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A leucine to proline mutation in puroindoline b is frequently present in hard wheats from Northern Europe

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Abstract Endosperm hardness in wheat (*Triticum aestivum* L.) is determined by one major genetic factor, the *Hardness (Ha)* gene on the short arm of chromosome 5D. Grain hardness has previously been reported to result from either a failure to express puroindoline a (*Pina–D1b*) or a glycine to serine mutation at position 46 in puroindoline b (*Pinb–D1b*). In this study, which involves a large survey of 343 wheat genotypes of mostly Northern European origin, we report two new mutations in puroindoline b associated with hard endosperm. These were characterized as involving a leucine to proline change at position 60 (*Pinb–D1c*), and a tryptophan to arginine change at position 44 (*Pinb–D1d*), respectively. While the former seems to be widely distributed in germplasm around the world, the latter was only found in three winter wheats from Sweden and Netherlands. As discussed in the paper, the three known mutations in puroindoline b can be considered "loss-of-function" mutations (i.e. soft to hard), and structural analysis may serve to predict that their dramatic effect on wheat grain texture is a result of reduced lipid–binding ability.

Key words *Triticum aestivum* L. · Wheat · Puroindoline · Mutations · Endosperm hardness

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Introduction

Endosperm hardness is one of the most important quality characteristics of cultivated wheat (*Triticum aestivum* L.) and has a profound effect on milling, baking and the quality of the end product. Soft wheat kernels fracture more easily, release numerous intact starch granules and produce finer-textured flours with less starch damage. Hard wheats produce coarser textured flours in which fracture planes produce broken starch granules and hence higher levels of starch damage. Because broken starch granules absorb more water, hard wheats are better suited for yeast-leavened bread baking, while soft wheats are preferred for cookies, cakes and pastries (Morris and Rose 1996).

Barlow et al. (1973) and Simmonds (1974) showed that there was no difference in the absolute hardness of either the starch or the protein between soft and hard wheat genotypes. Scanning electron micrographs of starch granules prepared by solvent sedimentation also showed that starch granules from hard wheats had a considerable amount of material adhering to them, whereas soft wheat starch was relatively free of adhering materials. Soft and hard wheat texture, therefore, most likely results from differences in the binding strength between starch granules and the protein matrix in the endosperm. Although several theories have been proposed to explain the difference in binding strength between the starch granules and the protein matrix in soft and hard wheat (see Autran 1996 for review), little is known about the underlying biochemical processes.

Although the biochemical basis for endosperm hardness is poorly understood, the genetic inheritance of the character is well-established. Wheat hardness is mainly controlled by the *Hardness (Ha)* locus on the short arm of chromosome 5D and is inherited as a single genetic factor (Symes 1965; Mattern et al. 1973; Sampson 1983). Greenwell and Schofield (1986) reported the existence of a 15-kDa protein, termed friabilin, that was abundant on the surface of water-washed soft wheat starch but scarce on hard wheat starch and absent on

starch from durum wheat (*Triticum turgidum* L. var. *durum* 2n = 28 = AABB). This correlation between the quantitative level of friabilin and endosperm hardness has remained unbroken among hundreds of wheats from around the world and among reciprocal hard by soft F_2 grains (Bettge et al. 1995 and references therein).

Friabilin is not a single protein, but consists of two major polypeptides (Morris et al. 1994; Rahman et al. 1994; Oda 1994). Oda and Schofield (1997) showed that these components of friabilin correspond to the basic cysteine-rich proteins puroindoline a and b (Gautier et al. 1994). Puroindolines are unique among plant proteins because of their tryptophan-rich domains, which have an apparent high affinity for binding lipids (Wilde et al. 1993; Dubreil et al. 1997). Giroux and Morris (1997, 1998) have previously reported that hard wheat is associated with the failure to express puroindoline a or with a glycine to serine mutation in the tryptophan-rich domain of puroindoline b.

