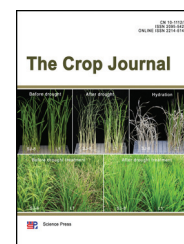
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Effects of sulfur fertilization and short-term high temperature on wheat grain production and wheat flour proteins

Zhiqiang Tao¹, Xuhong Chang¹, Demei Wang, Yanjie Wang, Shaokang Ma, Yushuang Yang, Guangcai Zhao*

Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing 100081, China

Key Laboratory of Crop Physiology and Ecology, Ministry of Agriculture, Beijing 100081, China

ARTICLE INFO

Article history:

Received 29 October 2017

Received in revised form 11

December 2017

Accepted 10 February 2018

Available online xxxx

Keywords:

Triticum aestivum L.

Sulfur fertilization

Strong gluten

Climate warming

ABSTRACT

The content of wheat flour proteins affects the quality of wheat flour. Sulfur nutrition in wheat can change the protein content of the flour. The inconsistency and instability of wheat grain quality during grain filling under high temperature stress (HTS) are a major challenge to the production of high-quality wheat. The effects of sulfur fertilization and HTS on wheat flour protein and its components are unknown. In this study, treatments varying two factors: sulfur fertilization and exposure to short-term HTS, at 20 days post-anthesis, were applied to two wheat cultivars with differing gluten types. Plants of a strong-gluten wheat (Gaoyou 2018) and a medium-gluten wheat (Zhongmai 8) were grown in pots in Beijing in 2015–2017. HTS significantly increased the contents of total protein, albumin, gliadin, glutenin, Cys, and Met in wheat kernels, but reduced grain yield, grain weight, protein yield, globulin content, and total starch accumulation. The HTS-induced increase in total protein amount was closely associated with nitrate reductase (NR) and glutamine synthetase (GS) activities in flag leaves. Sulfur fertilization increased grain and protein yields; grain weight; total protein, albumin, gliadin, glutenin, and globulin contents; protein yield; total starch; Cys, Met; and NR and GS activities. HTS and sulfur fertilization had larger effects on the strong- than on the medium-gluten cultivar. Sulfur fertilization also alleviated the negative effects of HTS on grain yield, protein yield, and starch content. Thus, growing wheat with additional soil sulfur can improve the quality of the flour.

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1. Introduction

Wheat (*Triticum aestivum* L.) is one of the world's staple crops. In 2014, the total global output of wheat was approximately

850 Mt [1]. Maintaining consistency in wheat flour quality is necessary to meet the demands of a growing population for more high quality wheat. Wheat quality is determined by gluten strength and is affected by the proteins in wheat flour.

* Corresponding author.

E-mail address: zhaoguangcai@caas.cn (G. Zhao).

Peer review under responsibility of Crop Science Society of China and Institute of Crop Science, CAAS.

¹ These authors contributed equally to this work.

<https://doi.org/10.1016/j.cj.2018.01.007>

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Please cite this article as: Z. Tao, et al., Effects of sulfur fertilization and short-term high temperature on wheat grain production and wheat flour proteins, *The Crop Journal* (2018), <https://doi.org/10.1016/j.cj.2018.01.007>

Total protein content and protein components determine flour processing quality and the commercial value of flour products. Genes, environmental factors, and cultivation methods control the content of protein and its components in wheat grain. During the grain filling stage, high temperature stress (HTS) can not only reduce grain yield, but also affect flour protein components and its functional properties, and in turn, worsen the rheological properties and baking quality of the flour [2,3]. Nuttall et al. [4] reported that owing to climate change, the daily frequency of extreme high temperatures is increasing; hence, the frequency of HTS occurring in the late growth stages of wheat will increase and will reduce the yield and quality of wheat.

