Starch and Starch-Associated Proteins: Impacts on Wheat Grain Quality

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Abstract Wheat storage proteins have been historically examined and periodically established to be the major determinant of wheat quality. Gluten proteins largely contribute to the formation of viscoelastic network in a dough, enabling processing of wheat to food products including bread. More recently starch, the major component constituting 60–70% of wheat grain, is understood to play key roles in flour quality, dough functionality and end product and nutritional quality. Starch is composed of two neutral macromolecules of glucose, amylose and amylopectin. The structural differences between amylose and amylopectin are predominantly dependent on the extent and distribution of α-1,4 and α-1,6 linkages that connect the glucose units to form these two polymers. The functional properties of starch as governed by its structure, molecular organisation, granule morphology and size distribution influence dough behaviour during processing, differentially impacting the end product qualities. Also, varyingly important are the roles of starch granule associated proteins, comprised of both surface proteins and granule-integral proteins with enzyme functions, in driving starch responses in a complex dough matrix system. This chapter aims to provide an extensive review on how starch, associated proteins and starch-protein interactions influence functional properties of food systems.

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G. Igrejas et al. (eds.), *Wheat Quality For Improving Processing And Human Health*, https://doi.org/10.1007/978-3-030-34163-3_3

1 Introduction

Our planet occupies a myriad of edible plants. Amongst these, more than 50% of energy intake by humans is from the three so called mega crops, rice, wheat and maize. Ancient domestication, wider geographic and climatic adaptability, and versatility in grain utilization have made wheat the most widely grown crop and the most traded cereal in the world. For more than a third of the world's population wheat is a staple food. Wheat primarily laid the foundation for most of the foods enjoyed by humans over centuries across the globe. Wheat flour is an essential ingredient for an umpteen number of food products developed to suit the diverse taste buds of human race. It inherently possesses quality attributes (that are also constantly being improved through breeding) that enable processing to produce a range of products including, but not limited to breads, cakes, cookies, biscuits, pastries breakfast cereals and noodles. Wheat quality attributes required for different products are different and generally, wheat is categorised into different classes to reflect the different end use quality. The classification standards followed by different countries vary and these are generally dependent on various parameters such as protein content, morphological features, milling and end use quality and the region and season of growing.

With globalisation and diversification of diets, wheat based product consumption has spread widely across the world to a large extent. Moreover, innovative products are being developed to suit the fast changing lifestyle and convenience of the growing population. While the primary breeding target for wheat improvement continues to be yield enhancement, increasing demand is there to incorporate targets for grain quality trait enhancement in breeding program to cater for unique and distinct quality feature requirements for various types of wheat based products. Understanding the influence of each of the grain components on quality is essential to drive the efforts on improving wheats for superior quality. In this chapter, we discuss how starch, the major component of wheat grain, is synthesised, the impact of altered starch biosynthetic pathway on starch structure and functionality, the influence of starch granule associated proteins on quality, and the impact of abiotic stresses on starch properties.

2 Starch Biosynthesis in Wheat

The quantity and structure of starch synthesised and deposited in plant tissues are dictated by the participating enzyme machinery. The process of starch synthesis fundamentally involves; (1) provision of carbon flux to produce ADP glucose, the precursor molecule of starch, (2) building linear glucan chains with α-1,4 linkages (3) creating α-1,6 branches by cleaving specific portions of the linear chains and re-attaching at specific intervals along the chains and (4) removal of excess branches to generate the semi-crystalline insoluble homopolymer of starch. While there is a significant level of redundancy in the nature of enzymes involved in the synthesis of both transitory starch contained in the vegetative tissues and reserved starch deposited in storage tissues such as the wheat grain endosperm, fine differences exist in the biosynthesis of the two. As the focus of this book is on wheat grain quality, this chapter will cover only starch synthesis in the endosperm of wheat grain.

Supply of a hexose phosphate (glucose 1-phosphate (G1P) being the preferred) is important for starch synthesis to take place (Emes et al. 2003; Keeling et al. 1988; Zi et al. 2018). This supply comes from the initial conversion of sucrose, the widely transported photoassimilate in wheat, into uridine diphosphate glucose (UDPG) and fructose by sucrose synthase (SUS: EC 2.4.1.13). G1P is subsequently synthesised from these carbon precursors, catalysed by enzymes such as UDP-glucose pyrophosphorylase, hexokinase and phosphoglucomutase. A recent study on waxy wheat has highlighted the importance of sucrose conversion for starch synthesis, through the demonstration that the low kernel weight and total starch accumulation of the waxy wheat is caused by the reduced conversion of sucrose to starch in the late grain filling stage (Zi et al. 2018).