Based on the study presented here, which involved a large survey of wheat germplasm of mostly Northern European origin, we report two additional mutations in puroindoline b associated with hard wheat.

Materials and methods

Germplasm

Most of the wheat germplasm included in this study is maintained at the Department of Horticulture and Crop Science, Agricultural University of Norway, and consists mostly of Northern European varieties, breeding lines and landraces, but also includes some CIMMYT materials and germplasm from China. Part of the collection is germplasm previously obtained from the Nordic Gene Bank in Sweden. Samples were harvested from field plots at Vollebekk Research Farm (Ås, Norway) during the 1998 growing season. Seed of 15 additional wheat varieties currently grown in Norway (Table 1) was provided by the Seed Testing Station at the Norwegian Agriculture Inspection Service.

Hardness measurement

Single-kernel hardness was measured on 100-kernel samples of each genotype using the Perten single kernel characterization system (SKCS) 4100 (Perten Instruments, Springfield, Ill.) following the manufacturer's suggested operation procedure. Due to the wet harvest season of 1998, each sample was inspected, and obviously sprouted or damaged kernels were removed prior to hardness measurement.

Isolation of Triton X-114 soluble proteins and SDS-PAGE

Triton-soluble proteins were extracted by phase partitioning using Triton X-114 as described by Giroux and Morris (1998). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed by standard methods using 13.5% T and 2.6% C and 0.75-mm-thick SE600 gels (Bio-Rad) and silver-stained by a trichloroacetic acid fixation method as described by Morris et al. (1994).

DNA isolation and PCR amplification of puroindoline a and b

DNA was isolated from single wheat kernels using a slightly modified procedure of Dellaporta et al. (1983). Full-length puroindoline a and b were amplified using primers described by Gautier et al. (1994), purified from agarose gels after electrophoresis and sequenced using the amplification primers. Amplification of puroindoline b sequences specific for the Gly-46 or Ser-46 has been described by Giroux and Morris (1997). Annealing temperature for all sets of primers was maintained at 58°C.

Site specific cleavage of polymerase chain reaction (PCR)-amplified puroindoline b

As a method for detection of the Pro-60 mutation, 2 U of the restriction enzyme *Pvu*II (Promega) together with supplied reaction buffer and bovine serum albumin (BSA) was added directly to PCR-amplified puroindoline b in a final reaction volume of 30 µl and incubated at 37°C for 1 h, without prior purification or precipitation of the PCR product.

Results

Soft wheats

The natural, wild-type state of wheat is to have soft endosperm and functional puroindoline a and b. Among the collection of 15 currently grown cultivars and 328 germplasm lines, 34 were soft (Tables 1 and 2). All exhibited

Table 1 SKCS Hardness Index and allelic constitution of puroindoline b for wheat cultivars currently grown in Norway. Kalle, the only of these cultivars classified as soft by the SKCS, has the "soft" wild-type puroindoline b, while the other hard cultivars all have a mutation in puroindoline b

[a] Hardness Index (± SD) was measured on seeds from different sources and is reported here to illustrate the profound effect of puroindoline mutations on hardness

[b] For description of *Pinb-D1* alleles, see Fig. 1

	Cultivar	Origin	Hardness Index[a]	*Pinb-D1* allele[b]
Spring wheats	Avle	Sweden	71 ± 16	*c*
	Bastian	Norway	72 ± 14	*b*
	Brakar	Norway	67 ± 15	*b*
	Polkka	Sweden	71 ± 16	*b*
	Reno	Norway	63 ± 14	*c*
	Sport	Sweden	74 ± 16	*b*
	Tjalve	Sweden	64 ± 16	*c*
Winter wheats	Bercy	Netherlands	61 ± 18	*d*
	Bjørke	Norway	67 ± 15	*c*
	Folke	Sweden	55 ± 18	*b*
	Kalle	Norway	24 ± 15	*a*
	Mjølner	Sweden	60 ± 13	*d*
	Portal	Germany	74 ± 23	*c*
	Rudolf	Sweden	70 ± 15	*b*
	Terra	Denmark	54 ± 20	*c*