The nutritional status of the wheat plant is one of the most important factors affecting the protein content of the grain. Studies have characterized the effects of macronutrients, such as nitrogen and sulfur, on wheat grain protein [5,6] and the effect of copper deficiency on dough extensibility [7]. The macronutrient sulfur is known to play an important role in grain protein formation and nitrogen assimilation in winter wheat [8]. Sulfur is an essential mineral nutrient for plant growth. It is the fourth major nutrient after nitrogen, phosphorus, and potassium. Ninety percent of the sulfur in the plant body is used to synthesize sulfur-containing amino acids, which are the major component of proteins [9]. Sulfur is an important component of nitrogen metabolism enzymes such as nitrate reductase (NR) [10]. A sulfur deficit will reduce the absorption of nitrogen, affecting the content of protein and the proportions of glutenin and gliadin in total protein, all of which affect the quality of flour. However, the mechanisms driving these effects in wheat flour are not clear [9,11]. In the North China Plain, the humid Mediterranean region of northern Spain, and other regions, soil sulfur is low in some areas, owing mainly to a decrease in the use of S fertilization and of atmospheric deposition [9–12]. Increasing sulfur fertilization application in S-limited soils could increase the content of protein and its components in wheat flour [8,13]. Zhao et al. [14] reported that the activity of NR and glutamine synthetase (GS) in wheat flag leaves significantly influence the content of protein in wheat flour. Sulfur fertilization can increase the activity of NR and GS in flag leaves and thereby affect the content of protein components [15]. Furthermore, sulfur fertilization can increase the plant content of sulfur amino acids, which favors synthesis of proteins rich in cysteine (Cys) and methionine (Met) [16], and the disulfide bond (–S–S–) in the sulfur-bearing protein Cys helps to improve the elasticity of gluten, which affects the processing quality of wheat [8].

To date, we lack research on how sulfur regulates the protein content of wheat grains that have experienced high temperature stress. Accordingly, this study examined the response of wheat grain protein and of four different gluten components in strong- and medium-gluten wheat cultivars grown with sulfur fertilization and exposed to short-term HTS after anthesis. The aim was to provide empirical data to help elucidate the physiological mechanisms involved in response to additional sulfur fertilization and HTS effects, and also to provide a theoretical basis and technical support to food producers and researchers for improving grain yield and flour from wheat grown under more stressful conditions imposed by climate change.

2. Material and methods

2.1. Cultivars

Seeds of two cultivars of winter wheat were obtained from wheat fields in northern North China. The strong-gluten wheat, Gaoyou 2018 (GY2018), is considered a high-quality wheat and the medium-gluten wheat, Zhongmai 8 (ZM8) is considered of average quality. The optimal temperature range for growing these cultivars is 15–32 °C (Department of Plant Management, Ministry of Agriculture of the People's Republic of China 2016). In northern North China, recorded average, maximum, and minimum daytime temperatures were 11.2, 42.0, and –16.0 °C, respectively, between 2000 and 2017.

2.2. Experimental design

The experiment was conducted in test fields at the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences (39°57'40"N, 116°19'23"E). The soil for pot experiments was silt loam (12.74% clay, 9.03% sand, and 78.23% silt), collected from the top 30 cm of the soil in the test field. The contents of organic matter, total nitrogen, available nitrogen, available phosphorus, available potassium, and available sulfur in the soil were 16.51 and 0.92 g kg⁻¹, and 55.22, 35.13, 189.62, and 26.05 mg kg⁻¹, respectively. Ten kilograms of sieved (2-mm mesh) dry soil was placed in each of 128 pots that were 25 cm in height and 25 cm in diameter. Seeds were sown on October 15, 2015 and October 12, 2016, and harvested on June 5, 2016 and June 3, 2017, respectively. The pot soil was fertilized with urea, superphosphate, and potassium chloride at respective concentrations of 90 mg N, 90 mg P, and 80 mg K per kg soil before sowing. When plants reached the three-leaf stage, seedlings were thinned to 10 per pot. Each pot was topdressed with 1 g of urea dissolved in de-ionized water at the jointing stage of wheat growth. Sulfur fertilization consisting of S granules (98% S) was applied after fertilization with urea, superphosphate, and potassium chloride. All fertilizers were applied only once. Two sulfur fertilization levels were used: 0 (S0) and 45 (S45) kg ha⁻¹. To maintain soil field capacity at 75%, plants were watered with the control of a TZS-1K soil moisture analyzer (Zhejiang Top Cloud Agricultural Polytron Technologies Inc., Zhejiang province, China) used to measure soil moisture content every three days. The daytime temperature range was 15–38 °C, and the relative humidity reached an average of 50 ± 15% by the time the wheat plants began to flower.

Over the last ten years in the winter wheat region of northern North China, daily maximum temperatures reached approximately 38 °C for 1.8 consecutive days during the grain filling period in wheat growth. Thus, plants at 20 days after flowering were exposed to a two-day HTS treatment at 38 °C to simulate the potential growing trend of increased days of high temperature in northern North China occurring during the wheat grain-filling period. Plants were transferred to an artificial climate chamber, where they were exposed to heat stress for 5 h from 11:00 to 16:00 each day at a relative humidity of 45% ± 5%. After the 2-day heat treatment, all pots were returned to their previous locations in the field to continue growing until seed set. Control (NT) plants were

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