Investigation on the relationship of proline with wheat anti-drought under soil water deficits

Shao Hong-Bo^{a,b,c,*}, Chen Xiao-Yan^{d,g}, Chu Li-Ye^c, Zhao Xi-Ning^{b,*}, Wu Gang^{h,*},
Yuan Yong-Bing^e, Zhao Chang-Xing^e, Hu Zan-Min^f

^a Key Laboratory of Molecular Biology, Bio-informatics College, Chongqing University of Posts & Telecommunications, Chongqing 400065, China

^b Northwest A&F University and Center of National Water-Saving and Irrigation at Yangling, Centre of Soil and Water Conservation and Eco-Environmental Research, The Chinese Academy of Sciences, Yangling 712100, China

^c Biology Department, College of Molecular and Chemical Engineering, Qingdao University of Science and Technology, Qingdao 266042, China

^d Centre of Soil and Water Conservation and Eco-Environmental Research, The Chinese Academy of Sciences, Yangling 712100, China

^e Qingdao City Key Laboratory of Modern Agriculture Quality and Safety Engineering, Laiyang Agricultural University, Qingdao 266109, China

^f Institute of Genetics & Developmental Biology, the Chinese Academy of Sciences, Beijing 100101, China

^g Graduate School of the Chinese Academy of Sciences, Beijing 100039, China

^h State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-environmental Sciences, the Chinese Academy of Sciences, Beijing 100085, China

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Abstract

Proline (content) is closely with plant anti-drought, especially under soil water deficits. Many reports from crops and other plants have proved this. Wheat is the second important crop on the globe, whose research in this aspect of importance for food quality, safety, and yield in field. The related difference in physiological indicators and proline content for different soil water treatments among wheat with different genotypes is not clear, which has limited deep study of wheat anti-drought molecular biology and related anti-drought biotechnological breeding. Our current study was focused on the physiological relationship of proline and different genotype wheat anti-drought under soil water deficits. Main results showed that different wheat genotype had different soil water stress threshold. Pro content had closed relationship with soil water stress threshold and wheat anti-drought. Developmental course also impacted Pro content for different wheat genotypes.

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1. Introduction

Humankind is dependent on crops for most of our food and feed, as well as for many other important plant materials. Cur-

rently crops occupy nearly one fifth of the planet's vegetated surface, by far the biggest imprint of man upon the planet and its landscapes. Cropping is also the world's largest source of employment and livelihood, with well over 1 billion small farmers in developing countries [1–3,18,31]. Drought is a worldwide problem, constraining global crop production and quality seriously. Water drives agricultural production in many parts of the world. Indeed, the “Green Revolution” was so successful in reducing hunger because the increased use of irrigation was one of the factors behind the successful increase in crop

Abbreviations: FC, field capacity; POD, peroxidase; SOD, superoxide dismutase; CAT, catalase

* Corresponding authors. Tel.: +86 532 84022506; fax: +86 532 84022757.

E-mail addresses: shaohongbochu@126.com (S. Hong-Bo), Xiningzhao@yahoo.com.cn (Z. Xi-Ning), wug@rcees.ac.cn (W. Gang).

production. However, in the 21st century, demand by industry, demand by urban populations and demands to maintain environmental flows and water quality will reduce the water available for irrigated agriculture. Moreover, emerging climate change is predicted to reduce rainfall and increase rainfall variability in many agricultural systems [4–9,26–32,60,78–80,85]. Thus, the issue for crop science is how to increase production with less water available for irrigation and less reliable rainfall that limits dryland agriculture in many parts of the world [10–14]. 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. In addition, drought is also related to salt stress, cold stress, high temperature stress, acid stress, alkaline stress, pathological reactions, senescence, growth, development, cell cycle, UV-B damage, wounding, embryogenesis, flowering, signal transduction and so on [15–19,20–25,82,83]. 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 [14,49]. Just 2 months 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 [13]. Many advances in relation to this hot topic, including molecular mechanism of anti-drought and corresponding biotechnological breeding have taken place [33–48,62,64–67,81–83]. Although the obtained transgenic crops (mainly, wheat) by different types of gene technology all exhibit drought resistance to some extent, they have some shortfalls related to agronomical performance and/or development [3,6,19,26,32,43,45,46,48,60,62,65,81]. These results imply that systematical, 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 [49–54,80–85]. Towards this aim, many promising methodologies appear, but they should also be linked with field practice [1,10,23,28–30,36,46,60,61,68]. Wheat is a staple food for more than 35% of the world population and wheat is also the second main grain crop in China, whose production status is directly related to social stability, Chinese survival and sustainable development [25,38,52–59,79]. With progressive global climate change and increasing shortage of water resources and worsening eco-environment, wheat production is influenced greatly [3,8,11,12,15,18,25,26,31,35,39,42,44,60,73–80,85].

