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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).

2.4. Lipid quantification

The polar lipids targeted for analysis were: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylglycerol (PG), lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), lysophosphatidylglycerol (LPG), digalactosyldiglycerols (DGDG), monogalactosyldiglycerols (MGDG), digalactosylmonoglycerols (DGMG) and monogalactosylmonoglycerols (MGMG).

An automated electrospray ionization tandem mass spectrometry approach was used, and data acquisition and analysis and acyl group identification were carried out as described previously (Devaiah et al., 2006; Finnie, 2009; Finnie et al., 2009).

2.5. Statistical analysis

For all polar lipid determinations (meal, flour and starch extracts), five replicated lipid extractions were conducted on each sample. Differences in means were assessed using the Scheffé multiple-comparison procedure, $P = 0.05$. To determine whether wheat kernel hardness was a significant source of variation in polar lipids extracted in whole-meal, flour and starch-surface samples, a one-way ANOVA was conducted. Data for each fraction were determined to be normally distributed by observations of residual plots and normal probability plots. All statistical analyses were conducted using Proc GLM and Proc Univariate in Statistical Analysis System (SAS) software (SAS version 9.1; SAS Institute; Cary, NC). Type III sums of squares were reported.

3. Results and discussion

3.1. Total polar lipid class variation among wheat fractions

The sample statistics and wheat endosperm hardness *F*-values for total polar lipids are summarized in Table 2. Total polar lipids are additive values of all the bound polar lipid classes analyzed (DGDG, MGDG, PC, PE, PI, PA, PS, PG, LPC, LPG, LPE, DGMG and MGMG). The sample statistics for the bound polar lipids from both the whole-meal and flour fractions contained similar ranges with 2750–3600 and 2550–3600 nmol/g, respectively (Table 2). The similarities between the whole-meal and flour fractions were expected, because the majority of polar lipids are located in the endosperm of wheat (Carr et al., 1992; Morrison, 1988).

When compared to previous work, these ranges are slightly lower, ~0.33%, when converted from nmol/g to per cent (Chung, 1986). Chung (1986), reported values of 0.4–0.7% of the flour are bound polar lipids. The slightly lower values are most likely caused by not quantitatively determining the *N*-acylphosphatidylethanolamine class of phospholipids in this study. The method for quantitatively determining *N*-acylphosphatidylethanolamine lipid class using lipid profiling has not been fully developed and more work is needed.

Table 2
Sample statistics[a] and *F*-values from one-way ANOVA[b] of total bound polar lipids in one-way ANOVA for wheat endosperm hardness.

Statistic/source	Whole-meal	Flour	Starch-surface
Minimum	2750	2550	35
Mean	3150	3150	200
Maximum	3600	3600	500
Hardness (*F*-value)	ns	10*	724**

[a] Sample statistics are expressed as nmol/g of sample.
[b] ns = not significant, * and ** represent *F*-values significant at $P = 0.05$ and 0.0001, respectively. *F*-values derived from Type III sums of squares.

The range in total bound polar lipids in the whole-meal and flour samples was relatively minor (0.3- and 0.4-fold increase, respectively) compared with the range in the starch-surface lipids (14-fold increase, 35–500 nmol/g; Table 2). These results were expected because the samples analyzed were nearly genetically identical to each other but had different endosperm hardness phenotypes. The one-way ANOVA for endosperm hardness source of variation further emphasized the genetic similarities of the samples. Wheat endosperm hardness had no significant effect on total bound lipids from the whole-meal fractions and a relatively minor effect on the flour samples (*F*-value of 10 with a significance level of $P = 0.05$), but, starch-surface bound polar lipids were significantly related to endosperm hardness ($P < 0.0001$ level, *F*-value of 724; Table 2). These results were expected because it has been shown that the surface of wheat starch isolated from soft wheat flour contains greater amounts of polar lipids than wheat starch isolated from hard wheat flour (Finnie, 2009; Finnie et al., 2009; Greenblatt et al., 1995). Overall, results indicate that whole-meal and flour from soft and hard wheat had relatively similar quantities of bound polar lipids, but the starch-surface from those same flours had dramatically different amounts of bound polar lipids.

