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Wheat genome specific granule-bound starch synthase I differentially influence grain starch synthesis

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ABSTRACT

Wheat grain development is a complex process and is characterized by changes in physicochemical and structural properties of starch. The present study deals with endosperm starch physicochemical properties and structure during development in different granule-bound starch synthase I (GBSSI) null also known as waxy (Wx) genotypes. The study was conducted with pure starch isolated from wheat grains at 3–30 days post anthesis (DPA), at 3-day intervals. Amylose concentration increased throughout grain development in non-waxy (7.2–30.5%) and partial waxy genotypes (6.0–26.8%). Completely waxy genotype showed 7.0% amylose at 3 and 6 DPA, which declined during development and reached non-detectable quantities by 30 DPA. Amylopectin structure had a higher content of short chains at 3 DPA, which decreased continuously until 12 DPA, after which there were only minor changes in amylopectin chain length distribution. Similarly, the average degree of polymerization (DP) increased from 3 DPA (12.3) to 12 DPA (15.0), and then did not differ significantly up to 30 DPA (15.0). This suggests the formation of basic amylopectin architecture in wheat by 12 DPA. Wx-B and Wx-D affected amylopectin short chains mostly of DP 6–8 at 3 and 6 DPA. Wx-A affected the same fraction of chains at 9 and 12 DPA, and Wx-D affected DP 18–25 chains from 18 to 30 DPA, suggesting differential effect of waxy isoproteins on amylopectin structure formation.

1. Introduction

Biochemical properties of starch have been extensively utilized in the food industry for uses such as thickeners for soups, sauces and dairy products, starch-based fat replacers to maintain texture, flavor and mouth-feel of low- or no-fat products. The relative proportion and structure of starch constituents, amylose and amylopectin, greatly influence its end-use. For instance, waxy wheat starches possess exceptional freeze-thaw stability, water absorption capacity, peak viscosity, and low gelatinization temperatures; hence they are useful in the baking industry (Abdel-Aal, Hucl, Chibbar, Han, & Demeke, 2002). Amylopectin chain length distribution also affects gelatinization, retrogradation, and pasting properties of starch. Short glucan chains reduce gelatinization temperatures (Jobling, 2004), while very-long branch chains influence starch-pasting properties (Jane et al., 1999); thus making them valuable in food industry applications.

Structurally, starch is a glucan homopolymer composed of one-quarter amylose (linear with few branches) and three-quarters amylopectin (highly branched). Linear chains are formed by α-1,4 glucosidic linkages, while branches are formed by α-1,6 glucosidic linkages. The structures of amylose and amylopectin, and their organization in the intact granule have been widely discussed (Buléon, Colonna, Planchot, & Ball, 1998). Amylopectin shows a polymodal distribution of chains, organized into clusters, giving the molecule its architectural specificity (Hizukuri, 1986). Briefly, A-chains (DP 6–12) are the outermost chains, B-chains named B1, B2 and B3 depending on their degree of polymerization (DP 13–24 up to 50) support A-chains or other B-chains. They are attached via α-1,6 glucosidic linkage, to the central C-chain, which within a granule is oriented toward the center or hilum of the granule (Manners, 1989). Packing of these chains, or rather the amylopectin molecule in the granule determines the basic structure of the starch granule.

The first step in the synthesis of amylose and amylopectin is brought about by ADP-glucose pyrophosphorylase (AGPase). Elongation and branching of amylopectin is a complex process and it

Abbreviations: DPA, days post anthesis; DP, degree of polymerization; GBSSI, granule-bound starch synthase I; SEM, scanning electron microscopy.

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requires an array of enzymes viz., starch synthases (SS), starch branching enzymes (SBE) and debranching enzymes (DBE) (Buléon et al., 1998). Synthesis of amylose, however, is brought about solely by the enzyme granule-bound starch synthase I (GBSSI) or waxy protein. Plants lacking GBSSI are termed 'waxy'. Recently, GBSSI has also been postulated to have a role in amylopectin synthesis (Ral et al., 2006). Incorporation of [^{14}C] glucose from ADP [^{14}C] glucose by GBSSI in granules from different species has been reported to be mainly into the long chain fraction of amylopectin (Denyer, Clarke, Hylton, Tatge, & Smith, 1996). However, the precise contribution of GBSSI to amylopectin synthesis is still unclear.

Contrasting reports exist on the differences in amylopectin chain length distribution due to the absence of waxy protein. In *Chlamydomonas* and wheat waxy mutants that lack GBSSI, the amylopectin molecule has been shown to differ from that of their non-waxy counterparts (Delrue et al., 1992; Li, Feng, et al., 2007). In contrast, no obvious difference in the glucan chain-length distribution profiles of amylopectin molecules between waxy and non-waxy starch within three chain-length groups were reported (Yasui, Matsuki, Sasaki, & Yamamori, 1996). These studies were conducted on mature grains. To better understand changes in amylopectin chain length distribution profiles, some studies have been conducted at different developmental stages (Kalinga et al., 2014; Waduge, Xu, Bertoft, & Seetharaman, 2013). It was reported that the content of A- and short B-glucan chains increased in the waxy, and decreased in the non-waxy type of wheat during grain development. Also, the content of mid-length B-glucan chains decreased in both waxy and non-waxy starch during grain development. It was also suggested that GBSSI elongates the A- or short B-glucan chains to produce long B-glucan chains of amylopectin (Cao, Hu, & Wang, 2012).