Table 2 All 328 wheat germplasm lines in the survey sorted by geographical origin and puroindoline alleles. *Pina-D1a*, *Pinb-D1a* corresponds to the "soft" wild-type puroindoline a and b, and *Pina-D1b* to the puroindoline a null-mutation as described by Giroux and Morris (1998). The hardness alleles of puroindoline b are described in Fig. 1

Geographical origin	Number of genotypes analyzed	Soft	Hard			
		Pina-D1a *Pinb-D1a*	*Pina-D1b* *Pinb-D1a*	*Pina-D1a* *Pinb-D1b*	*Pina-D1a* *Pinb-D1c*	*Pina-D1a* *Pinb-D1d*
Spring wheats:						
Norway	69	3	–	48	18	–
Sweden	56	2	–	29	25	–
Finland	18	2	–	7	9	–
Rest of Western Europe	21	4	–	5	12	–
Eastern Europe	15	2	1	11	1	–
China	22	1	2	9	10	–
Latin America	29	6	12	10	1	–
USA and Canada	40	3	–	33	4	–
Africa	5	–	–	5	–	–
Others and unknown	27	2	2	14	9	–
Total spring wheats	302	25	17	171	89	–
Winter wheats:						
Northern Europe	26	8	1	14	2	1

PCR product using the Gly-46 (soft)-specific puroindoline b primers (Giroux and Morris 1997). In addition, both puroindoline a and b from a control group of 10 randomly selected soft wheat genotypes were sequenced and all possessed the "soft" wild-type sequences (data not shown).

Screening for known puroindoline mutations

As reported by Giroux and Morris (1998), the friabilin components puroindoline a and b can easily be separated by SDS-PAGE of Triton X-114 soluble proteins in the absence of any reducing agents in the sample buffer. In this survey, all genotypes with hard endosperm were screened for the presence or absence of puroindoline a by this method. Of the 15 spring and winter wheat cultivars currently grown in Norway, none possessed the *Pina-D1b* null-mutation (Table 1). Screening the 328 germplasm lines revealed that the puroindoline a null-mutation (*Pina-D1b*, Giroux and Morris 1998) in this germplasm is most prevalent among wheats from Latin-America (12 of the 16 wheats carrying the *Pina-D1b* mutation, Table 2). Most of these genotypes are breeding lines from CIMMYT, Mexico. The only Northern European genotype with this allele and the only winter wheat was the Finnish cultivar Vakka. Sequencing of puroindoline b in 3 randomly selected genotypes having the puroindoline a null-mutation showed that these lines all have the "soft" wild-type puroindoline b.

Hard genotypes expressing puroindoline a protein on SDS-PAGE were further evaluated for the presence of the glycine to serine mutation in puroindoline b *(Pinb-D1b*, Giroux and Morris 1997) by the use of sequence-specific PCR primers. The "hard" or serine 46-specific 3' puroindoline b primer ends with T and the "soft" or glycine 46-specific 3' primer ends with C (see above). Differential amplification of a 250-bp fragment with either of the two 3' primers indicates which of the two possible sequences are present. Of the currently-grown cultivars in Norway, four of seven spring wheats, but only two of eight winter wheats carry the *Pinb-D1b* Ser-46 mutation (Table 1). Of the 328 germplasm lines, approximately half of the hard wheats carry this mutation (Table 2). This allele appeared in wheats of all geographical origins but was particularly prevalent in wheats from Eastern Europe and Latin America.