3.2. Whole-meal and flour polar lipid class differences among the wheat genotypes

In both the whole-meal and flour samples, the genotype class totals were similar, with no apparent pattern for the polar lipid class totals among the wheat genotypes. In the whole-meal samples, the hard-textured genotypes contained the greatest quantities of DGDG and MGDG lipids, whereas the soft-textured genotype contained the greatest quantities of PC, LPC, PG, DGMG, MGMG, PA and LPE (Table 3). PI and PS class totals were statistically similar for all genotypes (Table 3). The flour samples had little differences among the genotypes. The hard-textured genotypes contained the greatest quantities of PC, LPC, PE, DGMG, LPG, PA, and PS, whereas the soft-textured genotype contained the greatest quantity of DGDG lipids. Within each fraction, the predominant lipid classes for all the genotypes were the same, with the order of decreasing abundance for all the class totals in the whole-meal

Table 3
Mean content of different bound polar lipid classes extracted from wheat whole-meal of the six near-isogenic wheat lines.

Lipid class	Alpowa	Alpowa/Canadian Red//7*Alpowa	Alpowa/ID377s//7*Alpowa	Alpowa/Mjølner//7*Alpowa
DGDG	1138 b ± 33	1348 a ± 34	1278 ab ± 24	1347 a ± 62
MGDG	541 c ± 14	774 a ± 31	692 ab ± 5	675 b ± 21
PC	859 a ± 25	786 ab ± 28	749 b ± 20	727 b ± 24
LPC	116 a ± 3	64 b ± 2	67 b ± 1	73 b ± 5
PE	103 ab ± 3	108 a ± 2	105 ab ± 2	92 b ± 4
PG	71 a ± 3	52 b ± 1	50 c ± 2	52 b ± 2
DGMG	21 a ± 1	14 b ± 0	12 b ± 1	15 b ± 2
MGMG	10 a ± 0	7 b ± 0	6 b ± 0	7 b ± 1
LPG	10 a ± 1	6 b ± 1	5 b ± 1	6 b ± 0
PA	22 a ± 1	16 bc ± 1	13 c ± 1	18 ab ± 2
PI	172 a ± 20	222 a ± 6	145 a ± 36	151 a ± 20
PS	11 a ± 2	14 a ± 1	9 a ± 2	11 a ± 2
LPE	8 a ± 0	6 c ± 0	6 b ± 0	7 ab ± 1
Total	3083 a ± 87	3416 a ± 73	3138 a ± 57	3181 a ± 121

Each value represents nmol/g of sample ± SE, $n = 5$. Mean values (within the same row) from each lipid extract followed by the same letter are not significantly different between genotypes at $P = 0.05$ (based on Scheffé multiple-comparison procedure). 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.

Table 4
Mean content of different bound polar lipid classes extracted from wheat flour of the six near-isogenic wheat lines.

Lipid class	Alpowa	Alpowa/Canadian Red//7*Alpowa	Alpowa/ID377s//7*Alpowa	Alpowa/Mjølner//7*Alpowa
DGDG	1984 a ± 40	1764 b ± 34	1808 b ± 17	1851 b ± 16
MGDG	684 a ± 13	691 a ± 15	649 ab ± 4	628 b ± 5
PC	475 b ± 7	489 ab ± 18	534 a ± 7	470 b ± 5
LPC	82 ab ± 2	80 ab ± 4	90 a ± 2	78 b ± 1
PE	55 bc ± 1	58 b ± 2	67 a ± 1	49 c ± 1
PG	32 a ± 1	31 a ± 2	32 a ± 1	30 a ± 0
DGMG	20 ab ± 1	19 ab ± 1	21 a ± 1	18 b ± 0
MGMG	14 a ± 0	15 a ± 1	14 a ± 1	14 a ± 1
LPG	11 bc ± 0	10 c ± 1	13 a ± 0	12 ab ± 0
PA	9 b ± 0	9 b ± 1	12 a ± 0	9 b ± 0
PI	8 c ± 0	16 b ± 1	26 a ± 1	15 b ± 0
PS	6 b ± 0	4 c ± 0	7 a ± 0	7 ab ± 0
LPE	5 b ± 0	5 b ± 0	7 a ± 0	5 b ± 0
Total	3385 a ± 61	3192 ab ± 60	3281 ab ± 23	3185 b ± 11

Each value represents nmol/g of sample ± SE, $n = 5$. Mean values (within the same row) from each lipid extract followed by the same letter are not significantly different between genotypes at $P = 0.05$ (based on Scheffé multiple-comparison procedure). 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.

fraction being DGDG > MGDG > PC > PI > LPC > PE and the flour class totals being DGDG > MGDG > PC > LPC > PE > PG. Interestingly in the whole-meal fractions, PI class totals were relatively large when compared to the flour and starch-surface lipids (Tables 3–5). The PI results are similar to results from Finnie et al. (2009), which showed that PI lipids were localized predominantly in the bound lipids from the whole-meal fractions.