Since wheat is an allohexaploid ($2n = 6x = 42$), it has three doses of GBSSI, one from each genome, namely Wx-A, Wx-B and Wx-D. The lack of waxy protein from each genome has been shown to affect the end-use of starch. Durum wheat, which possesses waxy protein from two genomes, is the best candidate for pasta production (Sharma, Sissons, Rathjen, & Jenner, 2002). Similarly, double null-waxy mutants of wheat result in better texture of noodles (Zhao et al., 1998). Considering GBSSI potentially contributes to amylopectin synthesis or elongation, its absence from each genome could affect the glucan chain length distribution of amylopectin and/or organization of glucan chains within the granule, resulting in changes in the morphology of starch granules during wheat grain development. Therefore, the present study focused on studying starch characteristics during wheat grain development in non-waxy and waxy-null genotypes.

2. Materials and methods

2.1. Plant material

Near-isogenic partial-waxy and non-waxy genotypes including four lines each of Wx-$A^+B^+D^+$, $A^-B^+D^+$, $A^+B^-D^+$, $A^+B^+D^-$, $A^-B^-D^+$, $A^-B^+D^-$, $A^+B^-D^-$ and $A^-B^-D^-$ were used for the developmental study. Plants were grown in a growth chamber in pots containing Redi-earth (W. R. Grace and Co. of Canada, ON, Canada) at 23 °C/16 h light (350 μmol m^{-2} s^{-2} PPFD) and 19 °C/8 h dark, and were fertilized every 3 weeks with a slow release fertilizer, Nutricote-14–14–14:N–P–K (Plant Products Co., Ltd., Brampton ON, Canada). Spikes, in triplicate, were tagged at anthesis, collected and frozen in liquid N_2 at selected days post anthesis (DPA), viz., 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 days, and stored at −80 °C for further experiments. Spikes were collected in mid-afternoon consistently for all developmental stages. Starch was purified from freeze-dried seeds from the middle of the spikes using a modified method (Peng, Gao, Abdel-Aal, Hucl, & Chibbar, 1999) involving cesium chloride density gradient centrifugation (Asare et al., 2011).

2.2. Starch granule morphology by scanning electron microscopy

Prior to imaging under scanning electron microscope (SEM), starch granules were gold coated to make them electrically conductive. Starch granules were spread on, or attached to a metal stub with double-sided carbon tape and gold coated using a sputter gold coater (S150B, Edwards, Wilmington MA, USA). Granule morphology was observed using a scanning electron microscope (SU6600 Hitachi High-Technologies Europe GmbH, Krefeld, Germany) at 5 kV.

2.3. Total starch concentration determination

Total starch concentration was determined using an American Association of Cereal Chemists International (AACCI) approved method (AACC method 76–13.01), in which ground meal samples were hydrolyzed to dextrins and finally to D-glucose using α-amylase and amyloglucosidase respectively. Total starch concentration was determined as free glucose by measuring the absorbance at 510 nm (Hucl & Chibbar, 1996) and calculated on a percent dry weight basis (McCleary, Gibson, & Mugford, 1997).

2.4. Amylose concentration determination by HPSEC

High performance size exclusion chromatography (HPSEC) was used to determine the amylose concentration (Demeke, Hucl, Abdel-Aal, Båga, & Chibbar, 1999). Gelatinized starch samples (1 mg/mL) were debranched using isoamylase one unit for each mg of starch (1000 U/mL Megazyme International Ltd., Wicklow, Ireland), freeze-dried, suspended in dimethyl sulfoxide (DMSO) and injected (40 μL) into the column (PL gel MiniMix 250 mm × 4.6 mm ID column, Polymer Laboratories Inc., Amherst, MA, USA) attached with Waters 410 differential refractometer. (Asare et al., 2011). Starch samples, column, and detector were maintained at 40, 100, and 45 °C, respectively. DMSO (99%) with 0.4% Lithium bromide was used as an eluent at a flow rate of 0.2 mL/min. Data was collected and analyzed using Empower 1154 chromatography software (Waters Corporation, Milford, MA, USA).

2.5. Protein detection and immunoblotting

Proteins were extracted from the starch granules by suspending 10 mg starch in 300 μL denaturing electrophoresis buffer (Demeke, Hucl, Nair, Nakamura, & Chibbar, 1997). GBSSI was separated using polyacrylamide {15% (w/v) 1 mm thick gel} denaturing gel electrophoresis (Protean II, Biorad, Hercules, CA, USA) (Demeke et al., 1997). Separated polypeptides were visualized by silver staining (Gromova & Celis, 2006). The gel-electrophoresis separated polypeptides were transferred on a nitrocellulose membrane at 10 V for 4 h (Gao & Chibbar, 2000). The membrane with transferred polypeptides was processed and incubated with primary antibodies raised against wheat GBSSI, SSI, SSII and SBE (Gao & Chibbar, 2000; Peng, Hucl, & Chibbar, 2001). The antigen-antibody complex was then detected with goat anti-rabbit alkaline phosphate conjugate using a 5-bromo-4-chloro3′-indolyphosphate p-toluidine/nitro-blue tetrazolium chloride immunoscreening color development kit (Biorad, Hercules, CA, USA).