Some reports have been related to proline (content) relationship with wheat and other crop plant drought resistance, but the data related to the relationship in different genotypes of wheat have been lacking and contrary [11,15,25,42,44,68–70,74], especially little is involved in such aspect of dif-

ferent wheat genotypes [54–58]. It has been well known that osmotic regulators include many important small molecules such as potassium, soluble sugar, proline and betaine [2,5,6,21,25,34,42,52,64,67,70,74,75,77]. These small molecules are also important physiological indicators for evaluating osmotic adjustment ability [62–67,71–73,82–84] and drought resistance in wheat species and genotypes. 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, we chose 10 promising wheat genotypes (field experiments have proved) as experimental materials, applied stimulation natural drought and potting cultivation methods, collected proline data of two growth stages (seedling stage and tillering stage) and primarily evaluated the relationship between their anti-drought and these genotypes and proline.

2. Materials and methods

2.1. Plant materials

Ten wheat genotypes (Xinong 9-1-1-13, Xinong-1, Xinong 3-2, Xinong 4-2, Xinong 9337-1, YB0738, Xiaobingcao 7, Jinmai 47, Yumai 49, Xiaoyan 22, and labeled 1–10, respectively) are provided kindly by Professor Zhang ZM from Yangling Breeding Center of National Wheat Engineering Research Center of China [55].

2.2. Experimental fields

The outward potting cultivation field is affiliated to experimental plots of State Key Laboratory of Soil Erosion and Dryland Farming, the Center of Soil and Water Conservation and Ecoenvironmental Research, Chinese Academy of Sciences. Pots are made of plastics, whose empty weight was 2 kg and the full pot weight was 24 kg according to [20]. The physical and 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, effective P 52.4 mg/kg, available K 222.4 mg/kg [56].

2.3. Experimental design

Each genotype was conducted in three level soil water treatments controlled by weighting (75% FC, 55% FC, and 45% FC, respectively) according to [24], each of which is six times repeated and matched with one empty control, correspondingly [57,58].

2.4. Proline content measurement

According to Gao [20], proline content in the wheat plant leaves of different genotypes was detected (flag leaves of different wheat genotypes at seedling stage and tillering stage). All the data were measured three times at the same time and the mean used for result analysis and discussion.

3. Results

3.1. Proline content comparison of 10 wheat genotypes at soil water deficits at seedling stage

According to Fig. 1, under the condition of level 1 (75% FC), genotypes 2, 3, 4, 8 and 9 (A group) had higher Pro content. Genotype 2 had the highest content of Pro and genotype 8 the lowest among these five genotypes. Under the condition of level 2 (55% FC), genotypes 1, 5 and 7 possessed higher content of Pro (B group). Genotype 5 exhibited the highest and 7 the lowest among the above genotypes. At level 3 (45% FC), genotypes 6 and 10 expressed higher content of Pro (C group). Generally, half of the tested genotypes were classified into A group, which indicated that most wheat genotypes had the anti-drought performance under appropriate condition of soil water and further demonstrated that the drought resistance character was an accumulated one, needing one concerted environment in vivo for its better performance. Seemingly, genotypes 6 and 10 have the potential to be selected for planting and breeding in arid and semi-arid areas.