3.3. Variation within starch-surface polar lipid classes

Starch-surface bound polar lipid class totals varied significantly between the NILs, with the Alpowa cultivar containing the greatest concentrations for all the class totals. The hard-textured genotypes contained considerably fewer bound polar lipids than the soft-textured genotype (Table 5). LPG was the only polar lipid class that was not significantly different among genotypes (Table 5). Similar to results presented in Finnie et al. (2009) and Finnie (2009), the most predominant lipids located on the surface of wheat starch were DGDG, MGDG, PC and LPC (Table 5).

Table 5
Mean content of different bound polar lipid classes extracted from wheat starch of the six near-isogenic wheat lines.

Lipid class	Alpowa	Alpowa/Canadian Red//7*Alpowa	Alpowa/ID377s//7*Alpowa	Alpowa/Mjølner//7*Alpowa
DGDG	271.6 a ± 3.9	31.6 c ± 3.4	58.4 b ± 0.4	18.6 d ± 0.5
MGDG	115.7 a ± 2.4	16.2 c ± 1.6	27.8 b ± 0.3	8.9 d ± 0.2
PC	64.8 a ± 1.6	6.1 c ± 0.5	14.4 b ± 0.3	3.4 c ± 0.2
LPC	11.5 a ± 0.3	6.7 b ± 0.6	6.3 b ± 0.10	6.2 b ± 0.2
PE	2.1 a ± 0.1	0.1 b ± 0.0	0.3 b ± 0.0	0.0 b ± 0.0
PG	3.8 a ± 0.1	0.5 c ± 0.0	1.0 b ± 0.0	0.3 c ± 0.0
DGMG	0.8 a ± 0.0	0.3 b ± 0.0	0.2 b ± 0.0	0.2 b ± 0.0
MGMG	0.4 a ± 0.0	0.2 b ± 0.0	0.1 c ± 0.0	0.2 b ± 0.0
LPG	0.5 a ± 0.1	0.4 a ± 0.1	0.4 a ± 0.0	0.5 a ± 0.1
PA	2.5 a ± 0.1	0.6 c ± 0.0	1.2 b ± 0.0	0.1 d ± 0.0
PI	0.9 a ± 0.0	0.2 c ± 0.0	0.3 b ± 0.0	0.1 d ± 0.0
PS	0.6 a ± 0.1	0.0 b ± 0.0	0.1 b ± 0.0	0.0 b ± 0.0
LPE	1.2 a ± 0.1	0.0 c ± 0.0	0.3 b ± 0.0	0.1 c ± 0.0
Total	476.5 a ± 7.7	62.9 c ± 5.9	110.8 b ± 0.9	38.8 d ± 0.9

Each value represents nmol/g of sample ± SE, $n = 5$. Mean values (within the same row) from each lipid extract followed by the same letter are not significantly different between genotypes at $P = 0.05$ (based on Scheffé multiple-comparison procedure). 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.

Results from the hard-textured wheat lines provide interesting insight into the effect of lipid binding on the starch-surface between the different puroindoline haplotypes. All the bound polar lipid class totals were significantly different within the hard-texture sample except LPC, PE, DGMG, LPG and PS (Table 5). Among the hard-textured samples, the Alpowa/ID377s//7*Alpowa (pin-A null) sample contained the greatest quantity of DGDG, MGDG, PC, PG, PA, PI and LPE class totals, Alpowa/Canadian Red//7*Alpowa (pin-B null) contained intermediate quantities, and Alpowa/Mjølner//7*Alpowa (pin-A wild-type and pin-B Trp-44 to Arg) contained the least amount of those same bound polar lipids (Table 5). These results indicate that starch granule surfaces that are pin-A null and contain pin-B in its wild-type state are able to associate with approximately twice as many polar lipids as starch granule surfaces that are pin-B null and contain pin-A in its wild-type state and approximately three times as much as starch granule surfaces that contain pin-A wild-type and pin-B Trp-44 to Arg (Table 5). It appears that pin-B alone (when associated with the surface of starch granules) binds more polar lipids than when pin-A is associated with the surface of starch granules (Table 5).

