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Salt stress increases content and size of glutenin macropolymers in wheat grain

Xiaxiang Zhang [a], Zhiqiang Shi [a], Youjia Tian [a], Qin Zhou [a], Jian Cai [a], Tingbo Dai [a], Weixing Cao [a], Hanchun Pu [b,*], Dong Jiang [a,*]

[a] National Technology Innovation Center for Regional Wheat Production, National Engineering and Technology Center for Information Agriculture, Key Laboratory of Crop Physiology and Ecology in Southern China, Ministry of Agriculture, Nanjing Agricultural University, PR China
[b] Lianyungang Academy of Agricultural Sciences, Jiangsu Province, PR China

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ABSTRACT

Addition of salt solution in making wheat dough improves viscoelasticity. However, the effect of native salt fortification on dough quality is unclear. Here, wheat plants were subjected to post-anthesis salt stress to modify salt ion content in grains. The contents of Na^+ and K^+, high-molecular-weight glutenin subunits (HMW-GS), glutenin macropolyers (GMP) and amino acids in mature grains were measured. As NaCl concentration in soil increased, grain yield decreased while Na^+ and K^+ contents increased. The contents of amino acids, HMW-GS and GMP in grains also increased, especially when NaCl concentration exceeded 0.45%. Fraction of GMP larger than 10 μm was also increased. Na^+ and K^+ contents were significantly positively correlated to GMP and total HMW-GS contents, and to large GMP fraction.

1. Introduction

Soil salinity is a worldwide adverse environmental factor for crop productivity and quality in arid and semiarid regions and in coastal areas (Francois, Maas, Donovan, & Youngs, 1986; Katerji et al., 2005; Rengasamy, 2010). About 20% of the irrigated crop land in the world was reported to have been affected by salinity, and the situation is deteriorating (Gao et al., 2011; Hasanuzzaman, Nahar, & Fujita, 2013). It is true in the North China Plain where farmers have had to irrigate their land with saline water in the past decades due to the shortage of fresh water (Wang, Yang, Liu, Yao, & Yu, 2015). However, salinity decreases water osmotic potential, causes ion imbalance, disturbs most physiological progresses, inhibits plant growth, and lowers crop yield and quality (Hauser & Horie, 2010; Katerji et al., 2005). Wheat, the extensively grown crop in the world, has suffered from severe salt stress resulting in low grain yield and poor quality (Asseng, Foster, & Turner, 2011; Francois et al., 1986; Zhang et al., 2013).

Storage protein in grains is one of the most important components determining the processing quality of wheat flour (Dupont et al., 2006). Glutenin is a storage protein that plays a key role in controlling dough elasticity (Goesaert et al., 2005). Glutenin is further divided into soluble glutenin (SG) and insoluble glutenin (IG) according to its dissolving capacity in sodium dodecylsulphate (SDS) solution (Wieser, 2007). Glutenin macropolymer (GMP) is an IG fraction and is considered to be the most important glutenin fraction for dough quality (Don, Lichtendonk, Plijter, & Hamer, 2003a). The quantity and particle size of GMP are proved to be two key indicators of flour quality (Don, Lichtendonk, Plijter, & Hamer, 2003b), and to be influenced not only by genetics but also by environment factors, such as fertilizer, temperature and water conditions (Don, Mann, Bekes, & Hamer, 2006; Spiertz et al., 2006).

GMP consists of high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) which are linked via disulfide bonds (Long et al., 2005). Although HMW-GS accounts for only about 5% - 10% of the total protein in grains, its composition and content play principle roles in gluten structure formation and determine the processing quality of wheat flour (Altpeter, Juan, & Wieser, 2004; Don et al., 2006; Dupont & Altenbach, 2003). Very close relationship between the content of GMP and the composition and content of HMW-GS has been documented (Don et al., 2006; Yue et al., 2007; Zhang et al., 2013).

In addition, the content and composition of amino acids have a great effect on the nutritional value of wheat products and can be regulated by environment and cultivation measures, such as nitro-

* Corresponding authors at: College of Agriculture, Nanjing Agricultural University, No. 1 Weigang Road, Nanjing 210095, PR China (D. Jiang).
E-mail addresses: phcxj@sohu.com (H. Pu), jiangd@njau.edu.cn (D. Jiang).

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gen application and irrigation (Fernandez-Figares, Marinetto, Royo, Ramos, & Garcia del Moral, 2000; Howarth et al., 2008). Meanwhile, cysteine, a sulfur (S) containing amino acid, is important for GMP formation, since disulfide bonds formed between the two cysteine residues are necessary for the aggregation of HMW-GS with LMW-GS (Iqbal, Khalil, Ateeq, & Sayyar Khan, 2006; Wieser, 2007). However, knowledge of the effect of salinity on wheat grain quality, especially in terms of GMP and amino acid profiles, is very limited.

In this study, wheat plants were subjected to different levels of salt stress in a pot experiment. The contents of Na^+ and K^+, HMW-GS, GMP and amino acids, and the size of GMP particles in grains were measured. The objectives were to elucidate the relationship between accumulation of salt ion and quality traits of wheat flour under salt stress and to better understand the effect of salt on wheat quality.

