Journal of Cereal Science 66 (2015) 66–73

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

Effects of drought on the morphological and physicochemical characteristics of starch granules in different elite wheat varieties

Cheng Li [a, b, c, 1], Chun-Yan Li [b, 1], Run-Qi Zhang [b], Wei Liang [b], Xing-Long Kang [b], Yan Jia [b], Yu-Cai Liao [a, c, *]

[a] College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, PR China
[b] College of Agriculture, Shihezi University, Xinjiang 832000, PR China
[c] Molecular Biotechnology Laboratory of Triticeae Crops, Huazhong Agricultural University, Wuhan 430070, PR China

ARTICLE INFO

Article history:
Received 23 December 2014
Received in revised form
11 August 2015
Accepted 28 October 2015
Available online 2 November 2015

Keywords:
Drought
Starch hydrolysis
Triticum aestivum
Ultrastructures

ABSTRACT

In this study, scanning electron microscopy (SEM) revealed the formation of pits and pores on the surfaces of starch granules in response to drought stress, with substantially more pronounced effects in the ordinary yield potential wheat cv. Xindong 23 than the excellent yield potential wheat cv. Xindong 20. Drought induced a significant reduction in starch granule sizes in both wheat varieties, though the reduction observed in Xindong 23 was six times more pronounced than that observed for Xindong 20. Amyloglucosidase and α-amylase treatment of starch from wheat grown in drought conditions released significantly more reducing sugars compared with samples from irrigated controls. SEM and confocal laser scanning microscopy (CLSM) revealed that starch granules from the two wheat varieties grown under drought conditions had substantially increased fluorescence after treatment with proteolytic enzymes and staining with methanolic merbromin and 3-(4-carboxybenzoyl) quinoline-2-carboxaldehyde dyes. Analysis of pasting properties showed significant increases of peak viscosity, trough viscosity, break down, and setback following drought stresses. Furthermore, drought induced a significant reduction in the water binding capacity and increased damage to starch only in Xindong 23. These results provide insight into the potential mechanisms through which drought influences the ultrastructures and physicochemical properties of starch in wheat.

1. Introduction

Starch is the most important polysaccharide produced from cereals to supply calories for human diets. Approximately 70% of the dry weight of grains of wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), maize (*Zea mays* L.), barley (*Hordeum vulgare* L.), sorghum (*Sorghum bicolor* (L.) *Moench*), millet (*Setaria italica* L.), oat (*Avena sativa* L.), and rye (*Secale cereale* L.) consist of starch (Jung et al., 2008). In wheat, the endosperm accounts for about 80–85% of the grain; starch exceeds 80% of the endosperm dry weight. Drought is a major growth-limiting factor that affects global wheat production (Dixon et al., 2009). Investigation of the morphological and physicochemical characteristics of starch granules in elite wheat varieties under drought stresses may provide insight into the mechanisms and regulation of wheat starch biosynthesis and can potentially provide information beneficial for the breeding of drought tolerant wheat varieties with good starch properties and high yield potentials.

Starch is laid down in the form of granules in wheat grains. One population of lenticular-shaped starch granules with a size of larger than 10 μm in diameter is referred to as A-type granules, while spherical granules smaller than 10 μm in diameter are designated as B-type granules (Salman et al., 2009). Native A- and B-type starch granules in wheat are thought to have different compositions, relative crystallinities, amylopectin molecular structures, and different microstructures including surface pores, channels, and cavities (Kim and Huber, 2008, 2010). Crystallinity plays a critical role in starch granule architecture and the physicochemical characteristics of starch, affecting the susceptibility of starch granules to

Abbreviations: DPA, days post anthesis; SEM, scanning electron microscopy; CLSM, confocal laser scanning microscopy; WBC, water binding capacity; SP, swelling power.

* Corresponding author. College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, PR China.
E-mail address: yucailiao@mail.hzau.edu.cn (Y.-C. Liao).

[1] Cheng Li and Chun-Yan Li contributed equally to this work.

http://dx.doi.org/10.1016/j.jcs.2015.10.005
0733-5210/

enzymes and indissolubility in cold water (Tang et al., 2006). Furthermore, the characteristic differences of A- and B-type granules lead to variations in swelling power, gelatinization, and pasting properties (Kim and Huber, 2010). An increased proportion of B-type granules is usually accompanied by a reduction in the peak and final viscosities of wheat starch (Shinde et al., 2003). Thus, granule type and associated factors are important considerations in the evaluation of the physicochemical characteristics of starches in wheat.

The biosynthesis and accumulation of starch is known to be affected by external environmental factors including drought (He et al., 2012), heat (Hurkman and Wood, 2011), salinity (Chen et al., 2008) and soil acidity (Mishra and Dubey, 2008). Among these, drought is believed to be the most important environmental factor. Drought stress accelerates grain filling and reduces total starch accumulation; these changes are directly related to wheat productivity (He et al., 2012). Various wheat starch characteristics are known to change in response to drought, including granule size, pasting, and thermal properties. He et al. (2012) reported that drought conditions decreased the number of small granules and that this decresase was accompanied by an increase of A-type granules. Although changes to wheat starch granule characteristics induced by drought have been well documented (Kim and Huber, 2008; Tang et al., 2006; Li et al., 2011; He et al., 2012), our understanding of how drought stress influences the physicochemical characteristics of starch granules is still limited. In this study, we investigated starch granule morphology, enzymatic digestibility, and the pasting and swelling properties of starch from wheat plants grown under drought conditions. These findings provide insight into the effect of post anthesis drought on the development and the physicochemical characteristics of wheat starch granules.

