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Variation in polar lipid composition among near-isogenic wheat lines possessing different puroindoline haplotypes

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ABSTRACT

The exact mechanism underlying wheat (*Triticum aestivum* L.) kernel hardness is unknown. Similar to puroindoline proteins, polar lipids are present on the surface of starch granules. The objective of this research was to determine the specific polar lipid species present on the surface of wheat starch from near-isogenic wheat lines that have different puroindoline haplotypes and endosperm hardness. Four near-isogenic wheat lines were used in this study, all derived from the soft cultivar Alpowa. Direct infusion tandem mass spectrometry was used to identify the lipid species in whole-meal, flour and starch samples. Endosperm hardness had no significant effect on the polar lipid contents in wheat whole-meal, a slight influence on the polar lipid contents of the flour fractions and a significant influence on the polar lipid composition of the polar lipids located on the surface of wheat starch. The greatest quantities of polar lipids on the starch-surface occurred when both puroindoline proteins were present in their wild-type form. Starch-surface polar lipid content dramatically decreased when one of the puroindoline proteins was null or if pin-B was in the mutated form. The least amount of polar lipids was present when pin-B was in its mutated form and pin-A was in its wild-type form.

1. Introduction

Wheat (*Triticum aestivum* L.) kernel physical hardness, often referred to as texture, is the most important trait used for end-use classification. An extensive amount of research has investigated wheat kernel hardness, but the exact mechanism underlying this phenomenon remains unknown. Despite the lack of understanding regarding the specific mechanism of kernel hardness, the molecular source has been located at the interface between the starch granule surface and storage proteins of the wheat endosperm. Using a micropenetrometer, Barlow et al. (1973) measured the hardness of starch granules and storage protein of wheat kernels from different hardness classes and found no significant difference in the hardness of the starch granules and that of the surrounding storage protein between the hard and soft-textured wheat samples. Barlow et al. (1973) concluded that differences between soft- and hard-textured wheat varieties must be in the adhesive characteristics at the interface between the starch granule surface and the storage proteins.

By evaluating the surface components of water-washed starch granules, Greenwell and Schofield (1986) discovered an unbroken molecular pattern between soft- and hard-textured wheat. A group of ~15 kDa proteins (friabilin) was found in greater quantities on water-washed starch from soft wheat than on equivalently treated starch from hard wheat. These proteins were absent from water-washed starch from durum wheat, the hardest wheat class. These results established a foundation for the molecular basis of wheat endosperm hardness.

Further investigation of the friabilin proteins revealed the existence of two protein isomers, puroindoline A (pin-A) and puroindoline B (pin-B), which together compose friabilin (Jolly et al., 1993; Morris et al., 1994). The name puroindoline is derived from their unique tryptophan-rich domain (indoline) and the Greek word for wheat (Puro). Pin-A contains five tryptophan residues in the sequence WRWWKWWK, whereas that region in pin-B is truncated to three tryptophan residues in the sequence WPTKWWK (Gautier

Abbreviations: Pin-A, puroindoline A; pin-B, puroindoline B; DGDG, digalactosyldigylceride; MGDG, monogalactosyldigylceride; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PA, phosphatidic acid; PS, phosphatidylserine; PG, phosphatidylglycerol; LPE, lysophosphatidylethanolamine; LPG, lysophosphatidylglycerol; DGMG, digalactosylmonoglycerols; MGMG, monogalactosylmonoglycerol.

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et al., 1994). Kooijman et al. (1997) suggested the tryptophan-rich domains in puroindolines form loop structures at the exterior of the protein.

Puroindolines in their wild-type state (*Pina-D1a/Pinb-D1a*) express a soft wheat phenotype (Morris, 2002 and references therein). Pin-A and pin-B act complementarily to each other to form friabilin, and both must be present in their wild-type state for the expression of soft-textured wheat (Martin et al., 2006). When either of the puroindoline proteins is mutated or absent, the resulting phenotype will be hard in texture. Several puroindoline mutations are known to result in hard endosperm texture. Pin-A null and pin-B wild-type (*Pina-D1b/Pinb-D1a*), pin-A wild-type and pin-B Gly46 to Ser (*Pina-D1a/Pinb-D1b*) (Giroux and Morris, 1997), pin-A wild-type and pin-B Lue60 to Pro (*Pina-D1a/Pinb-D1c*) (Lillemo and Morris, 2000), pin-A wild-type and pin-B Trp-44 to Arg (*Pina-D1a/Pinb-D1d*) (Lillemo and Morris, 2000), pin-A wild-type and pin-B Trp-39 to stop codon (*Pina-D1a/Pinb-D1e*) (Morris et al., 2001) pin-A wild-type and pin-B Trp-44 to stop codon (*Pina-D1a/Pinb-D1f*) (Morris et al., 2001) and pin-A wild-type and pin-B Cys56 to stop codon (*Pina-D1a/Pinb-D1g*) (Bhave and Morris, 2008a,b; Morris, 2002; Morris and Bhave, 2008).