2. Materials and methods

2.1. Experimental design

The pot experiment was conducted at the Tangquan Experimental Station of Nanjing Agricultural University, Nanjing (32°08′ N and 118°51′ E), Jiangsu Province, P. R. China, using a locally broadly planted wheat cultivar, Yangmai 16 (*Triticum aestivum* L.). The plants were grown in plastic pots (30 cm in length, 25 cm in width and 27 cm in height) filled with 14 kg of clay soil. The soil was pre-mixed with 0.9 g N, 0.36 g P_2O_5, and 0.9 g K_2O per pot. Another 1.6 g N per pot was top-dressed at the jointing stage. The sowing rate was 40 seeds per pot, and the seedlings were thinned to 15 per pot at the three-leaf stage. Six salt (NaCl) concentrations, 0% (as control), 0.15%, 0.3%, 0.45%, 0.6% and 0.75% (w/w, dry soil weight base), were established from 10 days after anthesis (DAA) till maturity. The experiment was a randomized complete block design, with at least three biological replicates for each treatment.

2.2. Contents of Na^+ and K^+

One hundred mg of whole grain meal was incubated in 5 ml 95% (V/V) sulphuric acid for complete digestion using 30% H_2O_2 as catalyst. The digested solution was transferred into a 50 ml volumetric flask and diluted, and ion contents were determined using a TAS 986 atomic absorption spectrophotometer (Beijing Purkinje General Instrument Co., Ltd, Beijing, China).

2.3. Quantification of HMW-GS

The content of HMW-GS was measured with our previous methods (Jiang et al., 2009; Yue et al., 2007). Briefly, HMW-GS was separated by SDS-PAGE, and total HMW-GS content was the sum of all subunits. The wheat cultivars Chinese Spring and Marquis were used as standards to identify HMW-GS types in Yangmai 16. A representative SDS-PAGE image was shown in the supplementary figure (Fig. S1).

2.4. Content of GMP and GMP particle distribution

Fifty mg of whole grain meal was suspended in 1 ml of 1.5% SDS (w/v) solution and was centrifuged at 15,500*g* at 20 °C for 30 min. The sediment was washed twice with 2 ml 0.2% NaOH (w/v), and was dissolved using 3 ml Biuret reagent to evaluate the N content as GMP content (Gornall, Bardawill, & David, 1949; Weegels, van de Pijpekamp, Graveland, Hamer, & Schofield, 1996; Zhang et al., 2013).

GMP isolation was performed according to the method of Don et al. (2003b). In brief, 1.4 g sample was dissolved in 28 ml 1.5% SDS solution. GMP gel was collected by centrifugation twice at 75,500*g* at 20 °C for 25 min. The collected GMP gel was resuspended with 5 ml of 1.5% SDS, followed by analyzing GMP particle size distribution using a Saturn DigiSizer-5200 digital particle size analyzer (Micromeritics Instrument Corporation, Norcross, GA, USA).

2.5. Content of amino acids

Amino acids analysis was done following Du's method (Du et al., 2012). Whole grain meal (100 mg) was hydrolyzed with 10 ml 6 M HCl for 24 h at 110 °C. The solution was transferred and dissolved in water in a 50 ml volumetric flask. Then the solution was filtered with 0.45 μm micropore filter, a 200 μl sample was collected and dried at 60 °C, and the residue was dissolved in 1 ml 0.02 mol l^{-1} HCl. The amount of each amino acid was determined with an automatic amino acid analyzer (L8900, Hitachi, Tokyo, Japan).

2.6. Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) to determine the significant differences between treatments using the SAS statistical analysis procedures (SAS Institute, USA). The SYSTAT SigmaPlot Suite V 12.0 (SYSTAT Software Inc., Chicago, USA) was used to analyze the correlation between grain quality traits and contents of Na^+ and K^+ in both soil and grain. The *t*-test was used to check the significance.

3. Results

3.1. Yield and yield components

As compared to control, post-anthesis NaCl stresses did not affect the number of spikes per pot and kernels per spike, but decreased the 1000-kernel mass, reducing the grain yields (Table 1). The differences in yield between salt stress treatments and control became significant when soil NaCl concentration exceeded 0.15%. The yield losses were 6.39%, 9.20%, 11.78%, 19.30 and 26.05% when the NaCl levels in soil were 0.15%, 0.30%, 0.45%, 0.6% and 0.75%, respectively.

3.2. Contents of Na^+ and K^+ in grains

Salt stress obviously increased the Na^+ content in grains, especially when the NaCl concentration was above 0.45% (Fig. 1). Here, the Na^+ contents under the NaCl concentrations of 0.45%, 0.60% and 0.75% were 7.60, 13.51 and 32.05 times that in control, respectively. The K^+ content in grains decreased firstly under the NaCl level of 0.15% and then increased under the NaCl level of above 0.45%. The K^+ contents were 0.51%, 2.82% and 10.24% higher than the control when the NaCl concentrations were 0.45%, 0.60% and 0.75%, respectively. These situations caused obvious imbalance between K^+ and Na^+ in grains. Namely, the K^+/Na^+ ratio was much lower under salt stress compared with the control.

3.3. Contents of GMP and HMW-GS in grains

Salt stress increased the content of GMP in matured grains, though the difference was not significant between the treatments with the NaCl concentration lower than 0.30% and the control (Fig. 2). NaCl concentrations of 0.45% and 0.75% significantly increased the content of GMP by 16.81% and 36.14%, respectively.