2.6. Amylopectin chain length distribution by capillary electrophoresis

Chain length distribution of amylopectin molecules was determined by fluorophore assisted capillary electrophoresis (FACE)

(O'Shea, Samuel, Konik, & Morell, 1998) using Proteome Lab PA800 (Beckman Coulter, Fullerton CA, USA) equipped with a 488 mm laser module. A modified debranching protocol (Asare et al., 2011) was used to obtain unit amylopectin chains, which were labeled using 8-aminopyrene 1, 2, 6-trisulfonate in the presence of sodium cyanoborohydride/tetrahydrofuran. The N-CHO (PVA) capillary with pre-burned window (50 μm ID, 50.2 cm total length) was used to separate debranched glucan chains.

2.7. Statistical analysis

All biochemical determinations are expressed as average of three biological replicates. The analysis of variance (ANOVA) of the means was performed with SPSS univariate analysis (SPSS Inc. version 17). Multiple means comparisons were determined with the Duncan's multiple range test at $p < 0.05$ confidence level. For scanning electron microscopy at least nine areas (from three independent starch preparations) selected on random basis were analyzed and image selected average distribution observed for these preparations.

3. Results and discussion

Wheat endosperm consists of four cell types: the embryo surrounding cells, transfer cells, the aleurone layer and the starchy endosperm (Olsen, 2001). Starchy endosperm in wheat represents the largest body of cell mass in the endosperm consisting of an estimated 60,000 cells (Chojecki, Bayliss, & Gale, 1986). Development of endosperm is broadly divided into five phases (Simmonds & O'Brien, 1981). Phase I (0 DPA) is the fertilization phase. Phase II (1–5 DPA) is the grain growth stage, where the initial endosperm nucleus divides repeatedly forming coenocytic endosperm. Phase III (6–13 DPA) is the initial grain filling stage, when cellularization occurs via formation of radial microtubule systems and alveolation (Olsen, 2004). Phase IV (14–24 DPA) is the maximum grain filling stage, in which rapid starch and protein synthesis occurs in the endosperm (Bechtel, Zayas, Kaleikau, & Pomeranz, 1990). Phase V (25–38 DPA) is the drying stage, leading to desiccation. Morphological and biochemical changes occurring in starchy endosperm during wheat grain development will provide an insight into the factors affecting the final wheat grain quality.

3.1. Starch granule morphology during wheat grain development

In wheat endosperm, starch granules are produced individually in separate amyloplasts. Starch granules of different shapes and sizes are developed in the endosperm during different periods of grain maturation, and have been divided into different classes. The number of size classes, however, is debated. All reports agree that in the initial grain development period, the first population of starch granules (large A-type, >15 μm) emerges at 4–7 DPA (Bechtel & Wilson, 2003). These are initially spherical granules of diameter 0.5–1.0 μm, which grow radially to 2–4 μm. According to Evers (1971), an equatorial plate which encircles the granule is formed after the starch granule has reached a specific size. Active deposition of glucan molecules takes place over the equatorial plate, and gives the granule its characteristic lenticular shape. A second population of small starch granules (B-type, 5–15 μm) is initiated at 12–14 DPA, which develop inside amyloplasts protrusions and remain spherical throughout development (Bechtel & Wilson, 2003). Some reports suggest the occurrence of a third population of even smaller spherical starch granules (C-type, <5 μm) at 21 DPA (Raeker, Gaines, Finney, & Donelson, 1998).

The first population of small spherical starch granules (possibly A-type) was observed at 3 and 6 DPA, and it increased in size from 9 DPA onwards (Fig. 1A and B). This indicates starch accumulation at the coenocytic stage, before endosperm cell wall formation (Simmonds & O'Brien, 1981). Expression of GBSSI in wheat at this stage has been previously reported (Laudencia-Chingcuanco et al., 2007; Nadaud et al., 2010), suggesting starch formation as early as 3 DPA. In the non-waxy genotype, starch granules of all sizes ranging from 0.8 to 19.2 μm diameter were initiated at 3 DPA (Fig. 1A). However in the waxy genotype, only small starch granules with diameter maxima of 5.0 μm were observed at this stage (Fig. 1B). At 6 DPA, the starch granule population was dominated by granules up to 11 μm (non-waxy) and 13 μm (waxy) diameters, in addition to smaller starch granules. A second population of starch granules (possibly B-type) of diameter 1.2 μm (non-waxy, Fig. 1A) and 0.8 μm (waxy, Fig. 1B), was observed at 9 and 12 DPA. At 9 DPA, equatorial grooves (as reported by Evers, 1971) were observed for large growing starch granules (Fig. 1). Between 12 and 15 DPA, starch granule diameter maxima reached 23.3 μm (non-waxy, Fig. 1A) and 26.3 μm (waxy, Fig. 1B), developed a shallow equatorial groove, and assumed lenticular shape. Emergence of a new population of small starch granules (possibly C-type) was observed between 18 and 21 DPA as depicted by increased starch granule density of diameter 1.6 μm (non-waxy, Fig. 1A) and 1.7 μm (waxy, Fig. 1B). After this stage, large starch granules did not increase in size and maintained a maximum diameter of 22.5 μm for both non-waxy and waxy genotypes. Although both small and large starch granules were present at 30 DPA, the waxy genotype showed a higher number of small starch granules than the non-waxy genotype.

