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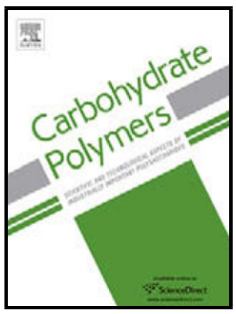

Title: Molecular structural differences between maize leaf and endosperm starches

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Molecular structural differences between maize leaf and endosperm starches

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Highlights

- Leaf starch has much shorter amylopectin chains than endosperm starch
- Leaf starch has much smaller molecules than endosperm starch
- Leaf starch granules are smaller than endosperm granules
- Differences between leaf and endosperm starch related to their biosynthesis process

Abstract

The morphology, whole molecular size distribution and chain-length distribution of maize leaf starch have been characterized and compared to its endosperm starch, to better understand differences between leaf and endosperm starch structure, and the relationship with the functions of starch in these organs. Leaf starch is found to have amylopectin with much shorter chains (virtually none with a degree of polymerization, DP, above 70) than the endosperm amylopectin, which has significant numbers of chains with DP up to ~120, and has much smaller molecular size (and is present at a much lower amount) than endosperm starch. It is postulated that these pronounced differences arise from the distinct starch synthesis pathways in these organs, and are consistent with the starches' distinct botanical functions: short-term storage requiring relatively rapid degradation for leaf starch, and high crystallinity and high energy density requiring slow degradation for endosperm starch.

Key words:

Leaf starch; structural characterization; molecular size distribution.

1. Introduction

Starch is divided into leaf starch (also termed transitory starch) and reserve starch (also termed storage starch). Reserve starch is present in storage organs for supplying energy during, for example, seed germination; leaf starch undergoes day-time synthesis and night-time degradation for the supply of sugars to sustain the metabolism of the whole plant and for export to sink organs throughout the night. Impairment of leaf starch structure affects the growth rate, grain yield and also the fruit growth and seed composition of the plants (Andriotis, Pike, Schwarz, Stephen, Wang & Smith, 2012; Yandeau-Nelson, Laurens, Shi, Xia, Alison & Guiltinan, 2011).

The focus of the present paper is to compare the structural characteristics of leaf and endosperm starch, and relate any observed difference to the different biological functions of leaf and reserve starch.

Both leaf and reserve starch have multiple levels of structure (Gilbert, 2011; Zeeman, Smith & Smith, 2004). Those considered here are the distribution of lengths of individual starch chains (first level), the branching structure of amylose molecules and amylopectin molecules (second level) and finally the granular morphology (fifth level). The individual branches of amylopectin, intertwining as helices, can form clusters (third level) and further alternating layers of crystalline and amorphous lamellae (fourth level). They are facilitated by a group of core enzymes, along with a few others. Starch synthase (SS) is responsible of forming linear chains with α-(1 → 4) glycosidic linkages by adding glucose units to glucan chains' non-reducing ends, while starch-branching enzyme (SBE) family, including SBEI, SBEII in plants, breaks an α-(1→4) glycosidic linkage of a glucan chain, then transfers the chain to attach another branch with an α-(1 → 6) branching linkage. SBEI transfers glucan

chains with DP 10 – 13, while shorter chains, DP 6 – 14, are transferred by SBEII (Kuriki, Stewart & Preiss, 1997; Nakamura, Utsumi, Sawada, Aihara, Utsumi, Yoshida & Kitamura, 2010). In plants, there are two SBEIIs, SBEIIa and SBEIIb, with distinct characteristics. In terms of expression patterns, SBEIIb with preference for glucan-length transfer in the range DP 6 – 7 is only expressed in storage tissue, while SBEIIa has a wider range of glucan-transferring length (DP 6 – 15) and is globally expressed in all the plant's tissues. Debranching enzyme (DBE) functions to trim widely-spaced chains, which would otherwise interfere with crystallinity. All these enzymes, including their isoforms, are critical to the plant physiology by influencing both leaf and reserve starch structures (Andriotis et al., 2012; Fujita, Yoshida, Kondo, Saito, Utsumi, Tokunaga, Nishi, Satoh, Park, Jane, Miyao, Hirochika & Nakamura, 2007; Mizuno, Kawasaki, Shimada, Satoh, Kobayashi, Okumura, Arai & Baba, 1993; Yandeau-Nelson et al., 2011).