3.2. Proline content comparison of 10 wheat genotypes at soil water deficits at tilling stage

From Fig. 2, it was observed that under the condition of level 1, genotypes 7–9 had higher Pro content (A group). Genotypes

Fig. 1. Proline content ($\mu\text{g/g dw}$) comparison of 10 wheat genotypes at soil water deficits at seedling stage (blue, red, and yellow color represents 75% FC (level 1), 55% FC (level 2), 45% FC (level 3), respectively. The below is the same).

Fig. 2. Proline content ($\mu\text{g/g dw}$) comparison of 10 wheat genotypes at soil water deficits at tilling stage.

Fig. 3. The proline content ($\mu\text{g/g dw}$) difference of 10 wheat genotypes for seedling and tilling stage under the condition of level 1.

7 and 9 had relatively higher Pro content of these three genotypes (A group). At level 2, genotypes 1–3, 5 and 10 (B group) exhibited higher Pro content. Genotype 5 had the highest Pro content in B group and genotype 1 the lowest Pro content of B group. At level 3, genotypes 4 and 6 (C group) had higher Pro content. Comparing Fig. 1 with Fig. 2, it was found that different genotype wheat had their different soil water suitable levels and they might change with progressive growth and development for different wheat genotypes. In most cases, most wheat genotypes (nearly 50%) could have higher potential to protect from soil water deficits regardless of developmental stages. From the viewpoint of application, genotype 6 is one better candidate for practice in arid and semi-arid zones in the world, which is in agreement with the above.

3.3. Proline content difference of 10 wheat genotypes for seedling and tilling stage under the condition of level 1

From Fig. 3, it was found that genotypes 1–4 and 6 had higher Pro content at seedling stage under level 1 condition than that at tilling stage. Genotype 2 had the highest Pro content at seedling stage and it reduced to 60% at tilling stage. These genotypes all decreased in terms of Pro content from seedling stage to tilling stage, showing that they had reducing ability to regulate osmotic substances for drought resistance to soil water deficits with progression of development. This fact was similar to A group mentioned above. Genotypes 5 and 7–10 had higher Pro content at tilling stage at level 1 than that at seedling stage. Genotypes 7 and 9 had the highest Pro content at tilling stage at level 1. These genotypes all had increasing Pro content from seedling stage to tilling stage, which was contrary to the first five genotypes. This fact showed that the latter five genotypes had increasing ability to synthesize osmotic regulators (Pro) for protect from the damage of soil water deficits, which should belong to B or C group mentioned above.

3.4. Proline content difference of 10 wheat genotypes for seedling and tilling stage under the condition of level 2

According to Fig. 4, genotypes 1, 3, 7 and 8 had higher Pro content at seedling stage at level 2 than that at tilling stage. Genotypes 2, 4–6, 9 and 10 had higher Pro content at tilling stage than that at seedling stage. Genotype 9 had the highest

Fig. 4. The proline content ($\mu\text{g/g dw}$) difference of 10 wheat genotypes for seedling and tilling stage under the condition of level 2.

Fig. 5. The proline content ($\mu\text{g/g dw}$) difference of 10 wheat genotypes for seedling and tilling stage under the condition of level 3. A 3.5 proline content difference of 10 wheat genotypes for seedling and tilling stage under the condition of level 3.

Pro content. These results indicated that with progression of soil water deficits (from levels 1 to 2) most genotypes expressed increasing anti-drought ability, which was also consistent with developmental pace.

From Fig. 5, it was found that at level 3, genotypes 1–3 had higher Pro content at seedling stage than that at tilling stage. Genotype 2 had the highest Pro content. These results demonstrated that the above genotypes performed better drought resistance at seedling than that at tilling stage under the condition of level 3, which needed precise field water management for full physiological realization and better productivity. Genotypes 4–10 had higher Pro content at tilling stage than at seedling stage. Genotype 6 had the biggest Pro content among these genotypes at tilling stage at level 3, showing that it had better anti-drought ability under the condition of severe soil water deficits and it perhaps had the lower soil water stress threshold. The above 7 genotypes exhibited better drought resistance potential at level 3 from seedling stage to tilling stage, implying that developmental course and soil water deficit level impacted on the expression of corresponding anti-drought-related genes, which would provide a valuable reference for molecular biology study and biotechnological breeding.