3.2. Carbohydrate accumulation during grain development

Free glucose concentration in the non-waxy genotype was 3.2% at 3 DPA, which decreased to 0.2% at maturity. Linear decreases in free glucose concentration, with minor differences, was recorded for all partial waxy and completely waxy genotypes (data not shown), concurring with observations in *Brachypodium* (Guillon et al., 2011).

Starch and amylose contents showed consistent increases with grain maturity, agreeing with previous reports for wheat (Ganeshan, Drinkwater, Repellin, & Chibbar, 2010), barley (McDonald, Stark, Morrison, & Ellis, 1991), and maize (Li, Blanco, & Jane, 2007b); however, differing with potato where apparent amylose did not significantly change with maturity (Liu, Weber, Currie, & Yada, 2003). In the present study, starch accumulation increased from 3 to 30 DPA, i.e. from 8.3 to 66.2% for non-waxy, and from 3.5–5.4% to 53.2–66.2% for partial-waxy genotypes. Total starch concentration in the completely waxy genotype increased up to 21 DPA (3.9–48.0%), after which it reached saturation (50.3%) at 30 DPA (Fig. 2). Starch concentration increased approximately 10% from 12 to 15 DPA in all genotypes, concurring with a previous transcriptional profiling study in wheat which suggested that the maximum accumulation rate of transcripts encoding storage proteins and starch biosynthetic enzymes, as well as maximum starch accumulation, occurs from 7 to 14 DPA (Laudencia-Chingcuanco et al., 2007).

In the present study, the rate of total starch accumulation depicted by linear regression values, in the completely waxy genotype ($r^2 = 0.90$) was higher than the non-waxy genotype ($r^2 = 0.86$), although the non-waxy genotype showed a significantly higher starch concentration throughout development (Fig. 2). Similarly, a high starch accumulation rate and higher enzyme activities of sucrose synthase, AGPase, SS, GBSS and SBE have been reported for a cultivar with higher starch content (Dai, 2010), supporting the higher total starch concentration in the non-waxy genotype in the present study. From 3 to 6 DPA, starch concentration in Wx-A$^-$ genotypes doubled, while a small increase was observed in other