Relatively little characterization has been conducted on the lower structural levels of cereal-plant transitory starch. Most work has focused on *Arabidopsis*, e.g. (Pfister, Lu, Eicke, Feil, Lunn, Streb & Zeeman, 2014), especially an elegant new study on crystallinity properties (Zhu, Bertoft, Wang, Emes, Tetlow & Seetharaman, 2015). However, *Arabidopsis* studies do not enable a comparison with the same plant's storage starch. Many studies used high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) for the characterization of the chain-length distribution (CLD) of leaf starch but this can only give information up to ~DP 50 and also suffers from a mass bias, which is very laborious to correct (Pfister et al., 2014; Wong & Jane, 1997). Fluorophore-assisted carbohydrate electrophoresis (O'Shea, Samuel, Konik & Morell, 1998) (FACE) is regarded as the best technique for the characterization of starch chains (first level) below degree of polymerization

(DP) of 180 (Wu, Li & Gilbert, 2014a) and thus is very useful in terms of revealing structural differences for longer amylopectin long chains as well as avoiding the mass bias of HPAEC. Quantitative labeling of the linear oligosaccharides with APTS has been proven to be size independent up to DP ~135 (O'Shea, Samuel, Konik & Morell, 1998); the method used here (Wu et al., 2014a) differs in the extraction technique from that of O'Shea and co-workers but the actual labeling step is the same. This technique has not previously been applied to the characterization of the CLD of leaf starch. Size-exclusion chromatography (SEC, which is a type of gel-permeation chromatography, GPC) is currently the most readily available and most developed technology for the size separation of branched polysaccharides and thus obtaining their size distribution (second level), and it can also characterize the full range of lengths of starch chains (first level), including the amylose chains. However, it suffers from band-broadening and shear scission effects, which can significantly affect the shape of a distribution (Gilbert, 2011).

In this study, a combination of SEC and FACE was used for the characterization of the first two levels of structure of leaf starch from B73 maize plants. Leaf starch granular morphology was also observed using scanning electron microscopy (SEM). These structures were compared to those of the corresponding endosperm starch to better understand the structural differences of leaf and storage starch in fulfilling their functionalities.

Amylose CLD is not considered here, as the method used for extracting starch from leaves cannot exclude contamination from small molecules with a hydrodynamic radius at ~ 5 nm because of a very low starch content in leaves. This contamination has been shown to elute in the amylose region in both the SEC weight distributions of whole starch molecules and the chain-length distributions (Li, Godwin

& Gilbert, 2015a; Syahariza, Li & Hasjim, 2010). Method optimization on starch extraction from leaves would be needed to overcome this problem.

2. Materials and methods

2.1. Plant material and growth conditions

The leaves and grains from B73 maize were provided by the National Key Laboratory of Crop Genetic Improvement, Huazhong Agriculture University, Wuhan, China. The plants were grown in the experimental (outdoor) field of Huazhong Agriculture University in the autumn of 2015. The leaf samples from the maize plants at the bell stage were harvested at 7 pm to obtain the highest starch content: daytime started at 5 am and ended at 7 pm. One leaf was collected from each maize plant for each time point (4 h intervals) over 36 h, flash-frozen in liquid nitrogen, and stored at –80 °C. All the samples of leaves were removed 3 cm from the tip of leaves to exclude the senescent part. There were 9 leaves from each maize plant harvested, and totally 45 leaves from 5 different maize plants (same status) were collected.

B73 maize grains were harvested from the same batch of plants after the grains were fully mature and the leaves were completely dried (the end of postulation period), then dehulled and frozen in liquid nitrogen before being ground into fine flour using a cryo-grinder (MM 400, Retsch GmbH, Haan, Germany) at 15 s^{-1} for 2 min. Leaf samples were freeze-dried overnight before the grinding using the same setting as for grains.

2.2. Starch Content

The starch content from the ground powder was determined using the ‘Total starch AOAC Method 996.11/AACC Method 76.13’ kit from Megazyme International Ltd.

(County Wicklow, Ireland) following the protocol recommended by the manufacturer for samples containing D-glucose and/or maltodextrins.