Starch is committed to be synthesised when G1P is converted to adenosine diphosphate glucose (ADPG) by ADPG pyrophosphorylase (AGPase). AGPase is a heterotetrameric protein complex with two small subunits and two large subunits with mostly catalytic and regulatory functions respectively. Wheat grain has AGPase present both in the cytoplasm and in the amyloplast, however the cytosolic isoform accounts for >90% of activity (Burton et al. 2002; Emes et al. 2003; Tetlow and Emes 2017). Evidence suggests that different genes encode the large and small subunits present in the cytosol and the amyloplast in wheat endosperm (Burton et al. 2002; Zhang et al. 2017b). AGPase is commonly found to be allosterically regulated, with activation by 3 phosphoglyceric acid (3PGA) and inhibition by inorganic phosphate (Pi) (Preiss et al. 1991). However the level of sensitivity to such regulation varies depending on the plant species and the enzyme localisation within the plant tissues. AGPase encoding genes are associated with grain attributes such as thousand kernel weight, total starch content and yield (Batra et al. 2017; Hou et al. 2017). Allosterically insensitive AGPase potentially could hasten starch accumulation and grain filling in wheat and help mitigate yield reduction under high temperature stress (Kaur et al. 2017).

As most of the glucosyl donor for starch synthesis comes from extraplastidially synthesised ADPG, there remains the need for transporters to transfer the ADPG from the cytosol into the amyloplast where most other starch biosynthetic enzymes are located, ready to activate the remaining stages of starch synthesis. ADPG transporters in maize (ZmBT1) and barley (HvNST1) are characterised and the respective defective proteins led to reduced uptake of ADPG into the amyloplast (Cao and Shannon 1997; Patron et al. 2004). Kinetic properties of a wheat ADPG transporter have also been characterised from isolated amyloplast (Bowsher et al. 2007).

The ADPG thus transported into the amyloplast now becomes the source of glucose moiety to be attached to the non-reducing end of a glucan primer through α-1,4 linkage resulting in the extension of glucan chains. This reaction is catalysed by a set of single polypeptide enzymes known as starch synthases (SSs) belonging to the glycosyl transferase family. SSs fall into two groups, one localised more or less exclusively to the starch granule matrix known as granule bound starch synthase (GBSS) or waxy protein and the other set present in the endosperm in a soluble form in the stroma as well as in starch granule, loosely termed as soluble SSs. Multiple isoforms are detected for most starch synthases however not all of them are involved in endosperm starch synthesis. GBSS is critical for synthesising amylose, although involvement of other enzymes such as starch branching enzyme (SBE) are indicated in some plant species (Regina et al. 2012). On the other hand, soluble SSs are involved in the synthesis of amylopectin. In wheat endosperm there are four types of soluble SSs, SSI, SSII, SSIII and SSIV, out of which the first three are known to be catalysing amylopectin chain elongation (Konik-Rose et al. 2007; Kosar-Hashemi et al. 2007; Li et al. 2011; Li et al. 1999; Li et al. 2000). Each of the SSs involved in chain elongation have specificity in the length of the chain it synthesises. While SSI generates shorter chains of ~8 to 12 degree of polymerisation (DP), optimal preferences of SSIIa and SSIIIa (the SSII and SSIII isoforms in wheat endosperm) are to produce much longer chains of ~11 to 30 DP and > 50 DP respectively (McMaugh et al. 2014). SSIV is functionally assigned to priming of starch granule formation influencing the number and size of the starch granules, rather than any involvement in the chain elongation, according to studies in other plant species and in wheat chloroplast (Guo et al. 2017a; Roldan et al. 2007). An extensive bioinformatics on SSs provided insight into sequence specificities that underscore the unique catalytic features and functional differences of various SSs in wheat (Leterrier et al. 2008).

