Changes of anti-oxidative enzymes and MDA content under soil water deficits among 10 wheat (*Triticum aestivum* L.) genotypes at maturation stage

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Abstract

Drought is a world-spread problem seriously influencing grain production and quality, the loss of which is the total for other natural disasters, with increasing global climate change making the situation more serious. Wheat is the staple food for more than 35% of world population, so wheat anti-drought physiology study is of importance to wheat production and biological breeding for the sake of coping with abiotic and biotic conditions. Much research is involved in this hot topic, but the pace of progress is not so large because of drought resistance being a multiple-gene-control quantitative character and wheat genome being larger (16,000 Mb). On the other hand, stress adaptive mechanisms are quite different, with stress degree, time course, materials, soil quality status and experimental plots, thus increasing the complexity of the issue in question. Additionally, a little study is related to the whole life circle of wheat, which cannot provide a comprehensive understanding of its anti-drought machinery. We selected 10 kinds of wheat genotypes as materials, which have potential to be applied in practice, and measured change of relative physiological indices through wheat whole growing-developmental circle (i.e. seedling, tillering and maturing).

Here, we reported the anti-oxidative results of maturation stage (the results of seedling and tillering stage have been published) in terms of activities of POD, SOD, CAT and MDA content as follows: (1) 10 wheat genotypes can be grouped into three kinds (A–C, respectively) according to their changing trend of the measured indices; (2) A group performed better resistance drought under the condition of treatment level 1 (appropriate level), whose activities of anti-oxidative enzymes (POD, SOD, CAT) were higher and MDA lower; (3) B group exhibited stronger anti-drought under treatment level 2 (light-stress level), whose activities of anti-oxidative enzymes were higher and MDA lower; (4) C group expressed anti-drought to some extent under treatment level 3 (serious-stress level), whose activities of anti-oxidative enzymes were stronger, MDA lower; (5) these results demonstrated that different wheat genotypes have different physiological mechanisms to adapt themselves to changing drought stress, whose molecular basis is discrete gene expression profiling (transcriptom); (6) our results also showed that the concept and method accepted and adopted by most researchers [T.C. Hsiao, Plant response to water stress, Ann. Rev. Plant Physiol. 24 (1973) 519–570], that 75% FC is a proper supply for higher plants, was doubted, because this level could not reflect the true suitable level of different wheat genotypes. The study in this respect is the key to wheat anti-drought and biological-saving water agriculture; (7) our research can provide insights into physiological mechanisms of crop anti-drought and direct practical materials for wheat anti-drought breeding; (8) the physiological study of wheat is more urgent up-to-date and molecular aspects are needed, but cannot substitute this important part. The combination of both is an important strategy and a key and (9) POD, SOD and CAT activities and MDA content of different wheat genotypes

Abbreviations: CAT, catalase; MDA, malondialdehyde; POD, peroxidase; SOD, superoxide dismutase; QF, field capacity

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had quite different changing trend at different stages and under different soil water stress conditions, which was linked with their origin of cultivation and individual soil water threshold.