2.3. Scanning Electron Microscopy

Leaf starch isolated for SEM was extracted from dry leaf powder as described in elsewhere (Dinges, Christophe, James & Myers, 2003) with slight modifications. 1 g ground leaf powder was dispersed in 5 mL of extraction buffer, which contains 500 mM 3-(N-morpholino)-propanesulfonic acid (pH 7.5), 5 mM EDTA, and 10% ethylene glycol. The whole suspension was then homogenized and filtered through Miracloth (Calbiochem) and centrifuged at 5000 g for 10 min. The pellet was suspended in 0.2 mL of extraction buffer and 1.8 mL of Percoll (catalog no. 17-0891-02; Amersham Biosciences, Piscataway, NJ). The Percoll gradient established by centrifugation at 10000 g for 30 min yields a starch pellet largely free of other cell components. The pellet, after washing by ethanol three times, was finally dried in a 30 °C drying oven overnight.

The maize grain flour and the extracted leaf starch were thinly spread onto circular metal stubs covered with double-sided adhesive carbon tape and sputter-coated with gold, and examined by SEM (model SU8010, HITACHI, Japan) at an accelerating voltage of 5 kV for grain flour and 3 kV for leaf starch. Multiple micrographs of each sample were examined at multiple magnifications and typical representative images were selected.

2.4. Starch Extraction and Size-exclusion Chromatography

The method for preparing starch samples for SEC was described elsewhere (Li et al., 2015a; Syahariza et al., 2010; Wu et al., 2014a). In brief, protein was removed as much as possible from the samples by using successive treatments by protease and

sodium bisulfite solution. The other non-starch components were removed by dissolving the samples in dimethyl sulfoxide (DMSO) and then precipitated by ethanol. The purified starch was finally dissolved in DMSO containing 0.5% (w/w) LiBr (DMSO/LiBr) at a concentration of 2 mg/mL, for SEC analysis.

The SEC weight distribution of whole branched starch molecules is the weight of chains as a function of the SEC size separation parameter, the hydrodynamic radius R_h. This quantity, denoted $w(\log R_h)$, was characterized using an Agilent 1260 infinity SEC system (Agilent, Santa Clara, CA, USA) with a refractive index detector (Optilab UT-rEX, Wyatt, Santa Barbara, CA, USA) following a previously described method (Cave, Seabrook, Gidley & Gilbert, 2009; Vilaplana & Gilbert, 2010b). A combination of a GRAM pre-column, GRAM 30 and GRAM 3000 columns (Polymer Standard Service, Mainz, Germany) were used for separation in a column oven at 80°C, and the starch molecules were eluted using DMSO/LiBr solution at a flow rate of 0.3 mL/min. Universal calibration was used to convert SEC elution time to R_h (Vilaplana & Gilbert, 2010a). This was implemented with pullulan standards (Polymer Standards Services, Mainz, Germany) with a molecular weight range of 342 – 2.35×10^6. The SEC weight distribution $w(\log R_h)$ gives (within arbitrary normalization) the total weight (not molecular weight) distribution of molecules as a function of $\log R_h$.

2.5. Fluorophore-Assisted Carbohydrate Electrophoresis (FACE)

Starch was extracted as described above and debranched using isoamylase following a previous method (Hasjim, Lavau, Gidley & Gilbert, 2010). The freeze-dried debranched starch was labeled using 8-aminopyrene-1,3,6,trisulfonic acid (APTS) following a procedure described elsewhere (Wu et al., 2014a), and then

separated with a carbohydrate separation buffer (Beckman-Coulter) in an N-CHO-coated capillary at 25 °C using a voltage of 30 kV. The debranched amylopectin number CLD, $N_{de}(X)$, which is the relative number of chains as a function of their degree of polymerization X, was characterized using a PA-800 Plus FACE System (Beckman-Coulter, Brea, CA, USA), coupled with a solid-state laser-induced fluorescence (LIF) detector and an argon-ion laser as the excitation source (Wu et al., 2014a).

3. Results

3.1. Starch Content

Leaf had much lower starch content (6.7%) than the endosperm (65.3%) based on dry ground powder weight. These leaves were sampled at the end of the day (7 pm) in order to obtain a high starch content. Recall that in leaves, starch is accumulated during the day and degraded during the night, while endosperm starch is accumulated over about two months until the seeds become mature, which leads to the much higher amount of starch in endosperm than in leaves.