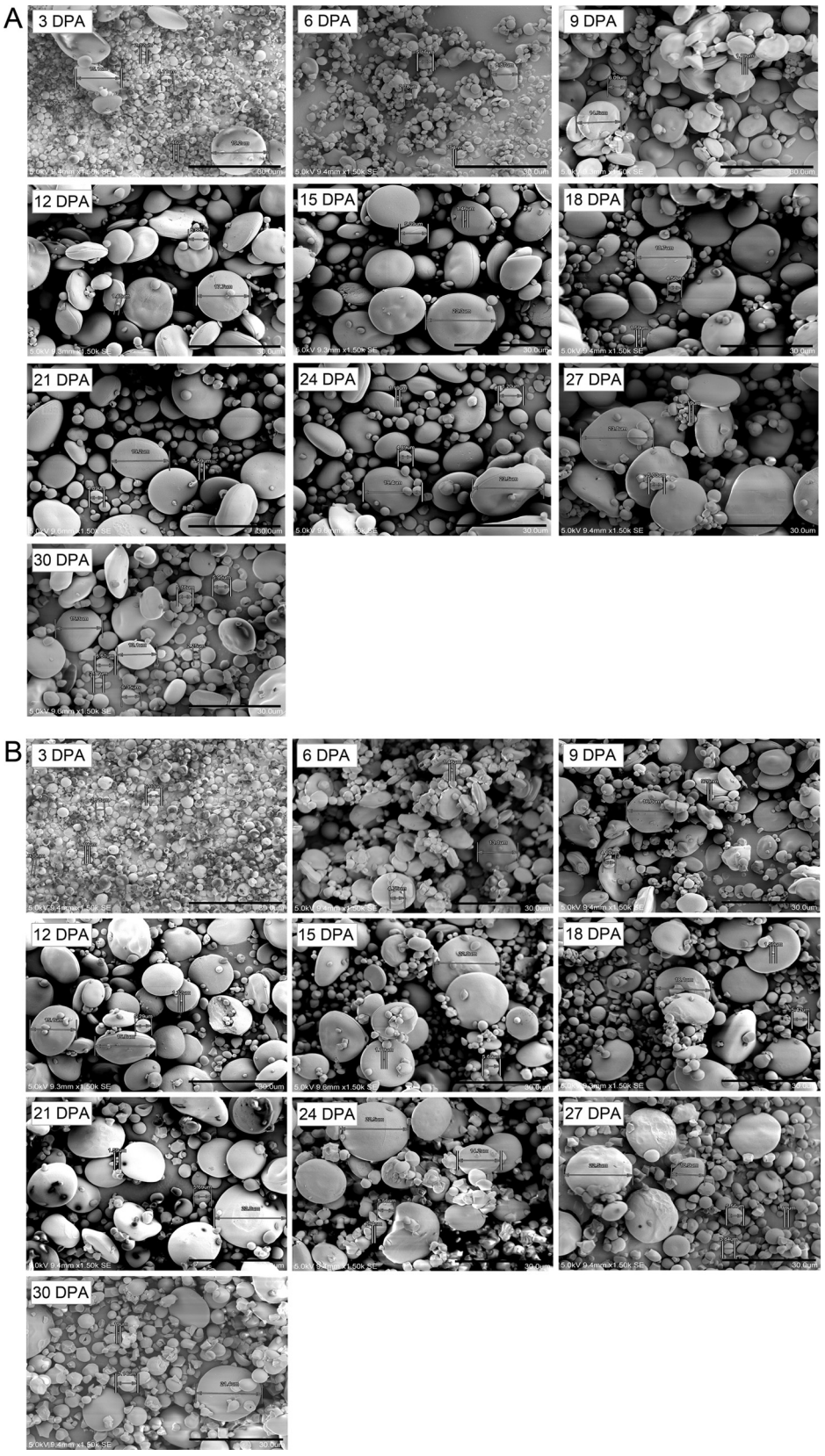

Fig. 1. SEM images of starch granules isolated at different development stages in non-waxy (A) and completely waxy (B) genotypes.

genotypes. This suggests that Wx-A could be playing a regulatory role in starch accumulation. At 15 and 21 DPA, starch concentration was higher in single nulls than double nulls, suggesting a significant influence of GBSSI dosage on starch accumulation.

The amylose accumulation sigmoid-curve during granule development showed a linear increase from 3 to 6 DPA, remained constant till 9 DPA, and increased again from 12 to 18 DPA reaching a stationary phase after 21 DPA (Fig. 3). It has been shown

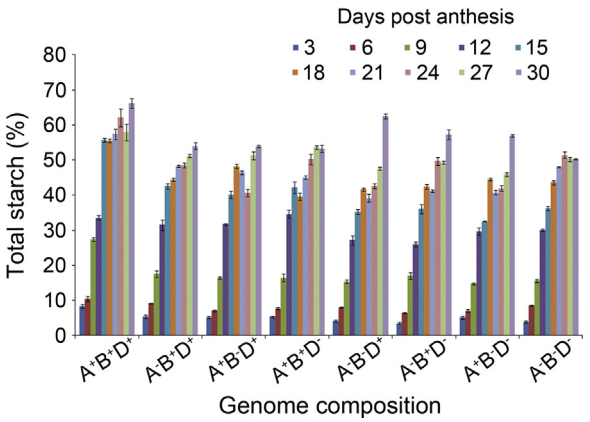

Fig. 2. Total starch concentration in near isogenic (waxy locus) lines during wheat grain development.

earlier that mRNA of rice GBSSI gene increased with the development of endosperm (Dian, Jiang, & Wu, 2005). Also, increase in GBSSI gene expression is directly related to amylose accumulation in maize endosperm (Li, Feng, et al., 2007). In the non-waxy genotype, amylose concentration increased from 7.2% at 3 DPA to 30.5% at 30 DPA, being higher than partial and completely waxy genotypes (Fig. 3). This was also depicted by higher GBSS activity in the cultivar with higher starch content, indicating that it had a greater capacity to synthesize amylose and amylopectin than the cultivar with low starch content during mid-late grain filling (Dai, 2010). In the present study, the rate of amylose accumulation among the partial waxy genotypes differed during grain development. From 6 to 9 DPA, it was higher in the single nulls (r^2 = 1.07) than double nulls (r^2 = 0.24); from 9 to 18 DPA, it was similar for all the genotypes (r^2 = 1.08–1.09). Thereafter single nulls had a higher concentration of amylose than double nulls, reaching 29.2% and 24.6% respectively at 30 DPA.

The exception to this trend was the completely waxy genotype, which showed a consistent reduction in the rate of amylose accumulation throughout grain development (7.0–0.8%) (Fig. 3). Ideally, it should have shown a negligible concentration of amylose at earlier stages of grain development. It is speculated that the detected amylose could be due to GBSSII from the pericarp, which is still photosynthetic at that stage. GBSSII is responsible for amylose synthesis in non-storage tissues (Vrinten & Nakamura, 2000), and since it is encoded by a separate gene, absence of GBSSI would not affect amylose accumulation by GBSSII. At the early stages of grain development pericarp could not be separated from the nascent endosperm, therefore amylose was also observed in the waxy starch genotypes.