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1. Introduction

Drought is a world-wide problem, constraining global crop production and quality seriously and recent global climate change has made this situation more serious [1–20]. Drought is a complex physical–chemical process, in which many biological macromolecules and small molecules are involved, such as nucleic acids (DNA, RNA, microRNA), proteins, carbohydrates, lipids, hormones, ions, free radicals, mineral elements [21–50]. In addition, drought is also related to salt stress, cold stress, high temperature stress, acid stress, alkaline stress, pathological reactions, senescence, growth, development, cell circle, UV-B damage, wounding, embryogenesis, flowering, signal transduction and so on. Therefore, drought is connected with almost all aspects of biology. Currently, drought study has been one of the main directions in global plant biology and biological breeding. NSF in USA established the program of Plant Genome Functions Under Stresses in 1998, and strengthened it in 2000, and drafted it towards 2010 [51,52]. Just 1 year ago, European Commission, who once kept conservative to biotechnological breeding, constructed a big project: Plants for the Future: A European Vision for Plant Biotechnology towards 2025, in which much is involved in resistance drought [5]. Many advances in relation to this hot topic, including molecular mechanism of anti-drought and corresponding molecular breeding have taken place [2,16,17,31,34,38,42,43,50,53–62]. Although the obtained transgenic crops (mainly, wheat) by different types of gene technology all exhibit resistance drought to some extent, they have many shortfalls related to agronomical performance and/or development [19,20,61,62]. These results imply that systemical, deeper and comprehensive understanding of physiological mechanism of crops under drought stresses is not enough to manipulate the physiological regulatory mechanism and take advantage of full this potential for productivity, whose study is the bridge between molecular machinery of drought and anti-drought agriculture, because the performance of genetic potential of crops is expressed by physiological realization in fields [1,6,30,31,50,63,64,77–79,83]. Towards this aim, many promising methodologies appear, but they should also be linked with field practice [3,4,15,28,37,54,64–67]. Wheat is a staple food for more than 35% of the world population and wheat is also the second grain crop in China, whose production status is directly related to social stability, Chinese survival and sustainable development [4,56]. With progressive global climate change and increasing shortage of water resources and worsening eco-environment, wheat production is influenced greatly [16,19,20,62]. To aim at making different wheat genotypes perform fully physiological potential under limited soil water conditions in fields, by selecting practical materials for direct breeding and establishing an efficient platform for deciphering molecular mechanisms of wheat drought resistance, especially, the link of the redox state with anti-drought, we chose 10 promising wheat genotypes (field experiments have proved) as experimental materials, applied stimulation natural drought and potting cultivation methods, collected related physiological data of whole growth circle (mainly, seedling stage, tillering stage and maturation stage) and primarily evaluated resistance drought of these genotypes. Here, we reported the results of maturation stage.

2. Materials and methods

2.1. Plant materials

Ten wheat genotypes (Xinong9-1-1-13, Xinong-1, Xinong93, Xinong6-2, Xinong937-1, YB0738, Xiaobingcao7, Jinmai47, Yumai49, Xiaoyan22 and labeled 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, respectively) are provided by Professor Zhang ZhengMao from Yangling Breeding Center of National Wheat Engineering Research Center of China.

2.2. Experimental fields

The outward potting cultivation field is affiliated to experimental plots of National Key Laboratory of Soil Erosion and Dryland Farming, The Center of Soil and Water Conservation and Eco-environmental Research, Chinese Academy of Sciences. Pots are made of plastic, whose empty weight is 2 kg and filled-soil weight is according to Gao [68]. The basic physical–chemical property of the selected soil is as follows: organic matter 11.5 mg/g, total N 0.94 mg/g, available N 122.2 mg/kg, available K 222.4 mg/kg.

2.3. Experimental design

Each genotype is conducted in three level soil water treatments controlled by weighting (75% QF, 55% QF and 45% QF, respectively), each of which is six times
repeated and matched with one empty control, correspondingly.

2.4. Collection and measurement of anti-oxidative enzymes

SOD (EC1.1.5.1.1), POD (EC1.1.1.7), CAT (EC1.1.1.6). Measurement of wheat seedling stage was conducted according to Zhang and Kirham [69] and Shao et al. (2005) [74, 81, 82]. MDA detecting was done by referring to Gao [68]. All data were measured three times at the same time and the mean used for result analysis and discussion.