3.2. The morphology of the starch granules from endosperm and leaves

The morphologies of maize endosperm and leaf starch granules were found to be quite different (Fig **1**). Endosperm starch granules had many pores on the surface while leaf starch has no pores (Fig **1**). Endosperm starch has polyhedral granules with much bigger size (~10 μm) than its leaf starch (~2 μm) which has lenticular granules. The much smaller granular size of leaf starch might be because leaf starch is formed on a diurnal cycle, and the total time available for a starch granule to grow is much less than that in endosperm starch.

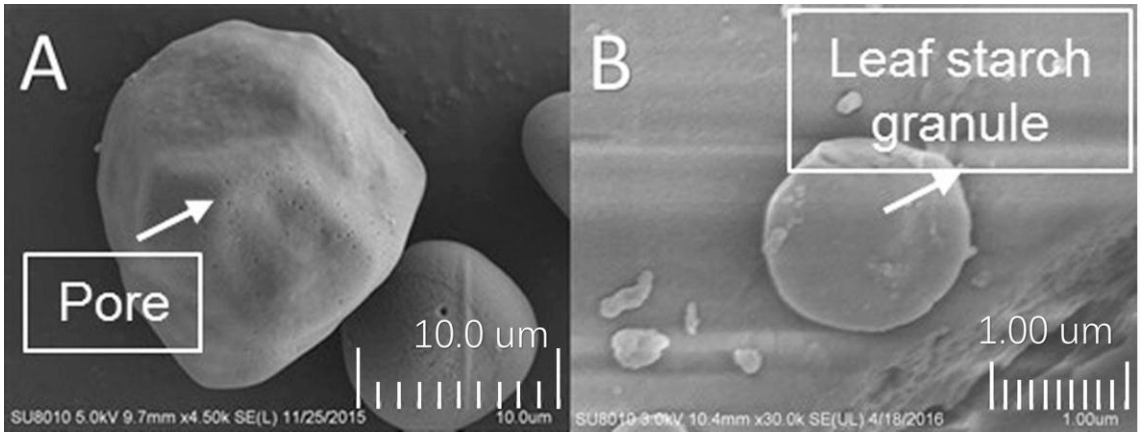

Figure 1. Morphology of starch granules from B73 maize endosperm (A), in which the scale bar is 10 μm, and B73 maize leaves (B), in which the scale bar is 1 μm. Pores are visible on the surface of the endosperm starch granules.

3.3. Size distributions of whole starch molecules from endosperm and leaves

SEC weight distributions of whole starch molecules from endosperm and leaves, as functions of molecular size, are shown in Fig **2**. Both distributions were normalized to the same maximum for comparison of the relative amount of large and small starch molecules. The molecules with R_h < 10 nm are considered to contain non-starch components, as suggested by previous studies (Li et al., 2015a; Syahariza et al., 2010). The contaminated component is separated from that containing only starch by a dashed line in the figure. The much higher contamination ratio in the leaf starch molecules is because of the very low starch content in leaves; however, the presence of this artifact has no effect on our overall conclusions.

For endosperm starch, there are the usual two peaks corresponding to amylose (peak R_h ~20 nm) and amylopectin (peak R_h ~130 nm) in Fig **2**, whereas there is only one peak (peak R_h ~ 35 nm) for leaf starch (except for the contamination peak). This has also been observed previously in sorghum (Li et al., 2015a). The smaller granular

and molecular sizes in leaf starch, compared with those in endosperm starch, might sterically inhibit the growth of amylopectin chains. Granule size has been observed to have some correlation with molecular structure (Dhital, Shrestha, Hasjim & Gidley, 2011; Kalinga, Bertoft, Tetlow, Liu, Yada & Seetharaman, 2014; Li, Liu, Liu, Godwin & Gilbert, 2015b). It may be the case that smaller granular and molecular sizes supply less binding sites for the starch-biosynthetic enzymes and results in shorter chains being synthesized. For example, in sorghum, it has been found that leaf starch has much smaller granular size and molecular size than endosperm starch, and the molecular size increases towards the outer parts of the granules, which could supply more binding sites for starch-biosynthetic enzymes (Li et al., 2015a; Li et al., 2015b). The factors determining the granular size and molecular size are unknown, but as suggested elsewhere (Li et al., 2015a), are related to the functions of leaf and endosperm starch. The diurnal growth pattern of transient starch means that the total time available for a starch molecule to grow is much less than that in storage starch, which could also contribute to the smaller molecular size of transient starch. Leaf starch mainly provides sugar to support the plant at night after daytime accumulation (Gibon, Bläsing, Palacios-Rojas, Pankovic, Hendriks, Fisahn, Höhne, Günther & Stitt, 2004).