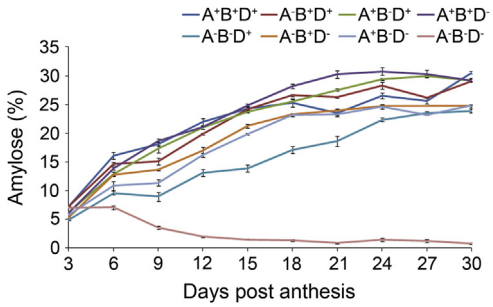

Fig. 3. Amylose concentration in near isogenic (waxy locus) lines during wheat grain development.

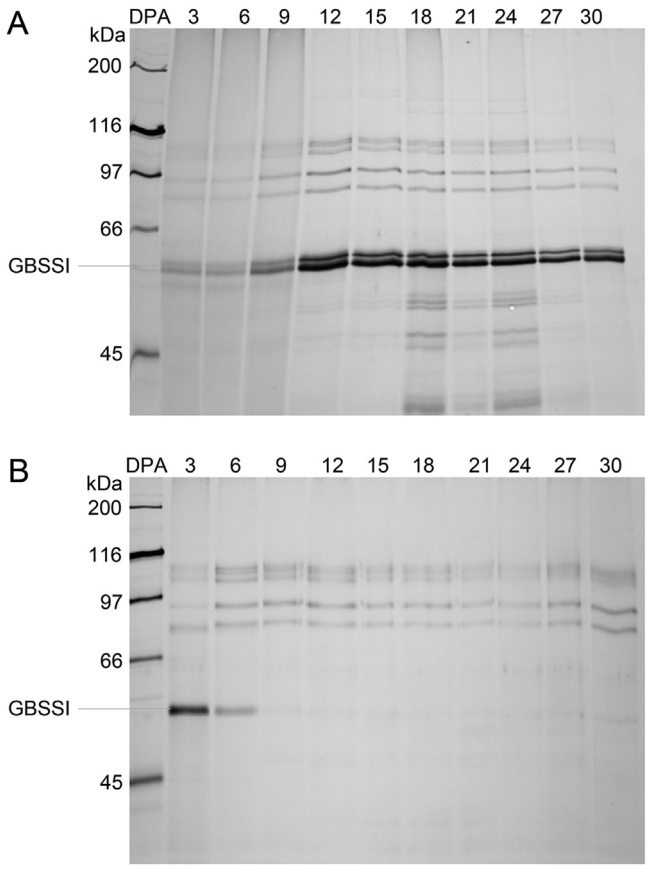

Fig. 4. Denaturing gel electrophoresis analysis showing the presence of starch granule-bound proteins (silver staining) during grain development (3–30 DPA) in non-waxy (A) and completely waxy (B) genotypes.

3.3. Starch granule polypeptides during grain development

Denaturing polyacrylamide gel analysis for non-waxy and completely waxy genotypes was performed to determine whether the amount of GBSSI accumulated within starch granules correlated with amylose content. For the non-waxy wheat genotype, reduced accumulation of GBSSI (~60 kDa) was observed at 3 and 6 DPA, i.e. at early grain filling stage, while normal accumulation levels were observed from 9 to 30 DPA, or the active grain filling period (Fig. 4A), as has been previously observed (Nadaud et al., 2010). In the non-waxy genotype, the level of accumulation of SSII (100–115 kDa), SBE (87 kDa) and SSI (77 kDa) was lower until 9 DPA, after which a constant level of accumulation of these polypeptides was observed. Very little accumulation of SBEIc (152 kDa) was observed from

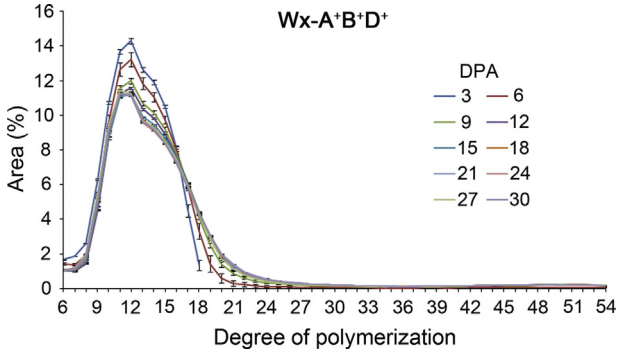

Fig. 5. Amylopectin chain length distribution during wheat grain development (3–30 DPA) in a non-waxy genotype.