3. Results

3.1. POD activities of 10 wheat genotypes at maturation stage

According to Fig. 1, genotypes 1 and 2 (A group) had higher POD activities at level 1, genotypes 4 and 5 (B group) possessed stronger POD activities at level 2, genotypes 3, 6, 7, 8, 9 and 10 (C group) had higher POD activities at level 3. Comparing A, B and C group, genotype 6 at level 3 had the highest POD activities (about 380 units) among 10 wheat genotypes under three level soil water conditions, which expressed increasing POD activities from level 1 to 3. In A group, genotype 1 had better POD activities and the top POD activities (about 330 units) of 10 wheat genotypes for level 1, which expressed decreasing trend from level 1 to 3, showing that this genotype was more sensitive to soil water deficits at maturation stage. The other member of this group had the similar changing trend. In B group, genotype 4 displayed higher POD activities (about 355 units), and had a little difference from those at level 3, showing that this genotype was more sensitive to soil water deficits at maturation stage. The other member of this group had the similar changing trend. In B group, genotype 4 displayed higher POD activities (about 355 units), and had a little difference from those at level 3, showing that this genotype was more sensitive to soil water deficits at maturation stage. The other member of this group had the similar changing trend. In C group, genotype 10 had the top POD activities (600 units) at level 3, genotype 10 had the top POD activities (500 units) at level 3, genotype 10 had the top POD activities (400 units) at level 3, genotype 10 had the top POD activities (300 units) at level 3, genotype 10 had the top POD activities (200 units) at level 3. In A group, genotype 1 expressed decreasing activities from level 1 to 3, and their total were not bigger. Genotypes 8 and 9 possessed relatively flat change, showing that they had better adaptable ability to respond to the environmental stress. In B group, all genotypes expressed similar changing pattern, in which they performed lower POD activities at level 1 and the lowest at level 3, showing decreasing redox ability with maturation. In C group, the members also reflected basically similar phenomenon: they had higher POD activities at level 1 than those at level 2, which demonstrated the expression of POD enzymes also was a course of soil water stress at maturation like POD mentioned above.

3.2. SOD activities of 10 wheat genotypes at maturation stage

From Fig. 2, it was observed that genotypes 1, 8 and 9 (A group) had higher SOD activities at level 1, genotypes 2, 3, 5 and 6 (B group) had stronger SOD activities at level 2, genotypes 4, 7 and 10 (C group) had higher SOD activities at level 3. Genotype 10 had the top SOD activities (600 units) at level 3 among three groups. In A group, genotype 1 expressed decreasing activities from level 1 to 3, and their total were not bigger. Genotypes 8 and 9 possessed relatively flat change, showing that they had better adaptable ability to respond to the environmental stress. In B group, all genotypes expressed similar changing pattern, in which they performed lower SOD activities at level 1 and the lowest at level 3, showing decreasing redox ability with maturation. In C group, the members also reflected basically similar phenomenon: they had higher SOD activities at level 1 than those at level 2, which demonstrated the expression of SOD enzymes also was a course of soil water stress at maturation like POD mentioned above.

3.3. CAT activities of 10 wheat genotypes at maturation stage

According to Fig. 3, genotypes 2, 6, 8 and 9 (A group) had higher CAT activities at level 1, genotypes 1, 3, 4 and 7 reached a certain degree. These results further demonstrated that performance of resistance and tolerance was a course of accumulation and development for different wheat genotypes, which is consistent with the concept that anti-drought character is a quantitative one.
ilar change, i.e. they had lower MDA content at level 1 than changing trend. Among C group, genotypes 1 and 7 had sim-
soil water stress degree. In B group, they displayed discrete increasing MDA content from level 1 to 3, demonstrating group, these members had quite the same trend; they had maturation and decreasing with maturation and their membrane integrity was reducing with maturation showing that they increased anti-drought with
of MDA content for these 10 wheat genotypes was lower development and maturation. But, different genotypes had
at maturation, showing that they increased anti-drought with
levels. Among A group, genotypes 3 and 4 and 7 had similar changing trend, i.e. CA T activities at level 1 was higher than level 1 to 3, demonstrating that suitable soil water deficiency (level 2) could lead to higher CAT gene expression and increase anti-drought with some wheat genotypes, which fur-
ther implied resistance drought and water-saving at the same time in agriculture has its biological basis and could be real-
ized possibly. Between C group, genotypes 5 and 10 both had better CAT at level 1 than that at level 2, showing that severe soil water stress could result in high CAT gene expression for some wheat genotypes to combat environmental conditions.

