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SHORT COMMUNICATION

Accumulation characteristic of protein bodies in different regions of wheat endosperm under drought stress

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Abstract

The structural characteristics of protein body accumulation in different endosperm regions of hard wheat cultivar (XM33) and soft wheat cultivar (NM13) under drought stress were investigated. Drought stress treatment was implemented from plant regreening to the caryopsis mature stage. Microscope images of endosperm cells were obtained using resin semi-thin slice technology to observe the distribution and relative area of protein body (PB). Compared with NM13, relative PB area of XM33 was significantly higher in sub-aleurone endosperm region. The amount of accumulation, including the size and relative area of PB, in two wheat cultivars was higher in sub-aleurone region than that in central region at 18 days post anthesis (DPA). Drought stress significantly enhanced the sizes and relative areas of PBs in the dorsal and abdominal endosperms in two wheat cultivars. Particularly for dorsal endosperm, drought stress enhanced the relative PB area at 18 DPA and NM13 (5.0% *vs*. 6.73%) showed less enhancement than XM33 (5.49% *vs*. 8.96%). However, NM13 (9.58% *vs*. 12.02%) showed greater enhancement than XM33 (10.25% *vs*. 11.7%) at 28 DPA. The protein content in the dorsal and abdominal endosperms of the two wheat cultivars decreased at 12 DPA and then increased until 38 DPA. Drought stress significantly increased the protein contents in the two main regions. From 12 to 38 DPA, the amount of PB accumulation and the protein content were higher in XM33 than those in NM13. The results revealed that PB distribution varied in different endosperm tissues and that the amount of PB accumulation was remarkably augmented by drought stress.

Keywords: wheat, protein bodies, distribution, accumulation, drought stress

1. Introduction

Wheat grain contains four types of proteins based on solubility: albumins, globulins, gliadins, and glutenins (Gupta *et al*. 1996). Gliadins and glutenins are storage proteins because their contribution to grain weight reach 8–10% during wheat caryopsis development (Shewry *et al*. 1995; Tosi 2012). Gliadins are monomeric proteins, whereas glutenins contain two subunits, namely, low- and high-molecular-weight glutenin subunits (Loussert *et al*. 2008).

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These two types of storage proteins are both deposited in organelles such as vacuoles; proteins stored in vacuoles are collectively named as protein bodies (PBs). Two types of PBs can be identified under an electron microscope: low- and high-density types. Gliadins are found in both types of protein bodies, whereas high-molecular-weight glutenins are mainly present in high-density PBs (Rubin *et al*. 1992). In wheat endosperm cells, PBs are sequestered into small vacuoles at approximately 9 days post anthesis (DPA). As the caryopsis develops, these small vacuoles subsequently fuse with each other to form a large vacuole and reach their maximum volume at approximately 26 DPA (Rubin *et al*. 1992; Galili *et al*. 1995).

Drought stress (DS) is an important environmental constraint in cereal crop cultivation. As reviewed by Barnabás *et al*. (2008), DS influences the physiology, development, morphology, and hence the yield of cereal crops. As a highly important cereal crop, wheat is greatly sensitive to DS regardless if it is in the vegetative or reproductive stage (Jamieson *et al*. 1995). Several reports have explored the effects of DS on the protein content and composition of wheat grain (Daniel and Triboï 2002; Gooding *et al*. 2003; Flagella *et al*. 2010; Lizana *et al*. 2010; Ge *et al*. 2012). Pierre *et al*. (2008) reported that DS increases the protein content of wheat grain at the grain filling stage, in which the content of monomeric proteins increases to a greater extent than the content of polymeric proteins. However, Zhang *et al*. (2014) pointed out that only the contents of albumins and gliadins increase in wheat under DS and not those of globulins and glutenins.

A previous research showed that the number and size of PBs increase in endosperm cells of DS-exposed wheat kernels at 14 DPA (Fábián *et al*. 2011). PBs accumulate in different endosperm regions, including the sub-aleurone and central regions, and present different patterns (Zheng and Wang 2014b). Environmental factors such as nitrogen application affect PB accmulation in these regions of wheat endosperm (Xiong *et al*. 2013, 2014). However, very little information is available concerning the spatial differences in responding to drought stress, furthermore, the exact mechanism by which DS affects PB accumulation in different regions of developing wheat endosperm is not clear. PB distribution in different endosperm tissues may directly affect the physical and nutritional properties of endosperm (Dombrink-Kurtzman and Bietz 1993). In the present study, two wheat cultivars, one hard and one soft, were subjected to DS from plant regreening to the caryopsis mature stage, and the microstructural changes of the effect of DS on PB accumulation in different regions of endosperm were observed. The storage protein contents in different regions of wheat endosperm were also determined, moreover, the main reason of PB distribution difference in different endosperm regions was also discussed. These findings may provide valuable information for verifying the morphological changes in wheat grain structure under DS.

2. Materials and methods

2.1. Plant materials and DS conditions

Two wheat cultivars, soft wheat Ningmai 13 (NM13, low protein content) and hard wheat Xumai 33 (XM33, high protein content), were evaluated under DS. The cultivars were provided by the Agricultural College of Yangzhou University, Jiangsu Province, China. Seeds were sown 2 cm deep in plastic pots (30 cm×30 cm, 20 seeds per pot), which were placed in rainproof shelters under DS simulation in the experimental field of Key Laboratory of Crop Genetics and Physiology in Yangzhou University from October 2013 to May 2014. The soil was sandy loam containing organic material (2.45%), available nitrogen (106 mg kg^{-1}), available phosphorus (33.8 mg kg^{-1}), and available potassium (66.4 mg kg^{-1}). Wheat seeds were well watered two times a week until growth to the two-leaf stage. Plants were thinned to eight plants per pot 2 weeks after sowing. A minupressure soil hygrometer (SP-11, Institute of Soil Science in Nanjing, China) was inserted into the soil at a depth of 20 cm to detect the soil water potential. Wheat seedlings were accurately irrigated with water from plant regreening (the stage before erecting and after wintering) to the caryopsis mature stage to maintain water potential at −20 and −60 kPa, which reflected the optimum level of control conditions (CC) and DS, respectively. Each treatment contained 30 pots. At the wheat flowering stage, two individual florets at the base of the center of the ears of wheat were marked with a marker pen and the plants were tagged with anthesis dates.

2.2. Endosperm microstructure observation

Wheat caryopses were collected at 12, 18 and 28 DPA. Each caryopsis was cut transversely into 2 mm-thin slices from the center. The slices were immediately immersed in 2.5% glutaraldehyde fixative (25% glutaraldehyde diluted 10 times with pH 7.2 phosphate buffer) at 4°C for 48 h. The samples were subsequently rinsed with phosphate buffer (pH 7.2), dehydrated with graded ethanol (20, 40, 60, 80, 90, 95, and 100%), embedded with low-viscosity embedding kit (SPI supplies, USA), and polymerized at 70°C for 12 h. Eventually, the samples were cut into 1 μm slices using an ultramicrotome (Ultracut R, Leica, Germany), pasted on glass slides, stained with 0.5% methyl violet, and observed and photographed under a light microscope (DMLS, Leica, Germany) equipped with a CCD camera (TruechromeII, Truechrome, China). Each treatment contained three rep-