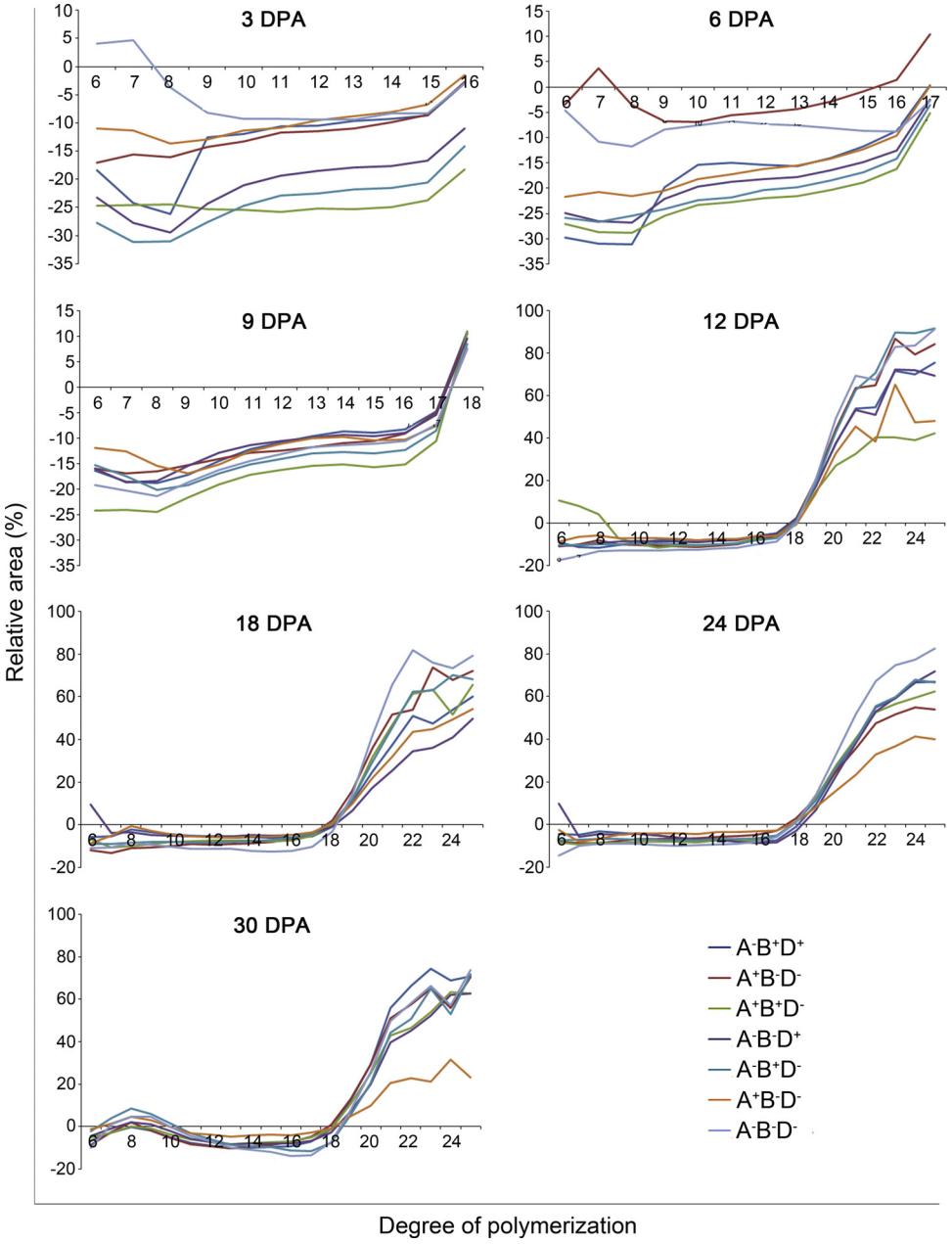

Fig. 6. Amylopectin chain length distribution in partial and completely waxy wheat genotypes during grain development (3, 6, 9, 12, 18, 24, and 30 DPA). Subtractive graphs depict relative area in a particular waxy-null genotype subtracted from that of normal genotype. Values are based on average of three biological replicates.

18 to 30 DPA (Fig. 4A). In the waxy genotype, a similar level of accumulation of SSII, SBE and SSI was observed throughout grain development. SBEIc could not be detected in the waxy genotype. As expected for the waxy genotype, GBSSI accumulation was observed at 3 and 6 DPA, but not from 9 to 30 DPA (Fig. 4B). Presence of GBSSI at early stages of development was confirmed by immunoblot analysis using polyclonal antibodies raised against wheat GBSSI (Supplementary Fig. 1), which was speculated to be GBSSII.

Since starch synthesizing enzymes in cereals have been shown to work in multi-protein complexes (Tetlow et al., 2008), immunoblotting was performed for other starch synthesizing enzymes to check whether the absence of GBSSI affects the accumulation of other starch granule proteins. SSI, SSII, SBEI and SBEII showed a similar pattern in non-waxy and waxy genotypes indicating that absence of GBSSI did not affect the accumulation of other starch granule bound proteins (Supplementary Fig. 1).

Supplementary Fig. 1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.08.004.