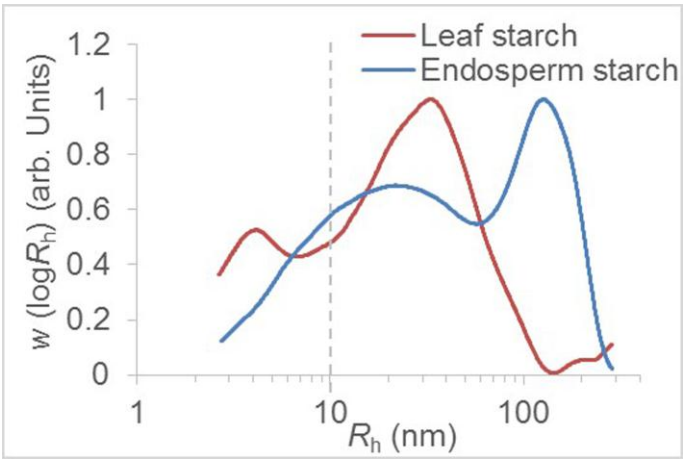

Figure 2. SEC weight distributions, $w(\log R_h)$, of whole starch molecules extracted from B73 maize endosperms and leaves. The distributions have been normalized to the maximum. The dashed line at R_h = 10 nm is used to separate the starch part from the contamination component. The data shown here are from one representative experiment chosen from multiple experiment repeats. All experiments showed the same trend. Fig. A in the Supporting Information gives the other experimental repeats.

3.4. CLDs of endosperm and leaf amylopectin

The CLDs of amylopectin chains were obtained using FACE. Fig **3** shows these as number distributions normalized to the global maximum. A number of obvious features can be observed in the endosperm starch, similar to what has been reported many times for rice and other starches. The first one is the global maximum at DP 12, with the peak covering DP 6 – 33; this is from the enzyme set producing chains spanning a single lamella (French, 1972; Nikuni, 1978). There is then a small shoulder or maximum at DP ~ 22, which corresponds to the component from a second enzyme set in this region. The second peak is from DP 34 – 67, with a local maximum at DP ~43 (Hizukuri, 1986; Mua & Jackson, 1997; Wang & White, 1994). This peak represents intermediate amylopectin chains spanning two crystalline lamellae. The chains from DP 68 – 100 span through at least three crystalline lamellae, and are termed B_3 or three-lamellae spanning chains (Wu, Morell & Gilbert, 2013). Chains

longer than DP 100 are normally assigned as amylose chains, although there are almost certainly a small proportion of extra-long amylopectin chains with lengths similar to the shortest amylose chains in this region (Horibata, Nakamoto, Fuwa & Inouchi, 2004; Matsugasako, Takeda, Yamazaki, Itoh, Kuratomi, Hanashiro & Igarashi, 2008).

As discussed below, the same basic features are observed for leaf starch, but the amount present for higher DPs (DP > 70) is so small that there is poor signal:noise.