Based on previous *in vitro* work that demonstrated that pin-A associates tightly to phospholipids and galactolipids but pin-B is loosely associated with galactolipids and preferentially binds to negatively charged phospholipids (Dubreil et al., 1997), it would be expected that the genotype with pin-A wild-type (Alpowa/Canadian Red//7*Alpowa and Alpowa/Mjølner//7*Alpowa) would contain greater amounts of bound polar lipids than the pin-B wild-type (Alpowa/ID377s//7*Alpowa) genotype. However, this was not the case when samples were analyzed *in vivo*. In this study, the pin-B wild-type sample contained the greatest quantity of bound polar lipids. One important difference between the *in vivo* and *in vitro* studies is that the *in vivo* study involved other flour components, such as the starch granule surface and storage proteins, which will compete for interactions with the puroindoline proteins and polar lipids.

The interactions between the puroindoline proteins, polar lipids, starch granule surface and storage protein were such that when both puroindoline proteins were present in their wild-type state on the starch granule surface, the greatest concentrations of polar lipids were present (476.6 nmol/g of total polar lipids) (Table 5). When only pin-B wild-type was present on the surface of the starch granules, 110.8 nmol/g of total polar lipids were present; and when only pin-A wild-type was present on the surface of the starch granule, 62.9 nmol/g of total polar lipids were present (Table 5). The least amount of polar lipids was present on the surface of the starch granule when pin-A wild-type and pin-B Try-44 to Arg were present (38.8 nmol/g).

3.4. Variation of specific polar lipid molecular species within hardness classes

Specific molecular species of the bound polar lipids on a mol% of total lipid basis from the DGDG, MGDG, PC and LPC classes are described in Fig. 1. Sample means were compared using Scheffé's procedure. Overall, 138 specific molecular species were identified from all the polar lipid species, with a range from 0.00 to 35.89 mol% of total polar lipids. The predominant molecular species were similar to previous results in which the acyl-carbon and double bond configurations are 18:2 and 16:0 fatty acids (34:2 and 36:4). PI and PG contained the only relatively substantial amounts