Both amylose and amylopectin components of starch are branched molecules, although the frequency of branches are approximately six times more in amylopectin compared to amylose. Hence amylose is more often referred to as ‘more or less a linear glucan’. Starch branching enzymes (SBEs) catalyse the building of α-1,6 linkages through cleavage of an internal α-1,4 chain and attaching the released reducing end to C6 hydroxyl group of a chain, thus converting linear chains into a branched structure. SSs then act on the non reducing ends created by SBEs to further elongate the chains, resulting in the growth of the molecule (Tetlow and Emes 2017). Two broad types of SBEs are present in cereals including wheat, SBEI and SBEII. In wheat endosperm, only one isoform of SBEI is detected while there are two isoforms of SBEII, SBEIIa and SBEIIb. The two classes of SBE enzymes functionally differ in cereals with regard to the length of the chains they transfer, the minimum chain length requirement to create a branch and the substrate affinity (Morell et al. 1997). SBEI prefers to act on longer chains than SBEII and has more affinity to amylose, while amylopectin is the preferred substrate for SBEII. Also, the two classes temporally differ in their time of expression during grain development and also spatially differ in their localisation within the amyloplast. In wheat SBEI is more expressed towards the later stage of endosperm development (>20 days post anthesis (DPA)), while SBEII is highly expressed earlier in the developmental stage (<15DPA) (Morell et al. 1997; Regina et al. 2005). Amongst the two isoforms of SBEII, there is 2–3 times more SBEIIa in the amyloplast stroma of wheat than SBEIIb, unlike in maize and rice where there is a predominance of SBEIIb over SBEIIa in the endosperm. SBEII in cereals, in general, plays more substantial roles than SBEI as revealed by studies in mutants of these enzymes (see following section of this chapter). A third class of SBE, SBEIII is also detected in wheat (as in some other plant species), which is constitutively expressed during the whole grain filling period. The function of SBEIII is not very clear, although an association with the synthesis of A and B starch granules is speculated (Kang et al. 2013a).

Role of debranching enzyme in starch biosynthesis is mainly to trim excess branches formed in the growing amylopectin molecules to establish an organised semi crystalline structure to starch. Out of the two (isoamylase and pullulanase) types of debranching enzymes detected in plants, the isoamylase types act on amylopectin to remove unfitting α-1, 6 linkages. Functionally, ISA1 and ISA2 are the isoforms of isoamylase that are involved in amylopectin trimming either in homomeric or heteromeric complex forms, while a third isoform ISA3 is mostly involved in starch degradation (Kubo et al. 2010; Lin et al. 2013; Nielsen et al. 2002; Utsumi et al. 2011; Yun et al. 2011). Involvement of more enzymes in reserve starch biosynthesis (that are not covered here) such as starch phophorylase, disproportionating enzyme, glucan water dikinase and phospho glucan water dikinase are suggested, although their precise roles are yet to be defined.

Concerted action of the multiple isoforms of starch biosynthetic enzymes are essential in the temporal and spatial management of starch biosynthesis. Evidences of protein-protein interactions and protein complexes existing in starch synthesising organelles supports the need for enzymes to act collaboratively to regulate starch biosynthesis (Crofts et al. 2017; Fushan et al. 2012; Tetlow et al. 2008). Also more insights are emerging on the regulation of starch synthesis and accumulation influenced by transcription factors and enzymes outside the core pathway. For example, TaRSR1 (reduced sugar response 1) in wheat appears to negatively regulate most of the key starch biosynthetic enzymes including AGPase, SSs and SBEs (Kang et al. 2013b). Stimulation of starch synthesis and its accumulation by Trehalose 6-phosphate, the precursor of the disaccharide trehalose, has been more recently demonstrated in plants (Kolbe et al. 2005; Paul et al. 2018; Zhang et al. 2017a). The effect is mainly through the post translational redox dependent activation of AGPase, the rate limiting enzyme of starch synthesis. In wheat external application of precursors of T6P during grain filling stage has shown to increase the grain size and total starch content (Griffiths et al. 2016). TaGW2-6A, a weight related gene allele that encodes a functional E3 RING-type ubiquitin ligase, is recently speculated to affect starch-related genes promoting accumulation of starch (Geng et al. 2017).

Readers are also referred to recent review articles on starch biosynthesis for further insights and understanding (Crofts et al. 2017; Jeon et al. 2010; MacNeill et al. 2017; Regina et al. 2016; Tetlow and Emes 2017).