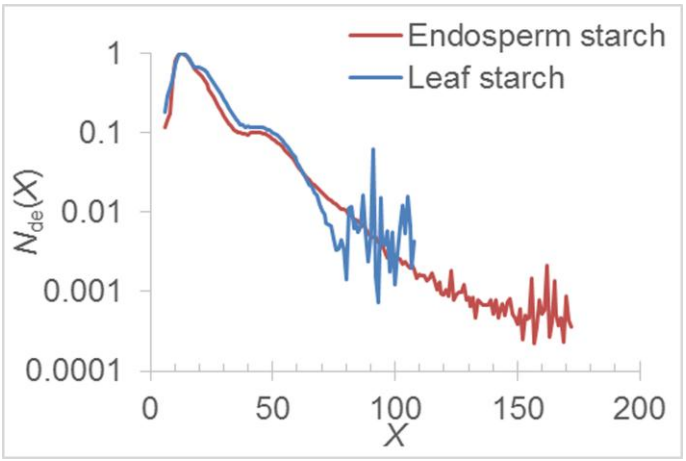

Figure 3. The number CLDs, $N_{de}(X)$, of B73 leaf starch (blue) and endosperm starch (red), obtained from FACE. All distributions were normalized to yield the same global maximum. (See Fig. B in Supporting Information for the other experimental repeats). FACE resolves individual DPs, the points for which are joined here with a line for visual ease. Note that the Y axis is logarithmic.

4. Discussion

Comparison between CLDs is best seen with a logarithmic Y axis, as shown in Fig. 3, because such plots bring out features which are often not apparent in the commoner ways of presenting as number or as difference distributions (Castro, Dumas, Chiou, Fitzgerald & Gilbert, 2005). The normalization of a CLD is arbitrary, and for convenience those in Fig. 3 are normalized to have the same maximum. It is essential to avoid “conclusions” which are in fact artifacts of the way one decides to normalize

a distribution (Castro et al., 2005); such artifacts are unfortunately not uncommon in the literature when using simple difference plots. Now, the best way of making a comparison between different CLDs is by fitting to the biosynthesis-based model for this (Wu & Gilbert, 2010; Wu et al., 2013; Wu, Ral, Morell & Gilbert, 2014b), to find differences between fitting parameters for the biosynthesis processes and the underlying genetics. However, in the present case, α- and β-amylases play a role in the biosynthesis and degradation cycle for transitory starch and the fitting is much more complex (Wu et al., 2014b), whereas they are not directly significant for storage starch (Wu et al., 2014b).

We adopt here a simpler approach. An obvious difference between leaf and endosperm starch is that there are only significant amounts of leaf starch chains up to ~ DP 70, which is much lower than that of endosperm starch (~DP 120). Now, care is required with such a conclusion, because this could either be because leaf starch has more chains at DP < 70 or has fewer > 70; however, the former is not the case in the present instance, because in fact the poor signal: noise ratio for DP > 70 for leaf starch is simply because there are so few chains in this region. Because of the limited length of the amylopectin seen in leaf starch, higher level structures (here crystallinity and crystalline-amorphous lamellae) should be different from those in native endosperm starch (Witt & Gilbert, 2014). Amylopectin chains in native starch can be classified into three types, A, B, and C chains, according to the lengths and branching patterns (Peat, Whelan & Thomas, 1952). The A chains are the group of outer chains with no branches attached (Hizukuri, 1985, 1986), while B chains are the inner chains with few branches attached, which can be further divided into B1, B2, B3 and B4 chains based on the number of clusters they traverse, which depends on their chain length (Tester, Karkalas & Qi, 2004). The DP of B1, B2, B3 and B4 chains are commonly

around 20 – 24, 42 – 48, 69 – 75 and 101 – 109 (Hizukuri, 1986; Mua & Jackson, 1997; Wang & White, 1994). It has been suggested that long B-chains do not influence the lamellar thickness, and that only the short B1-chains (and A-chains) are found in the crystalline lamellae (Bertoft, 2013; Witt & Gilbert, 2014). There is only one C chain for each amylopectin molecule, which is the chain with the reducing end. Within the semi-crystalline structure of starch, there are three polymorphisms, i.e. A-, B-, and C- types of crystalline structures. A type has monoclinic unit cells, while hexagonal unit cells are the feature of B type. C type is a mixture of monoclinic unit and hexagonal unit cells (Buleon, Colonna, Planchot & Ball, 1998; Cairns, Bogracheva, Ring, Hedley & Morris, 1997; Zobel, 1988). In general, amylopectin molecules of A-type starches have shorter chains in both the long- (DP > ~33) and short-chain (DP < ~33) fractions and larger amounts of the short-chain fractions than those of the B-type starches (Hizukuri, 1985). The chain lengths of amylopectin of the C-type starch are intermediate. The absence of long B chains observed in the present study, due to negligible amounts of amylopectin chains over DP 70, could thus have the potential to result in a different type of crystallinity from that of the endosperm starch, while the broader chain distribution of short chains (DP 6 – 40) would produce a thicker semi-crystalline lamella compared to endosperm starch. It would be of interest to measure the type of crystallinity and the lamellae thickness by X-ray diffraction to prove this hypothesis. However analysis of starch by this method requires considerable amounts of starch (typically ~0.1 g) and was beyond the scope of the present study, as leaves yield low quantities of starch.