4. Discussion

Osmotic adjustment is the main component of physiological machinery, by which plants respond to soil water deficits [8,15,24,36,38,44]. Osmotic regulators include ions (K^+), small

molecules (Pro), soluble sugar and others. Plants with higher osmotic regulators can absorb water from soil-water-deficit condition. In wheat, many reports showed that wheat cultivars with higher K^+ , Pro, soluble sugar and lower MDA at different growth stages performed better drought resistance [2,5,6–8,11,24,36,59]. Our experiments [Figs. 1–5] further proved such conclusion.

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 threshold (e.g. [56–58]). 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 in arid and semi-arid locations. Secondly, our experimental results further demonstrated the concept and method accepted and adopted by most scholars [24], 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. As for different wheat genotypes, they have quite different soil water stress thresholds. In our experiment and under our experimental condition, genotypes 1–3 (divided into A group) may have higher soil water stress threshold and 4–10 (divided into B or C group) have lower soil water stress threshold (Figs. 1–5). Of course, soil water stress threshold for wheat with different genotypes can also be influenced by developmental course and physiological activities (e.g. POD and SOD CAT). Thirdly, we also found that the changing trend of Pro under our condition of three stress levels (Figs. 1 and 2) is tightly linked with the location of their cultivation, which reflects the change of corresponding alleles of different genotypes under the pressure of natural and artificial selection [7,17,35,47]. Moreover, this might be linked with evolution of wheat root systems [8,38,39,41,47,68–72]. This point is of importance to plant species selection for eco-environmental construction and crop selection for agricultural sustainable development in arid and semi-arid regions. Knowing the refine physiological nature, in conjunction with natural rainfall status of different locations, is very important to popularize new wheat species and conduct wheat breeding [49–54,73–85]. Fourthly, evaluating wheat drought resistance need many indexes as possible as one can. Moreover, measurement of these indexes through different growth-development stages is also necessitated. Our results demonstrated that Pro content (Figs. 1–5) was a better indice for evaluating wheat drought resistance [11,18,47,71,74]. Finally, our results primarily showed that genotypes 5, 9 and 10 were potential wheat genotypes for popularizing in arid and semiarid locations.

In a word, the study of physiological mechanisms of wheat anti-drought has much work to do. Molecular biology aspects of wheat cannot substitute for this important part, but can strengthen such research and provide a broad future and platform. 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 [54–58,60]. The combination of molecular biology, plant physiology and other related disciplines (e.g. pedology) is the

key. Many achievements in biotechnological and traditional breeding of wheat are good examples. Although some progresses in terms of the exploration of molecular nature of wheat drought also have taken place, many problems exist. Currently, from the view of globe, sustainable development is the key point. The necessary way to solve the issue of sustainable development is by biological measures, in which plants will play greater roles and crops will play the greatest functions with no doubt [60]. To aim at taking advantage of full use of crop physiological potential for high production and safe food with better quality, the followed problems remain to be known, which we have laid a stress on for many times in the former reports [52–58,73–85]. What is the relationship of mineral elements (in particular, K^+ Na^+ in soils) with root signal transduction (pathways)? What is the exact soil water stress threshold of individual wheat genotype? This is of much importance to resistance drought breeding and saving-water 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 roles 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 by drought signal? What is the best allocation of different crops and grass-shrub-forest in worsening arid and semi-arid areas for obtaining economic and ecological effect simultaneously? What is the co-relationship between LEA (late-embryogenesis abundant protein) and drought resistance of different wheat genotypes? A widespread 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. physiological studies at different scales have a long way to go with the increasing climate change.

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