3 Impact of Starch Enzyme Mutations on Wheat Grain and Quality

Spontaneous occurrence of starch biosynthetic enzyme mutations in nature leading to a specific phenotype in wheat is hindered by the complex genetic make up of wheat. Being an allohexaploid by nature, most of the known genes encoding starch biosynthetic enzymes are triplicated with one copy present in each of the three genomes in wheat. The effect of mutation in any one of the three allelic forms will most often be masked by the functional redundancy of homoeologs, leading to lack of manifestation of a phenotypic effect in the grain. Multiple studies have been carried out to identify mutations in the wheat gene pool of starch synthesis enzymes. Probably, the waxy proteins have been the most intensively examined, with scores of studies describing lot of alleles in several wheat species (see Guzman and Alvarez 2016 for a review). Variability for SSII was also detected in wheat cultivars (Yamamori and Endo 1996) using SDS-PAGE gels. Simultaneous mutations of the three homoeo alleles of any specific gene to occur naturally is highly unlikely. The situation is more complicated with the presence of multiple isoforms for several of the starch biosynthetic enzymes (as detailed earlier). Lessons from diploid cereal species like maize, rice and barley led to speculative reflections on starch enzyme functionality in wheat; however traditional and advanced breeding and genetic techniques allowed further wheat specific insights on starch biosynthetic enzyme functionality and mutant phenotypes. These include, but not limited, induced mutagenesis using chemical mutagens such as ethyl methane sulphonate (EMS), sodium azide, followed by mutant detection and hybridisation, and transgenic technologies such as RNAi. High throughput allele detection techniques such as targeting induced lesions in genomes (TILLING) is increasingly being used in identifying allele variants in starch biosynthetic genes created through induced mutations (Regina et al. 2015a; Slade et al. 2012). The most modern techniques of targeted mutagenesis through gene editing (eg CRISPR/CAS9 mediated gene editing) is a powerful strategy for polyploid plants like wheat to create allelic variations (Uauy et al. 2017), and their use in modifying starch biosynthesis in wheat is optimistically a matter of time.

A short review on the impact of functionally impaired key enzymes in wheat starch biosynthetic pathway is presented in Table 1.

4 Starch and Nutritional Quality of Wheat

Glycemic carbohydrate (or available carbohydrate) is important for humans to support the normal functioning of several organs including brain, red blood cells and reproductive tissues (Hardy et al. 2015). Starch is the most abundant form of dietary carbohydrate and as such the main source of dietary energy. The most important functionality of starch that permits manipulation to serve a nutritional purpose is its digestibility. Approximately >99% of naturally occurring wheat starch is digestible and only the remaining very small proportion is resistant to digestion in the small intestine by human digestive enzymes (referred to as resistant starch (RS)). Within the digestible portion of starch, the rapidly digestible starch (RDS), the fraction that is digested within 20 min results in a rapid increase in postprandial blood glucose level, while the slowly digestible starch (SDS) that completely digests in the small intestine at a slower rate than RDS results in a sustained postprandial glucose level (Lehmann and Robin 2007). The relative proportion of RDS, SDS and RS in starch varies depending on the genotype, the form of the material containing starch (grain/flour/product) and the type of processing followed to transform the grain into a food product.

Table 1 Impact of impaired starch biosynthetic enzyme activity on wheat grain and quality

Target starch enzyme/s	Mode of impairment (transgenic/non transgenic)	Phenotype	Reference
AGPase	Non transgenic-mutation	Reduced total starch content	Guo et al. (2017b)
Isoamylase 1	Transgenic-down regulation	Reduced starch content, elevated phytoglycogen and beta-glucan, altered amylopectin chain length distribution, disrupted semi crystalline structure	Sestili et al. (2016)
GBSS1 (waxy wheat)	Non transgenic-mutation	Low to zero amylose, A-type X-ray diffraction pattern, higher crystallinity, lower gelatinisation temperature, higher peak viscosity and swelling power (lower peak viscosity and setback in some studies), higher resistance to retrogradation, higher flour water absorption, lower dough stability, desired for high quality white-salted noodles. Null4A GBSS desired for Udon noodles	Ahuja et al. (2013); Hayakawa et al. (1997); Hoshino et al. (1996); Miura et al. (1994); Nakamura et al. (1993a); Nakamura et al. (1993b); Yamamori et al. (1995)
GBSS1 & SSIIa (sweet wheat)	Non transgenic-mutation	Elevated maltose, sucrose and fructan, small and misshapen starch granules, altered crystallinity, lowered gelatinisation temperature, lower molecular weight for amylopectin, increased malt oligosaccharides and very short chains of DP 2 and 3	Nakamura et al. (2006); Shimbata et al. (2011); Vrinten et al. (2012)
SSIIa (SGP-1)	Non transgenic	Moderately elevated amylose content, higher protein content, lower starch content, increase in the amount of short amylopectin chains (6–10 DP) and decrease in intermediate chains of 11–25 DP, higher total dietary fibre, lower gelatinisation temperature, altered starch crystallinity, lower peak viscosity, lower kernel weight and flour swelling power, higher flour water absorption, lower bread loaf volume	Berky et al. (2016); Hogg et al. (2013); Hogg et al. (2017); Hung et al. (2006); Konik-Rose et al. (2007); Shimbata et al. (2005)
SBEII (predominantly SBEIIa, in combination with SBEIIb)	Non transgenic-mutation	Higher amylose, higher resistant starch, reduced total starch content, increased grain hardness	Hazard et al. (2012); Hazard et al. (2015); Regina et al. (2015a); Schonhofen et al. (2017); Sestili et al. (2015); Slade et al. (2012)
SBE 1	Non transgenic	No substantial alteration in any of the starch structural or functional properties	Regina et al. (2004)