2. Materials and methods

2.1. Plant materials

The Xindong 20 and Xindong 23 commercial winter wheat varieties were obtained from the Research Institute of Triticeae Crops of Shihezi University. Xindong 20 has an excellent yield potential (7500–8250 kg/hm^2) (Chen et al., 1998), while Xindong 23 has an ordinary yield potential (6000–6750 kg/hm^2) (Yao et al., 2010). The date of wheat flowering for each spike was tagged in an experimental field. Mature grains were collected from wheat plants that had been subjected to drought or normal watering treatments. These collected grains were used for the isolation of starch granules for the analyses of this study.

2.2. Drought treatment

Wheat varieties were planted in the experiment station of the Agricultural College of Shihezi University in Xinjiang, China. This experiment site (44°17′N/86°03′E, 461 m.a.s.l.) was located in an oasis located on the edge of the Gurbantunggut desert. Atmospheric precipitation was relatively scarce from the wheat heading stage to the maturation stage in this region. According to the Shihezi Meteorological Administration (http://www.shzqx.gov.cn/), there was 5.7 mm (from May 10 to June 15, 2012) and 19.7 mm (from May 10 to June 15, 2013) of atmospheric precipitation during the grain filling period. Rain-shelters were used to prevent atmospheric precipitation from impacting the drought treatments. The drought treatment was generated by withholding irrigation water; no water was applied from the flowering stage through to the maturation of grain. The control irrigatation treatment was applied (1125 m^3/hm^2 water) by drip irrigation; applied once at flowering and once at the middle of the filling stage. The same field management program was applied to all experimental plots prior to the flowering stage. All treatments were replicated three times (three units for drought treatment, 3 units for irrigation) and samples were pooled from across all of the experimental plots.

2.3. Starch isolation

Flour samples (15 g) were mixed with 150 ml of dH_2O and sieved with a 200 mesh sieve. The resulting slurry was centrifuged at 1700× *g* for 5 min, and the supernatant was then discarded. 50 ml of NaCl solution (2 mol/L) was added and the samples were centrifuged three times, as described above. The starch sediment was pooled and NaOH was added to 0.2% (w/w). After three further centrifugations, the supernatant was removed and the upper dark portion (tailings and protein layer) of the pellet was carefully scraped away and discarded. This procedure was repeated until no protein layer was observed. Starch sediment was re-suspended in washing buffer, gently stirred for 10 min, and centrifuged at 3000× *g* for 20 min. The supernatant was then discarded. The starch pellet was washed three times with an excess of dH_2O, re-suspended in 50 ml of 100% ethanol, recovered with a Buchner funnel, and allowed to air dry. Total starch content was determined as previously described (Zhao, 2005).

2.4. Scanning electron microscopy (SEM)

The starch granules were tiled onto aluminum stubs using double-sided conduction adhesive tape and coated with 20 nm gold-palladium (60: 40). Images of starch granules were obtained with a field emission SEM (JSM-6490LV, JEOL, Japan) at an accelerating voltage of 3–5 KV.

2.5. Confocal laser scanning microscopy (CLSM)

Protease-treated starch granules treated with fluorescent dyes were visualized using a Zeiss LSM 510 CLSM system (Zeiss, Oberkoche, Germany). Sample glass slides were prepared as described by Kim and Huber (2008). Excitation was achieved with an Argon laser (488 nm) operating at 30% power, and emission was detected through an LP505 emission filter. The images were processed using the Zeiss LSM Image Browser program (Zeiss).

2.6. Starch granule size distribution

The size distribution of starch granules was measured with a laser-diffraction particle-size analyzer (Microtrac S3500, Florida, USA) according to the manufacturer's instructions.

2.7. Enzymatic hydrolysis of starch granules

Amyloglucosidase and α-amylase treatment of starch granules was carried out as previously described (Tang et al., 2002). The extent of starch hydrolysis was determined by measuring reducing sugar concentrations by the colorimetric method (Bernfield, 1951). Dinitrosalicylic acid color reagent was prepared by dissolving 0.63 g of 3, 5-dinitrosalicylic acid in 26.2 ml of 2 mol/L NaOH. Sodium potassium tartrate tetrahydrate (18.5 g) was slowly added to the solution. Phenol (0.5 g) and sodium sulfite (0.5 g) were added to a final volume of 100 ml with dH_2O. 0.1 ml aliquots of the starch digestion solutions were removed from the reaction tubes at specific time intervals, 0.1 ml of dinitrosalicylic acid color reagent was added to these aliquots. The sample tubes were incubated in a boiling water bath for 5 min and then cooled to room temperature. 3 ml of dH_2O was added to each test tube and the absorbance at 540 nm was measured; reducing sugar concentrations were calculated based on a calibration curve prepared with glucose.