3.4. MDA content of 10 wheat genotypes at maturation stage
From Fig. 4, it was observed that genotypes 6, 9 and 10 (A group) had lower MDA content at level 1, genotypes 2, 5 and 8 (B group) had lower MDA content under level 2, geno-
types 1, 3, 4 and 7 (C group) had lower MDA content under level 3. Of A, B and C group, genotype 1 had the lowest MDA content (~5.2 units) at level 1. Overall performance of MDA content for these 10 wheat genotypes was lower at maturation, showing that they increased anti-drought with development and maturation. But, different genotypes had discrete change under different soil water stress levels. In A group, these members had quite the same trend; they had increasing MDA content from level 1 to 3, demonstrating their membrane integrity was reducing with maturation and soil water stress degree. In B group, they displayed discrete changing trend. Among C group, genotypes 1 and 7 had sim-
ilar change, i.e. they had lower MDA content at level 1 than that at level 2. Genotypes 3 and 4 had the same changing pattern, i.e. both of them had decreasing MDA content from level 1 to 3, implying that they had stable regulatory ability to respond to soil water stress with maturation progression, which was in concert with what was described in the results of SOD, POD and CAT to most extent.

4. Discussion
Higher plants have evolved a wide range of defence systems to survive continuous assault by an arsenal of biotic attacks, constantly changing weather and other envi-
ronmental conditions [3,9,16,17,19,39,49,59,63]. Unravel-
ing the physiological and molecular basis for the plasticity of plant defending metabolism not only provides access to a largely untapped resource of genes and selection mark-
ers for breeding enhanced stress tolerance and drought in crops but also ensures improved food security world-wide [2,5,10,14,20,29,31,37,44,58,69,70–74].

Living organisms can be viewed as reducing–oxidising (redox) systems in which catalytic, largely oxidative pro-
cesses produce energy and anabolic, principally reductive processes assimilate it [35,39,45,69,71]. Aerobic organisms exploit the redox potential of oxygen while controlling ox-
diation. A key feature determining the size of the plant "phys-
iological window" where metabolic functions can be main-
tained and regulated, is the extent to which oxidative reactions can be tightly controlled [72]. If environmental changes are too extreme to allow short-term metabolic controls to main-
tain fluxes through primary metabolism while preventing uncontrolled oxidation, then stress-induced damage ensues. Here, there is an obvious stress threshold [72,80–82]. In this situation acclamatory changes in gene expression are induced in attempt to restore redox homeostasis [16,17]. If the repertoire of genomic responses is not insufficient or not appropriate, then primary metabolism is impaired, oxidative stress becomes increasingly important and cell death and senescence responses are triggered [33,35,45,71]. One of the earliest responses of plants to pathogens, wounding, drought, extremes of temperature or physical and chemical shocks is the accumulation of active oxygen species (AOS) such as superoxide, hydroxyl radicals, hydrogen peroxide and singlet oxygen [39]. The oxidative stress that ensues is a wide-spread phenomenon [16,39,49]. It is observed in wheat exposed to most, if not all, biotic and environmental stresses. AOS are key components contributing to cellular redox poise. They participate in all processes controlled by redox reactions. These include signal transduction, gene expression, protein synthesis and turnover, thiol–disulphide exchange reactions and regulation of metabolism. AOS accumulation is sensed as an ‘alarm’ signal that initiates pre-emptive defense responses [12,17,24,31,37,39,47,50,73]. Common and linked signal transduction pathways are activated that can lead either to stress acclimation or to cell death depending on the degree of oxidative stress experienced [21]. Wheat responses to stresses
are therefore directed to acclimate and repair damage, which is the basic common feature of organisms [9,14,28,33,63,74].

POD, SOD, CAT and MDA are common and important indices for evaluating the status of wheat redox, whose higher activities of the former three and whose lower of MDA display higher anti-oxidative ability, reflecting higher resistance drought [2,12,27,59,69,71,74]. In our experiment, our results showed that different wheat genotypes clearly responded to soil water deficiency differently in terms of activities of POD, SOD, CAT and MDA content (Figs. 1–4).

This further implied that different wheat genotypes had discrete water stress thresholds and therefore they had different physiological adaptive mechanisms to regulate their redox status [39,50,73,81,82]. Obviously, the difference in this aspect exhibited the difference in their alleles of different wheat genotypes, which have been produced under the pressure of natural selection and artificial domestication for many years [6,12,13,31]. The emerging methodologies including genomic approaches, system-oriented ways, large-scale microarray technology, genome-metabolite profiling analysis and transcriptom profiling precise annotation, in combination with genetic mutant selection system, transgenic strategy, microRNA technology and plant physiology, will uncover this network regulatory system of wheat genotypes being adaptive to different soil water stresses [16,17,24,26,28,30,31,37,54,63,64,75].

