Available online at www.sciencedirect.com

ScienceDirect

Effect of nitrogen fertilizer on distribution of starch granules in different regions of wheat endosperm

Fei Xiong[a,b,*], *Xurun Yu*[a], *Liang Zhou*[a], *Jing Zhang*[a], *Yanping Jin*[a], *Dongliang Li*[a], *Zhong Wang*[a]

[a]*College of Bioscience and Biotechnology, Yangzhou University, Yangzhou 225009, China*
[b]*Department of Plant Science, University of Connecticut, Storrs, CT 06269, USA*

ARTICLE INFO

Article history:
Received 5 July 2013
Received in revised form
29 September 2013
Accepted 18 November 2013
Available online 28 November 2013

Keywords:
Wheat endosperm
Starch granules
Nitrogen
Distribution

ABSTRACT

This study provided visual evidence of a nitrogen effect on starch granules (SGs) in wheat endosperm. Winter wheat (*Triticum aestivum* L.) cultivar Xumai 30 was cultured under no nitrogen (control) and 240 kg ha^{-1} of nitrogen applied at the booting stage. The number, morphology, and size of A- and B-type SGs in subaleurone of dorsal endosperm (SDE), center of dorsal endosperm (CDE), modified aleurone (MA), subaleurone of ventral endosperm (SVE), and center of ventral endosperm (CVE) were observed under light and electron microscopes. (1) The distribution of SGs in SDE was similar to that in SVE, the distributions of SGs in CDE and CVE were similar, but the distribution of SGs in MA was different from those in the other four endosperm regions. The number of SGs in the five endosperm regions was in the order SDE > CDE > SVE > CVE > MA. (2) Nitrogen increased the number of A- and B-type SGs in SDE and SVE. Nitrogen also increased the number of B-type SGs but decreased the number of A-type SGs in CDE and CVE. Nitrogen decreased the numbers of A-type and B-type SGs in MA. The results suggest that increased N fertilizer application mainly increased the numbers of small SGs and decreased the numbers of large SGs, but that the results varied in different regions of the wheat endosperm.

1. Introduction

Among the cereals, wheat is the most widely grown in the world. Wheat starch is one of the primary food sources for humans, and the accumulation of starch in endosperm is a fundamental component of grain yield [1,2]. Starch is stored in the wheat endosperm as discrete semicrystalline aggregates called starch granules (SGs) [3]. Wheat SGs in mature grains are known to have a bimodal size distribution composed of larger A-type and smaller B-type SGs [4,5], which have been characterized structurally and evaluated for their functional properties [6]. In addition, a trimodal size distribution of A-, B- and C-type SGs

Abbreviations: CDE, center of dorsal endosperm; CVE, center of ventral endosperm; DAA, days after anthesis; MA, modified aleurone; PBs, protein bodies; SDE, subaleurone of dorsal endosperm; SGs, starch granules; SVE, subaleurone of ventral endosperm.

* *Corresponding author at:* College of Bioscience and Biotechnology, Yangzhou University, Yangzhou 225009, China.
E-mail address: feixiong@yzu.edu.cn (F. Xiong).

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

Production and hosting by Elsevier

2214-5141/$ – see front matter © 2013 Crop Science Society of China and Institute of Crop Science, CAAS. Production and hosting by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.cj.2013.11.005

has been observed by some researchers [7–9]. The distribution of SGs influences the starch-to-protein ratio in the endosperm, thereby affecting flour composition and quality [10].

Many studies have reported on SG development in wheat endosperm. A-type SGs are generally believed to be synthesized during the early stages of endosperm development and B-type SGs to develop during later stages [11]. A-type SGs are formed about 4 days after anthesis (DAA), and then continue to enlarge to their maximum at about 19 DAA, with diameters approaching 25–50 μm [7,12]. B-type SG formation begins at about 10–19 DAA [13], but these SGs do not enlarge until 21 DAA, with a diameter of only about 9 μm at maturity. The origin of B-type SGs has been debated during the history of starch research in wheat. Badenhuizen [14] demonstrated that B-type SGs are formed in mitochondria; however, many researchers have reported that B-type SGs form in vesicles budded off from outgrowths of A-type granules [15] or in protrusions emanating from A-type granules containing amyloplasts [9,13,16,17].