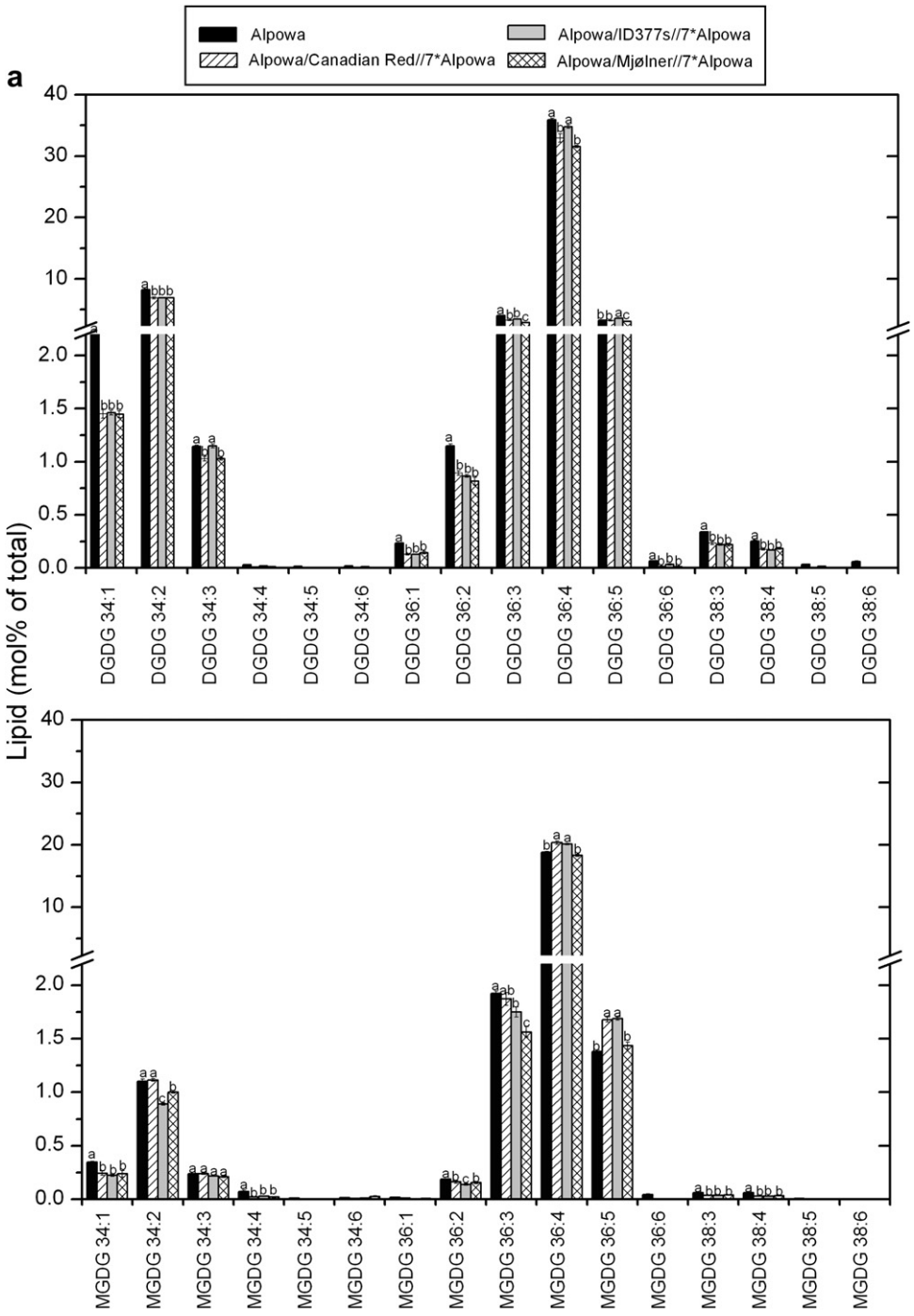

Fig. 1. Digalactosyldigylceride (DGDG), monogalactosyldigylceride (MGDG), phosphatidylcholine (PC), lysophosphatidylcholine (LPC) bound polar lipids molecular species means from starch isolated from the near-isogenic wheat lines. Values represent means ± SE in mol% of total lipids ($n = 5$). Different letters above column bars indicate significantly different means among the near-isogenic wheat.

of 32:0 acyl-carbons, whereas PS lipids were unique in their abundance of larger fatty acid molecules of 40:0, 42:2 and 42:3 molecular species (data not shown).

Even though Alpowa starch contained the greatest overall quantity of polar lipids on a nmol/g basis, it was slightly significantly greater on a mol% of total basis for the DGDG molecular species (34:1,34:2, 34:3, 36:2, 36:2, 36:3, 36:4, 36:6, 38:3, and 38:4), MGDG molecular species (34:1, 34:2, 34:3, 34:4. 36:2, 36:3, 36:4, 36:5, 38:3 and 38:4) and PC molecular species (32:0, 34:1, 34:2, 34:3, 34:4, 36:1, 36:2 36:3, 36:4, 36:5, 36:6, 38:2, 38:3, 38:5 and 38:6) (Fig. 1). These results indicate that the overall greater amount of polar lipids in the Alpowa starch was equally distributed across the DGDG, MGDG and PC lipid classes (Fig. 1). Comparisons between the class total data (nmol/g of sample) (Table 5) and molecular species data (Fig. 1) revealed that an increase in the total amount of lipid class resulted in a general uniform increase in the proportion of the molecular species of the DGDG, MGDG and PC lipid classes.

Fig. 1 demonstrates differences in the LPC molecular species mol% of total. The significant decrease in the proportion of starch bound LPC lipid species associated with the Alpowa samples is due