Rapid digestion of starch is important whenever a rapid energy conversion is required, as in the cases of infant diets and addressing severe undernutrition. In the current era of increasing incidence of diet related non communicable diseases such as Type II diabetes, gut diseases and cardiovascular disorders, SDS and RS are of much nutritional value, due to their physiological ability to address some of these health conditions. SDS is the fraction of starch that is converted into glucose only after 120 min of enzymatic digestion (Englyst et al. 1992). SDS enriched foods help in addressing diseases like metabolic syndrome and diabetes, due to the prolonged digestion and slow release of glucose (El Hindawy et al. 2018; Herrmann et al. 1995; Seal et al. 2003). SDS form of starch could be produced *in vitro* through several modification means such as cross-linking and enzymatic modification (Raigond et al. 2015; Shin et al. 2004). Amylopectin structure is significantly associated with SDS levels in starch. Studies on maize mutants showed a parabolic relationship between SDS content and weight ratio of amylopectin short chains ($DP < 13$, named SF) to long chains ($DP \geq 13$, named LF), suggesting starches with higher and lower SF/LF ratio producing high SDS levels compared to that with a medium SF/LF ratio (Zhang et al. 2008).

The potential of RS in preventing and retarding the most common diet-related diseases is increasingly being demonstrated (Bird and Regina 2017). Being resistant to enzymatic digestion, RS escapes digestion in the small intestine, thus not contributing directly to the spike of glucose in the blood stream assisting in Type II diabetes prevention and management (Behall et al. 2006). Once reaching the large bowel where it gets fermented by colonic microbiota resulting in the production of short chain fatty acid (SCFA). There is a growing body of evidence on the diverse benefits of SCFA for human health (Bird and Regina 2017; Bird et al. 2010; Conlon et al. 2012; Guilloteau et al. 2010; Keenan et al. 2015; Kim et al. 2016; Topping and Clifton 2001; Vetrani et al. 2018). The benefits include maintenance of bowel health through proliferation of beneficial gut microbial population at the same time suppressing the pathogenic bacterial species. Luminal SCFA levels, particularly the level of butyrate, are associated with protection of DNA damage caused by unhealthy diets, promotion of gut barrier function and suppression of epithelial inflammation, all of which are important in protection against bowel diseases such as colorectal cancer. RS also influences metabolism of skeletal muscle, adipose tissue depots and liver. The positive effect of RS on weight management is also suggested through promoting satiety and reduced food intake. Involvement of SCFA in regulating immune system function and responses to infection is also recently highlighted.

RS is significantly correlated with the contents of amylose and long chained amylopectin that is functionally similar to amylose (hence also called as amylose like molecule) (Regina et al. 2012). Thus enabling the necessary structural changes in starch to elevate one or the other or both these fractions in the grain is a major strategy adopted in generating RS enriched wheats. Mainly two mechanisms are demonstrated in wheat to elevate the amylose and amylose like molecules in wheat, once is suppressing SSIIa activity and the other is suppressing SBEIIa activity in combination with some of SBEIIb activity (Regina et al. 2015b; Regina et al. 2006; Yamamori et al. 2000). While SSIIa mediated elevation of amylose is only moderate

(<50% amylose), a higher level of amylose increase (>75%) is achieved through the SBEIIa mediated approach. The high amylose wheat developed through selective inhibition of SBEII is also enriched with RS, with a > ten-fold increase compared to a standard wheat. Flour from this wheat is suitable to produce bakery products such as bread that are of comparable quality to those from standard wheat flours, with the added advantage of having significantly elevated RS and TDF in the products (Berbezy et al. 2015). A high amylose durum wheat developed through SBEII mediated strategy was shown to produce pasta of acceptable quality with positive effects on pasta firmness (Hazard et al. 2015).