Our results firstly clearly showed that different wheat genotypes differently responded to soil water stress at different stages in terms of physiological mechanisms, implying that they have different soil water stress thresholds (Shao et al., 2005). Exploring its range and accurate amount of different genotypes is of importance to understanding physiological mechanisms of wheat resistance and tolerance drought and saving-water agriculture by the way of physiological regulation. Secondly, our experimental results further demonstrated the fact accepted and adopted by most scholars [7], that 75% FC, 55% FC and 45% FC is normal, light-stressed and severe-stressed water level, respectively, is needed to be modified in order to represent the practical level of more plants. Thirdly, the state of redox is exist with plant circle, but quite different at different stages and under the conditions of different soil water stresses [9,11,63,74,80–82]. In addition, POD, SOD, CAT and MDA have different changing time course, whose molecular basis is different expression–regulation machinery of corresponding genes, and cannot be regarded as only one criteria in evaluating wheat anti-drought. Fourthly, we also found that the changing trend of POD, SOD and CAT activities and MDA content under our condition of three stress levels is tightly linked with the location of their cultivation (domestication), which reflects the change of corresponding alleles of different genotypes under the pressure of natural and artificial selection [56,69]. Knowing the refine physiological nature, in conjunction with natural rainfall status of different locations of globe is very important to popularize new wheat species and conduct wheat breeding. Sixthly, different wheat genotypes have increasing ability to respond to adverse environment with progression of growth-development and maturation, and our results at seedling, tillering and maturation stages fully reflect this phenomenon, which is quite consistent with the “S” shaped curve of plant growth grand periodicity, implying that the performance of plant drought resistance is also a kind of plasticity (Shao et al., 2005), whose extensive and deeper investigation is of importance to dryland agriculture. Seventhly, the state of redox is exist with plant circle, but quite different with development and environmental changes. Different wheat genotypes have different changing scale, reflecting discrete physiological and molecular mechanisms.

In summary, anti-drought for crops is a comprehensive performance of many characters, and several indices are difficult to evaluate its resistance drought, what is the important, in combination with WUE and production (Shao et al., 2005, unpublished data). The study of physiological mechanisms of wheat anti-drought has much work to do. A practical, effective and comprehensive system for anti-drought evaluation (in particular, for crops) is urgently needed currently. Molecular biology aspects of wheat cannot substitute for this important part, but strengthen the research and provide a broad future. It is easy to see that one cell or molecule cannot be alive in natural fields and not provide any economic effect for human beings. The combination of molecular biology and plant physiology is the key. Many achievements in biotechnological and traditional breeding of wheat are good examples [16–18,20,55,75,76]. Although some progresses in terms of the exploration of molecular nature of wheat anti-drought also have taken place, many problems exist. What is the relationship of mineral elements (in particular, K+ and Na+) with root signal transduction (pathways)? Much of former study showed that K+ was little connected with anti-drought, but recent research and our results displayed that it was linked with wheat resistance drought [16–18,25,40]. What is the exact soil water stress threshold of individual wheat genotype? This is of much importance to resistance drought breeding and water-saving agriculture and precise agriculture under global climate change. What are the details that constitute the network regulatory system of drought, cold, UV-B, freezing, acidity, salty, wounding, pathogen, senescence, cell death? How is each linked with the other parts? What is the (transient) connection among different physiological adaptive regulatory pathways at different levels? What role do endogenous hormones play in this course? What is the crosstalk among them when abiotic or/and biotic stress happens? The redox state in plants is important, and how is it regulated? What is the relationship with microRNAs? A wide-spread use of data resources for fine gene functions and structure of different plants (species) is from model plants, Arabidopsis thaliana and rice, and how large is the reliability? No doubt, expanded detecting of plant range is more urgent. So, we think that physiological studies at different scales have much work to do with the increasing atmospheric change.
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