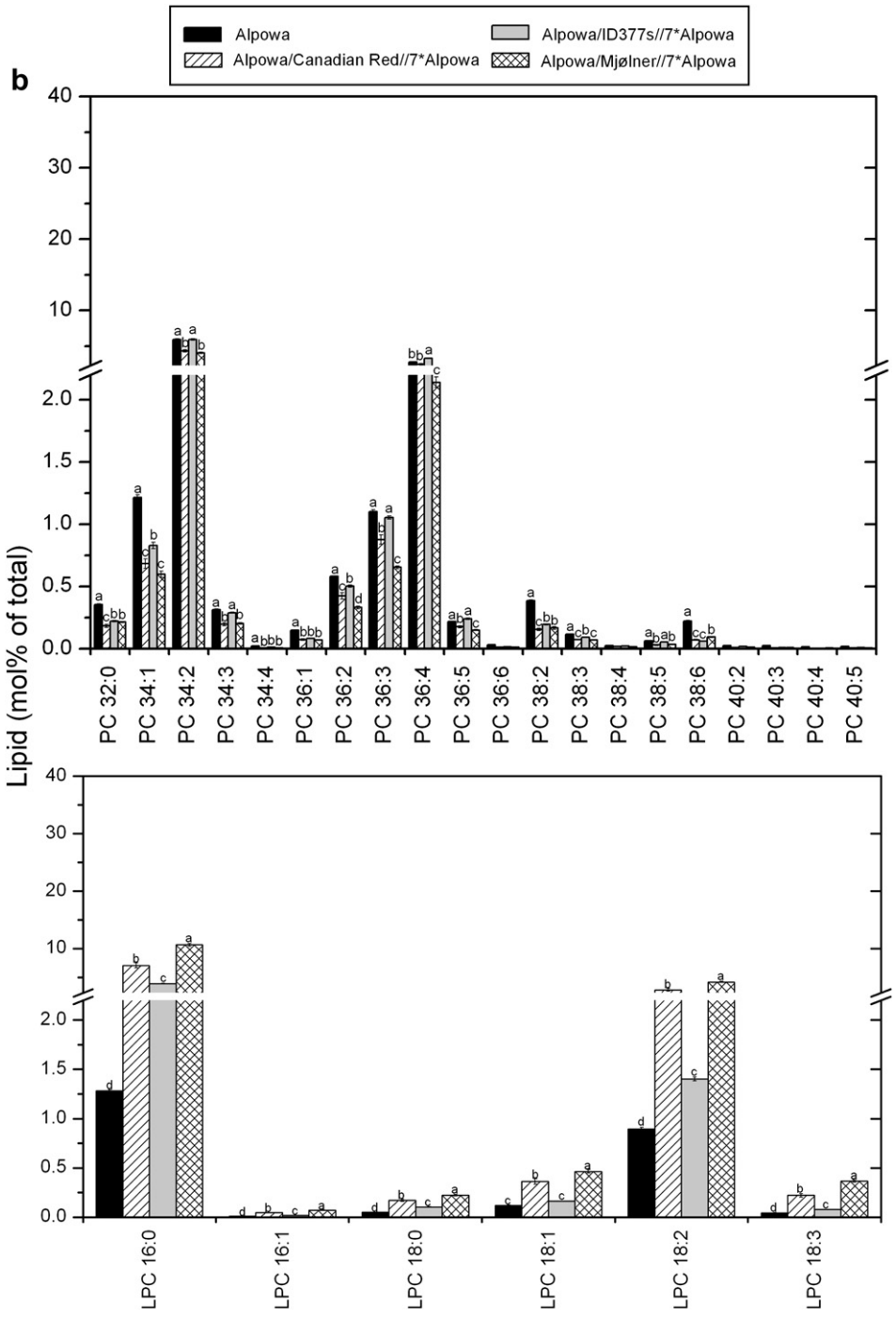

Fig. 1. (*continued*).

to a relatively significant quantity difference (nmol/g of sample) in the LPC lipids on the nmol/g basis when compared to the other major polar lipid classes (Table 5). Even though the overall content of the LPC lipids are greater in Alpowa, the increase was minor when compared to the DGDG, MGDG and PC classes. As a result the overall proportion of LPC lipids was reduced.

4. Conclusions

By using near-isogenic wheat lines, we were able to establish relationships between the polar lipid compositions of samples containing different puroindolines. 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 pin-B was in the mutated form (Trp-44 to Arg). Among the hard-textured samples, more polar lipids were present on the starch-surface when pin-B was in its wild-type form and pin-A was null than when pin-A was in its wild-type form and pin-B was null; the least amount of polar lipids were

present when pin-B was in its mutated form (Trp-44 to Arg) and pin-A was in its wild-type form, suggesting that pin-B is more important than pin-A in binding lipids. These results demonstrate that the mechanism of endosperm hardness is, at a minimum, a four-way interaction between the starch granule surface, storage proteins, puroindoline proteins, and polar lipid. It is unknown exactly how these components are configured, but it is evident that when puroindoline proteins are in their wild-type state, dramatically more polar lipids are associated with the puroindoline proteins. This strongly implies that the mechanism of endosperm hardness is a structure that provides an anti-adhesion characteristic between the starch granule surface and the storage proteins in the endosperm of the soft-textured wheat kernels.

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