5 Starch Granule Associated Proteins

Major macronutrient components in a cereal based food system are starch and proteins. While each of these individually influence the functional properties of the system, the interaction between these two components, also mediated by other compounds present such as lipids, plays an important role in determining the final quality of the grain and, in turn, the products. In wheat, starch granules are embedded in a protein matrix within the endosperm tissue (Marshall and Chrastil 1992).

There are mainly two types of proteins associated with starch in cereals (Baldwin 2001); (a) storage proteins such as the gluten proteins that remain adsorbed to the starch granule surface (storage proteins are outside the scope of this chapter, but reviewed comprehensively elsewhere in this book); and (b) starch granule associated proteins (SGAPs) that are either bound to the surface of starch granules or present as integral component of starch granules. SGAPs are mostly distinct from the storage proteins both structurally and functionally. Higher levels of basic and hydrophobic amino acids are characteristic features of SGAPs, which are attributable to their binding ability to starch granules. In a very broad sense, surface proteins are of low molecular weight (5 to 30 kDa) that are easily extractable using salt solutions or aqueous buffers, while integral proteins are of high molecular weights (60 to 149 kDa) that require stronger detergents and heat swelling of starch granules for their extraction. However, exceptions to this broad classification have also been demonstrated with certain low molecular weight proteins found integral to starch granules and vice versa. Major starch granule associated integral proteins and some surface proteins have enzymatic functions and are involved in starch biosynthesis (Rahman et al. 1995), taking either biosynthetic or degradative roles. Starch biosynthetic enzymes, their roles and their impacts on starch structure and quality are already covered in previous sections of this chapter.

Surface SGAPs, their quantities, orientation and the nature are known to influence several starch properties such as gelatinisation, viscosity, damage and enzyme resistance (Baldwin et al. 1997; Hamaker and Griffin 1993). Kernel texture of wheat grain is an important characteristic that determines its quality, milling performance, market classification and end use. The presence of ~13 kDa SGAPs known as friabilins or puroindolines (Pins) on the surface of starch granules modulates the adhesiveness of the protein-starch complex, and provides wheat kernel with a soft texture. Flour from such wheats with soft kernel texture is used for biscuits and cakes, while wheats with a harder kernel texture that are lacking or are lower in the levels of puroindolines on starch granule surface are better suited for breads, noodles and pasta. Two genes encoding puroindolines, *Pina-D1* and *Pinb-D1* when both present in the wild type forms in the endosperm imparts a soft kernel texture, while deletions or diverse mutations in either or both of the *Pin-D1* genes result in a harder kernel texture (Morris and Bhave 2008). A suggested reference for a detailed review on SGAPs is Baldwin (2001).

6 Environmental Effects and Starch Properties

Wheat grain starch properties, as most of the other grain characteristics, are related to the genotype and also are influenced by environmental factors during crop growth. In this regard, a significant number of research studies have been carried out to understanding the influence of specific environmental factors on wheat starch characteristics and properties (Vignola et al. 2016). In the current context of the Climate Change, understanding how different environmental conditions and, particularly, how abiotic stresses such as drought or heat modify starch composition and properties and accumulation in cereal endosperm is key. This knowledge will lead to improve the predictions of grain and flour quality and will be useful to breeding programs when deploying new germplasm that can tolerate extremes of environment (Thitisaksakul et al. 2012) and to the production of high quality wheat.