The development and distribution of SGs have been shown to be controlled largely by wheat genotype [18–20]. Environmental factors, such as drought or temperature during grain filling, also affect wheat grain development, SG size and SG features [21]. Tester et al. [22] reported that higher temperatures result in smaller SGs, but Hurkman et al. [23] reported that in conditions with high temperatures the proportion of A granules increases, while that of B granules decreases. Endosperm subjected to drought stress has lower numbers of B-type SGs per cell [24]. After drought and temperature, nitrogen (N) nutrition, an indispensable nutrient for wheat production, is considered the third most important environmental factor influencing starch composition and properties [21,25,26]. Blacklow and Incoll [27] showed that a moderate reduction in N leads to small increases in starch content in wheat. Increased N fertilization improves the ratio of A-type SGs while the ratios of B-type SGs in the endosperm of strong-gluten wheat cultivars decreases, but the opposite occurs in the medium-gluten and weak-gluten cultivars [28]. Although N application during endosperm development greatly affects the distribution of SGs and the properties of starch, very little information is available on the microstructure of N-treated wheat relative to the distribution of SGs in different regions of the endosperm. Visualizing the microstructure of SGs from immature and mature kernels will potentially allow the exploration of the interior of SGs. In the present study, we used image analysis software to investigate the distribution of both A- and B-type SGs under N treatment. Based on these primary measurements, the reasons for variations in the distribution of SGs in different regions of wheat endosperm are discussed.

2. Materials and methods

2.1. Plant materials and N treatment

Wheat (*Triticum aestivum* L.) cv. Xumai 30, a widely grown hard red winter wheat, was provided by the National Wheat Improvement Center. The experiment was conducted in the research fields of the College of Bioscience and Biotechnology, Yangzhou University, Jiangsu, China from November 1, 2011 to August 10, 2012. The sowing date was 1 November, 2011, and the wheat density was 2.4×10^6 seedlings per hectare. The sandy loam soil [*Typic Fluvaquent*, Entisols (US taxonomy)] contains 12.58 g kg^{-1} of organic material and 75.19, 45.52 and 99.3 mg kg^{-1} of available N, phosphorus and potassium, respectively. Plot dimensions were 4×5 m and plots were separated by an alley 1 m wide with plastic film inserted into the soil. Each of the treatment had three plots as repetitions in a complete randomized block. The treatment plots received 240 kg ha^{-1} at the booting stage. The control plots received no N at the booting stage. All other field conditions and cultivation managements were kept uniform. During the period of wheat anthesis, the anthesis dates were recorded by dotting the glumes and hanging time tags on the wheat plants. Caryopses that bloomed on the same day but developed on different days for the two treatments were chosen for experimentation. Samples were harvested at 15 and 45 DAA.

2.2. Preparation of materials for light microscopy

First, 2 mm cubic blocks were cut by cross-sectioning from wheat caryopses harvested at 15 DAA. The specimens were then fixed with 2.5% glutaraldehyde and 1% paraformaldehyde in a 0.05 mol L^{-1} cacodylate buffer solution (pH 7.2) and post-fixation treatment in 1% osmic acid in a 0.15 mol L^{-1} sodium cacodylate buffer solution (pH 7.2) for 3 h was applied. The blocks were washed, dehydrated through an ethanol series of 30%–100%, and embedded in Spurr's low-viscosity embedding medium. Sections of 1 μm thick were cut with a glass knife on a Leica Ultracut R (Leica Microsystems, Inc., Wetzlar, Germany), and stained with 0.5% toluidine blue O for 5 min. The sections were visualized and photographed with a Leica Dmls microscope (Leica Microsystems, Inc.). To reflect the nature of caryopsis structure, the findings were compared and confirmed in numerous sections made from developing grains. Five representative regions of transverse sections of the endosperm were observed for every specimen: subaleurone in dorsal endosperm (SDE), center in dorsal endosperm (CDE), modified aleurone (MA), subaleurone in ventral endosperm (SVE), and center in ventral endosperm (CVE), using three replications and 20 micrographs representing ten blocks from different regions.

2.3. SEM observation of caryopsis structure

Mature grains were harvested at 45 DAA and fractured by applying slight pressure on the middle of the caryopsis with a razor blade. The sample thickness was ~3 mm. Caryopses were mounted with the fractured surface facing upwards on a specimen stub and sputter-coated with gold before viewing with a scanning electron microscope (XL30 ESEM, Philips, The Netherlands) at 20 kV to observe the distribution of SGs.

2.4. Determination of number and percentage of SGs

The samples at 15 DAA were used to determine the numbers and percentages of SGs. SGs observed in the image were first marked with a specified color using Photoshop CS4 software (Adobe, U.S.A.) and the image was then analyzed to determine the numbers and percentages of SGs using software Image-Pro Plus 6.0 (Media Cybernetics, U.S.A.). Each treatment was represented by 4 kernels, 5 sections were chosen in each