (35/30 °C day/night) for 28 d (Du et al., 2013). Thus, the increase of antioxidant enzyme activity and the up-regulation of antioxidant enzyme-related genes play essential roles in resisting various stresses in plants.

Wheat (*T. aestivum* L.) is an important food crop worldwide and is a major source of calories for human nutrition (Altenbach, 2012). HT occurring in the late stage of growth will lead to reduce yield and quality (Hurkman et al., 2009), and hence screening HT-resistant cultivars at seedling stage is crucial for wheat. In addition, better understanding of the relation between antioxidant enzymes and HT tolerance will help in screening HT-resistant wheat cultivars and better breeding programs. The objectives of this study were to investigate the changes in antioxidant enzyme activity, osmotic adjustment substance content and transcript levels of antioxidant enzyme-related genes in response to HT stress in four wheat cultivars with contrasting levels of HT tolerance (two tolerant and the other two sensitive) at the seedling stage. The HT stress (38/28 °C day/night) in this study was similar to the HT stress used at filling stage in 2012. Based on these comparisons, predicting HT-resistant wheat cultivars which could be applied in field production will be possible.

2. Materials and methods

2.1. Plant materials and experimental design

The experiment was conducted with four wheat cultivars, two HT-resistant cultivars (Shannong 23 and Liangxing 77) and two HT-sensitive cultivars (Jimai 20 and Jinan 17), which were selected according to the changes in kernel weight (Supplementary Table 1) and morphology (Supplementary Fig. 1) after HT stress at the field level in 2012. Wheat seeds were grown in composite substrates containing vermiculite, peat moss and perlite. Wheat seedlings were cultured in a growth chamber (GXZ-280B, China) conditions (20/15 °C day/night, relative humidity 60–80%, the light intensity 300 μmol $m^{-2}s^{-1}$ during the day phase, and photoperiod of 12/12 h day/night) with 40 seedlings each cultivar per plate in a completely randomized design so that plants were randomly placed. After fifteen-day culture when seedlings were at the 3rd-leaf phase, HT stress (38/28 °C day/night) was imposed for three different treatment times: 1 d, 2 d and 3 d, while non-stressed seedlings were grown under the conditions (20/15 °C day/night) mentioned above. Wheat leaves were sampled and then were frozen in liquid nitrogen and stored at −80 °C until use.

2.2. Measurement of antioxidant enzyme activity

The frozen leaves (0.5 g) were ground in 5 mL of chilled buffer (50 mmol/L sodium phosphate buffer, pH 7.8) and the homogenate was centrifuged at 10 000 × *g* for 30 min. The supernatant was used for assaying antioxidant enzyme activity. All operations were conducted at 4 °C.

The activities of SOD, POD and CAT were measured according to the method described by Tan et al. (2008) with some modifications. SOD activity was measured on the basis of inhibition in the photochemical reduction of nitro blue tetrazolium (NBT). The 3 mL reaction mixture was in 50 mmol/L phosphate buffer (pH 7.8) containing 130 mmol/L methionine, 750 μmol/L NBT, 100 μmol/L EDTA, 20 μmol/L riboflavin and 20 μL of enzyme extract. The tubes were exposed under the illumination of 4000 lux for 20 min. Reaction was stopped by keeping the tubes in the dark. A non-irradiated complete reaction mixture served as a blank. The reaction mixture was measured at 560 nm using a spectrophotometer (UV-2450, Shimadzu Corp., Japan). One unit of SOD was defined as the quantity of SOD causing 50% inhibition of the photochemical reduction of NBT.

POD activity was determined by the method of guaiacol oxidation. The reaction mixture (3 mL) contained sodium phosphate buffer (pH 6.0, 50 mmol/L), guaiacol and 30% H_2O_2. The reaction was started by adding 20 μL of enzyme extract. The reaction mixture in which enzyme solution was replaced with phosphate buffer (pH 6.0, 50 mmol/L) served as a blank. Readings at 470 nm were recorded within 3 min after the start of the reaction at 1 min intervals. One unit POD activity was defined as the absorbance change of one unit per minute.

CAT activity was assayed by the decomposition of H_2O_2. The reaction was started by adding 100 μL of enzyme extract to 2.5 mL reaction mixture containing 50 mmol/L sodium phosphate buffer (pH 7.0) and 0.1 mol/L H_2O_2. The reaction mixture in which enzyme solution was replaced with phosphate buffer (pH 7.0, 50 mmol/L) served as a blank. Changes in absorbance at 240 nm were read once every 1 min for 3 min. One unit of CAT activity was defined as the absorbance change of 0.01 units per minute.

GR activity was measured according to the method of Wang et al. (2011) with some modifications. GR was assayed by the oxidation of NADPH (Molar extinction coefficient 6.2 $(mmol/L)^{-1}cm^{-1}$) at 340 nm every 1 min within the first 3 min. The 2 mL reaction mixture contained 50 mmol/L potassium phosphate buffer (pH 7.8), 2 mmol/L Na_2EDTA, 0.15 mmol/L NADPH, 0.5 mmol/L glutathione oxidized. The reaction was started by adding 500 μL of enzyme extract. The reaction mixture in which enzyme solution was replaced with phosphate buffer (pH 7.8, 50 mmol/L) served as a blank.

2.3. Measurement of osmotic adjustment substance content

Soluble protein content was estimated by the method of Bradford (1976) with bovine serum albumin as a calibration standard.

Table 1
Primer sequences for qRT-PCR analysis.

Gene	Accession no.	Forward 5′-3′/Reverse 5′-3′	Product size (bp)
Cu/Zn-SOD	U69632	TGGGAGAGCGTTTGTTGTTC GTCTTCCACCAGCATTTCCA	92
Fe-SOD	JX398977	CCTACTGGATGAGACGGAGAG GGACGAGGACAACGACGAA	124
Mn-SOD	AF092524	CGGACTACCTGACCAACATCT AACTCAAGAGCGAGCCAAGT	122
CAT	D86327	CCATGAGATCAAGGCCATCT ATCTTACATGCTCGGCTTGG	103
GR	AY364467	GCACACGACCAAGCACATAT ATATCCGCCACCAAGAATAACG	145
Actin	AB181991	CGAAGCGACATACAATTCCATC GAACCTCCACTGAGAACAACAT	84