The difference of CLDs between endosperm starch and leaf starch must arise from the distinct starch synthesis pathway in leaves. Soluble starch synthase (SSS) is the only enzyme that can elongate amylopectin branches in vivo (Ball & Morell, 2003).

Different SSS isoforms have been proved to be responsible for elongating starch branches with different chain lengths, e.g. SSSI has highest activity for chains with DP 6 – 12 (Fujita, Yoshida, Asakura, Ohdan, Miyao, Hirochika & Nakamura, 2006), lack of SSSII is associated with a deficiency of chains with DP 12 – 30 (Umemoto, Yano, Satoh, Shomura & Nakamura, 2002), while SSSIII plays a role in elongating longer chains with DP ≥ 30 (Fujita et al., 2007). A different expression pattern of SSS between leaf and grain might account for the CLD difference. Biochemical studies have shown that the starch biosynthetic enzymes have substrate specificity: for example, SSS cannot work at very high DP and SSSI is said to elongate very short glucan branches ($4 \leq DP \leq 7$), and the elongated short chains are subsequently elongated by SSSII (Tetlow, 2011). Transitory starch is mostly located in chloroplasts, a different physiological environment from that of the storage starch, which could result in the CLD difference. One, admittedly speculative, possibility is that if crystallization in leaf starch is slow compared to the rate of chain propagation, then a growing chain is "seen" by SSS as longer chains and so cannot propagate.

Steric hindrance might be another determinant of chain length. In sorghum, leaf starch has much smaller granular size and molecular size than endosperm starch, and the molecular size increases towards the outer parts of the granules (Li et al., 2015a; Li et al., 2015b). The smaller granular size and molecular size in leaf starch, compared with the sizes in endosperm starch, might sterically inhibit the growth of amylopectin chains. Granules of different sizes have been observed to have different molecular structure (Dhital et al., 2011; Kalinga et al., 2014; Li et al., 2015a) and different molecular size could also result in different chain length. It could be rationalized that smaller granular and molecular size supply less binding sites for the starch-biosynthetic enzymes and results in shorter chains being synthesized. For

example, in sorghum, it has been found that leaf starch has much smaller granular size and molecular size than endosperm starch, and the molecular size increases towards the outer parts of the granules, where there could be more binding sites for starch-biosynthetic enzymes (Li et al., 2015a; Li et al., 2015b). The factors determining the granular size and molecular size, as suggested elsewhere (Li et al., 2015a), are related to the functions of leaf and endosperm starch.

Leaf starch has a broader distribution of chains from DP 6 to 40 than that in the endosperm (Fig. **4**). This might be related to the different SBE expression pattern between these two organs. SBEIIb is usually specifically expressed in the endosperm, while SBEIIa is present in all tissues and appears to be relatively unimportant in endosperm (Mizuno et al., 1993; Nakamura, 2002). In vitro biochemical studies show that SBEIIa transfers a wide range of short chains with DP 6 – 15 while SBEIIb almost exclusively transfers chains of DP 6 and 7 (Nakamura et al., 2010). An increase in the number of amylopectin chains around DP 20 – 60 could mean thicker crystalline-amorphous lamellas in the leaf-starch granules (Witt et al., 2012; Witt & Gilbert, 2014), as these chains consist of A and B1 chains, which according to the starch backbone model (Bertoft, 2013) are the chains found in the crystalline lamellae.

It is seen in this study that leaves tend to have smaller starch granules and molecules than endosperm, and that the overall lengths of leaf amylopectin chains are much shorter than the endosperm amylopectin chains, as the chains with DP over 70 are of very low amounts. These differences must be favorable for fulfilling the functionalities of starch in the plant. While starches in leaf and grain both function as glucose storage reservoirs, for leaves, it is short-term glucose release, while for endosperm, it is long-term release, requiring high levels of crystallinity for compact and robust energy storage.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http:// …

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