Morris and King (2008) developed a series of unique puroindoline allele near-isogenic hexaploid wheat experimental lines. The soft white spring cultivar Alpowa (PI 566595) was used as the recurrent parent to which donor parents containing specific puroindoline haplotypes (pin-A null, pin-B Gly46 to Ser, pin-B Lue60 to Pro, pin-B Trp-44 to Arg, pin-B Trp-39 to stop codon, pin-B Trp-44 to stop codon and pin-B Cys56 to stop codon) were crossed as the male donor plant. These near-isogenic wheat lines provide an opportunity to study the molecular basis of endosperm hardness and have the potential to help solve the enigma of the mechanism of endosperm hardness.

Greenblatt et al. (1995) found that a pattern also exists among polar lipids present on the surface of starch granules. Galactolipids and phospholipids were found, via thin layer chromatography, in greater amounts on water-washed starch from soft wheat than from water-washed starch on hard wheat (Greenblatt et al., 1995). Konopka et al. (2005) further found a negative correlation between starch-surface lipids (polar and non-polar) and kernel hardness. However, a full profile of the lipid species found on the starch granule surface and the relationship of these molecules to endosperm hardness have not been reported.

The interaction between puroindoline proteins and polar lipids has been intensively researched with regard to endosperm hardness and gas cell stabilization effects (Bottier et al., 2008; Clifton et al., 2007, 2008; Dubreil et al., 1997; Wilde et al., 1993). The unique tryptophan-rich loop of puroindoline proteins plays a role in the proteins' interactions with lipids (Clifton et al., 2007). Wilde et al. (1993) demonstrated the ability of a single puroindoline molecule to bind ~5 lysophosphatidylcholine molecules. Dubreil et al. (1997) determined that pin-A associates tightly to phospholipids and galactolipids, whereas pin-B is loosely associated with galactolipids and preferentially binds to negatively charged phospholipids. In these *in vitro* studies, the puroindoline proteins have been extracted from the wheat and combined with either natural or synthetic polar lipids. It is unknown whether the results of the *in vitro* studies are valid *in vivo*. By studying the relationship between the polar lipids and puroindoline proteins located on the surface of wheat starch *in vivo* and implementing the relationships demonstrated with the *in vitro* studies, a clearer understanding of the potential mechanism of endosperm hardness may be established.

The objective of this research was to determine the specific polar lipid species present on the surface of wheat starch from near-isogenic wheat lines that have different puroindoline haplotypes and endosperm hardness. The near-isogenic wheat lines used in this study were developed and characterized by Morris and King (2008). Three hard-textured experimental lines, each with different puroindoline expressions, were used in this study. By using these near-isogenic wheat lines, we were able to establish relationships between the polar lipid compositions of different puroindoline haplotypes as they relate to endosperm hardness.

Table 1
Sample identification and corresponding source, puroindoline haplotype, molecular change and SKCS hardness value of the wheat samples.

Sample identification	Puroindoline haplotype	Molecular change from wild-type[a]	Hardness (SKCS)[b]
Alpowa	*Pina-D1a/ Pinb-D1a*	–	31
Alpowa/ID377s//7* Alpowa	*Pina-D1b/ Pinb-D1a*	Pina null	72
Alpowa/Mjølner//7* Alpowa	*Pina-D1a/ Pinb-D1d*	Pinb Trp-44 to Arg	59
Alpowa/Canadian Red//7*Alpowa	*Pina-D1a/ Pinb-D1e*	Pinb null (Trp-39 to stop)	68

[a] Wild-type defined as *Pina-D1a/Pinb-D1a* puroindoline haplotype.
[b] SKCS, single kernel characterization system hardness index value.

2. Experimental

2.1. Wheat samples

A series of near-isogenic wheat lines (NILs) that varied in their puroindoline haplotypes (Table 1) were collected. The near-isogenic wheat lines used in this study were developed in the cultivar Alpowa and characterized by Morris and King (2008). The use of these NILs, which contain different hardness phenotypes but are nearly identical genetically, provides a unique ability to analyze starch granule surface lipids from wheat lines that vary only in grain texture (i.e., all other genetically controlled wheat components are constant).

The wheat samples were grown in multiple locations near Pullman, WA, in the 2007 crop year. At each location, two field replications were used for each sample. Once the wheat lines were harvested and cleaned, single kernel hardness was determined with the Single Kernel Characterization System 4100 (Perten Instruments North America, Inc., Springfield, IL). To provide enough wheat for these proposed experiments, the two field replicates were bulked into one sample (hardness values were compared to ensure no combinations of multiple wheat lines).

2.2. Milling and starch isolation

The wheat lines were milled into straight-grade flour with a Bühler experimental mill per the American Association of Cereal Chemists International (AACC International) approved method 26–31 (AACC International, 2008). All wheat samples were tempered to 14% moisture content for 24 h, and the wheat was milled with a reduced feed rate of 100 g/min. Whole-meal samples were ground with a cyclone mill (UDY Corp., Boulder, CO) through a 0.5-mm screen. Starch was isolated from the flour using a modified batter method (Finnie et al., 2009).

2.3. Lipid extraction

Lipids were extracted from whole-meal, flour, and starch fractions. Because results from Finnie et al. (2009) indicated that starch-surface free polar lipids (hexane extractable) are extracted in minor proportions compared with the bound polar lipid extracts, only bound polar lipids were analyzed in this study. For a detailed description of the lipid extraction, see Finnie et al. (2009).