Different studies have analysed what starch characteristics are modified with different environmental conditions in general. Geera et al. (2006) targeted to identify the starch characteristics most influenced by the environment (under field conditions) that modulate the flour/starch properties, particularly the flour pasting properties. They found that total starch and A/B-type granule contents were affected by the environment while amylose content was minimally affected. These changes appeared to explain part of the environment-induced fluctuations in the flour pasting properties observed. Labuschagne et al. (2007), in an experiment conducted with grain from three different irrigated fields, also found that the total starch content was strongly affected by the environment but not the amylose content, which was more dependent on the genotype or genotype x environment interaction. Jing et al. (2003) did not report any significant changes in amylose content due to the environment too. On the other hand, Nhan and Copeland (2014) found the environment and genotype x environment as significant sources of variability in amylose content and amylopectin chain lengths. Starch pasting properties were also found to be affected by the environment (Konik et al. 1993; Morris et al. 1997). But, as described by Graybosch et al. (2003) in an experiment conducted with waxy wheat lines, stable starch properties (recorded in this case with the Rapid Visco Analyzer) responses over diverse environments are expected. This is contrary to what Vignola et al. 2016 showed in their study in which environment had a much greater impact on the starch properties than genotype for starch pasting parameters except for the pasting temperature. Ansari et al. (2010) showed that around 34% of the variation found in starch swelling power of wheat genotypes grown in different locations was determined by the environmental factors. In this study the differences in starch swelling power at different locations did not affect the ranking of the genotypes, which was quite consistent, which also agrees with the results of Nhan and Copeland (2014). In summary, it seems that in general, the environment affects the starch and its properties, but the magnitude of the impact will depend a lot on how contrasting are the different environments tested. Therefore, a breeding strategy considering multiples sites for evaluation of starch properties may not be always required.

One of the abiotic stresses most studied regarding its effect on starch and its properties is heat or elevated temperature during grain filling. It is well established that high temperatures during grain filling decrease starch accumulation in the grain leading to a reduction in the grain weight and yield (Gibson & Paulsen 1999; Hurkman et al. 2003; Matsuki et al. 2003; Wang et al. 2017). This is probably due to the reduction in the activity of enzymes involved in the starch synthesis pathway such as the soluble starch synthase activity (Keeling et al. 1993), the starch branching enzyme (Keeling et al. 1994) or the ADP-glucose pyrophosphorylase (Smidansky et al. 2002; Altenbach et al. 2003), and to reduced grain filling period. Other studies have showed that elevated temperature during grain filling may result in a qualitative change in starch: Shi et al. (1994) showed that amylose content was slightly increased and starch gelatinization temperature increased when wheat is grown at 40 °C compared with 15 °C while A-type granules concentration was reduced. In agreement with this, Panozzo and Eagles (1998) reported that environmental variation with accumulated temperatures above 30 °C led to an increase in amylose percentage and to an increase in A-type starch granules. Vignola et al. (2016) found higher amylose contents in the environments with higher temperatures during the grain filling period and also an increase in A-type starch granules. Opposite to these results, Stone and Nicolas (1995) reported a reduction in amylose content by heat treatment but limited and confined to small proportion of the genotypes of the experiment. On the other hand, Matsuki et al. (2003) reported that amylose contents were not significantly affected by elevated maturation temperature in several cultivars when wheat was grown at 15 °C and at 30 °C. Similar results were obtained by Wang et al. (2017), although in this last case the heat stress only represented 2.2 °C higher temperature than the normal environment. Therefore, the intensity of the heat stress and the susceptibility of the genotypes to heat determine the effect on amylose content (Thitisaksakul et al. 2012). In terms of end-use quality governed by starch properties, no significant changes in noodles swelling power in response to high temperature were found (Stone and Nicolas 1995).

The effect of drought stress on starch has been also studied. Wheat plants under drought stress have reduced yield, and this is due, in part, to alterations in starch biosynthetic enzyme activity (Jenner et al. 1991), mainly because of a loss of activity in starch synthases, which are the most sensitive enzymes to drought in the starch biosynthesis pathway (Ahmadi and Baker 2001). Amylose content is reduced in the case of drought stress and the proportion of A-type starch granules is increased (Fabian et al. 2011; Singh et al. 2008)

7 Conclusions

Starch is the major component of the wheat grain and it is an important determinant of both industrial and nutritional quality. Significant progress has been made in the last thirty years in understanding how starch is synthetized in the wheat grain and how starch composition can be manipulated through different approaches including classical breeding or more modern tools such as TILLING. This has allowed the development of commercial cultivars with modified starch properties, which are already available for cultivation and could emerge as protagonists in the coming years in farmers' fields due to their unique functional properties and nutritional profile.

Acknowledgments Carlos Guzman would like to greatly acknowledge to the Spanish Ministerio de Ciencia, Innovación y Universidades for a Ramon y Cajal grant (RYC-2017-21891).

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