3.4. Amylopectin chain length distribution during grain development

Most variation in amylopectin chain length distribution was observed from 3 to 12 DPA, after which there were no considerable differences up to 30 DPA (Fig. 5). In all genotypes, glucan chain of DP ≤ 14 decreased, while glucan chains of DP ≥ 19 increased during early stages of development. In the non-waxy genotype, glucan chains of DP 6–8, DP 9–11 and DP 12–14 decreased from 6.2, 30.7 and 38.9% at 3 DPA to 3.7, 25.2 and 32.9% at 9 DPA respectively. Glucan chains of DP 15–18 did not show significant variation across development stages, being 24.2% at 3 DPA, 27.1% at 12 DPA and 25.9% at 30 DPA. Glucan chains of DP 19–22, DP 23–36 and DP

37–45 increased from 2.6, 0.9 and 1.1% at 6 DPA to 6.6, 3.6 and 3.1% at 12 DPA respectively (data not shown). Glucan chains of DP ≥ 19 were absent at 3 DPA (Fig. 5). These results were also reflected in the increase in average DP from 3 to 12 DPA with no changes thereafter (Supplementary Table 1), suggesting a continuous change in the length and arrangement of glucan chains up to 12 DPA (Fig. 5). The content of short glucan chains was higher during the early endosperm development stage, mainly during the cellularization stage, and decreased consistently until the initial grain filling stage. From active grain filling onwards, the amylopectin chain length distribution changed only slightly (Fig. 5, Supplementary Table 1). This suggests that amylopectin attains its specific unit chain length distribution/structure by the grain filling stage. Similarly, in a previous study of endosperm starch properties during maize grain development, it was reported that average amylopectin branch chain length increased from 10 DAP to 14 DAP and then decreased on 30 and 45 DAP (Li, Feng, et al., 2007). It was suggested that the structure of endosperm starch was not synthesized consistently throughout grain development. It has previously been reported that the relative expression of GBSSI gene increases only after 5 DPA (Ganeshan et al., 2010). However other starch synthases (SSI, SSII) genes show a higher relative expression than GBSSI at earlier DPA. For the non-waxy genotype, therefore, the reduction in the content of glucan short chains (Fig. 5) could be a result of increased GBSSI gene expression. As GBSSI gene expression increases, it actively elongates the short glucan chains; hence their number decreases and the proportion of long glucan chains increases. In addition, 11 enzymes associated with starch synthesis, particularly GBSSII and soluble SS, have been shown to have expressed at the very beginning of wheat grain development (Nadaud et al., 2010), which could be the contributing factor for amylopectin synthesis in the absence of GBSSI, in the completely waxy genotype. Starch biosynthetic enzymes activity assays during endosperm development could provide a better understanding of amylopectin structure formation during grain development, as well as the precise function of GBSSI.

Supplementary Table 1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.08.004.

Subtle differences were observed in the amylopectin glucan chain length distribution with the reduction of waxy protein dosage during wheat grain development (Fig. 6). Compared to non-waxy starch, completely waxy starch at 3 DPA showed a higher proportion of short glucan chains of DP 6–8 (Fig. 6), suggesting a relatively earlier synthesis of amylopectin chains in the former as compared to the latter, concurring with a previous report (Cao et al., 2012). At 3 DPA, on DP 6–8 glucan chains, the influence of Wx-B, Wx-D alone or together was the highest, whereas, the influence of Wx-A was the lowest. Similarly at 6 DPA, Wx-B, D affected the glucan short chain fraction more than the other partial waxy starches. This suggests that Wx-B and Wx-D are playing a greater role in amylopectin synthesis during the initial stages of grain development. At 9 DPA, however, Wx-A, B showed a higher proportion of DP 6–15 glucan chains followed by Wx-B and Wx-D. However, Wx-A alone was the least effective in influencing DP 6–8 chains, suggesting that Wx-A only in combination with Wx-B or Wx-D has a stronger effect on the short glucan chains formation. Furthermore, at 12 DPA, Wx-A, B displayed a considerably higher proportion of short chains of DP 6–8 than the other partial waxy and non-waxy wheat starches, suggesting a positive influence of Wx-A on the short chain phenotype during grain filling. At 18 and 24 DPA, Wx-D showed an increase in DP 6–8 glucan chains relative to the non-waxy starch. In addition at 24 and 30 DPA, glucan chains of DP > 20 were negatively affected by Wx-A (Fig. 6). This suggests that Wx-B and Wx-D play a stronger role in influencing mid-length glucan chains than Wx-A, during later stages of grain development. The above observations suggest that different waxy isoproteins have different capacities for amylopectin structure formation during wheat grain development; Wx-B and Wx-D being the most effective during early grain development, while Wx-A being active as grain filling starts, followed by Wx-D during grain maturation.

4. Conclusions

Endosperm development is a complex process involving numerous physiological and biochemical transitions. Starch granule morphology continuously changed with each phase of development, resulting in consistent starch and amylose accumulation in non-waxy and partial waxy genotypes. Changes in amylopectin structure were observed until 12 DPA, suggesting the formation of a basic amylopectin skeleton by the grain filling stage. Various waxy isoproteins affected amylopectin structure differently during wheat grain development. In the early stages, Wx-B and Wx-D were the most effective, followed by Wx-A during the mid grain-fill stage and Wx-D during grain maturation. Thus, genome specific GBSSI in wheat considerably affects starch accumulation and glucan chain length distribution during grain development.

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