DNA sequencing reveals an additional mutation in puroindoline b

Although the two previously reported hardness mutations were quite common among hard wheats (Tables 1 and 2), exceptions were found. DNA sequencing of PCR-amplified puroindoline a and b from 2 hard genotypes expressing puroindoline a and not having the serine 46 mutation revealed an additional mutation, characterized as involving a leucine to proline change at position 60 in puroindoline b (Fig. 1). No mutations were found in puroindoline a. According to the revised Guidelines for Nomenclature of Biochemical/Molecular Loci in Wheat and Related Species (McIntosh et al. 1995) we designate the new mutation as *Pinb-D1c*.

Site-specific cleavage with *Pvu*II shows that the *Pinb-D1c* allele is widely distributed

As a screening method for the proline 60 mutation, specific PCR primers for the soft and hard sequence at the mutation site were developed, but since these primers did not yield the desired specificity (data not shown), an alternative strategy using the restriction enzyme *Pvu*II

Allele	Position 42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62
Pinb-D1a	AAA	TGG	TGG	AAG	GGC	GGC	TGT	GAG	CAT	GAG	GTT	CGG	GAG	AAG	TGC	TGC	AAG	GAG▼CTG		AGC	CAG
(soft)	K	W	W	K	G	G	C	E	H	E	V	R	E	K	C	C	K	Q	L	S	Q
Pinb-D1b	AAA	TGG	TGG	AAG	**A**GC	GGC	TGT	GAG	CAT	GAG	GTT	CGG	GAG	AAG	TGC	TGC	AAG	CAG▼CTG		AGC	CAG
(hard)	K	W	W	K	**S**	G	C	E	H	E	V	R	E	K	C	C	K	Q	L	S	Q
Pinb-D1c	AAA	TGG	TGG	AAG	GGC	GGC	TGT	GAG	CAT	GAG	GTT	CGG	GAG	AAG	TGC	TGC	AAG	CAG	C**C**G	AGC	CAG
(hard)	K	W	W	K	G	G	C	E	H	E	V	R	E	K	C	C	K	Q	**P**	S	Q
Pinb-D1d	AAA	TGG	**A**GG	AAG	GGC	GGC	TGT	GAG	CAT	GAG	GTT	CGG	GAG	AAG	TGC	TGC	AAG	CAG▼CTG		AGC	CAG
(hard)	K	W	**R**	K	G	G	C	E	H	E	V	R	E	K	C	C	K	Q	L	S	Q

Fig. 1 DNA and deduced amino acid sequence for a portion of puroindoline b. In addition to the previously reported glycine to serine mutation at position 46 (Giroux and Morris 1997), direct sequencing of PCR-amplified genomic DNA revealed two other mutations that also result in hard endosperm. These are a leucine to proline change at position 60 and a tryptophan to arginine change at postion 44. Mutation sites are *underlined* and in *bold*, and proposed allelic denotations for the new mutations are shown to the *left. Shaded areas* in the DNA sequence correspond to the restriction site of *Pvu*II, which was used for identifying the *Pinb-D1c* mutation. Sequence analysis among multiple members of all allelic classes showed no variation in either upstream (0–41) or downstream (> 63) sequences

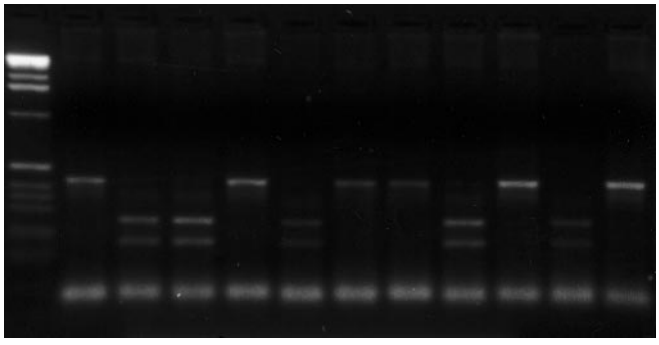

Fig. 2 Agarose gel electrophoresis of PCR-amplified puroindoline b cut with *Pvu*II. As described in the text, failure to cut puroindoline b (448 bp) into a 264-bp and 184-bp fragment can be taken as evidence for the proline 60 mutation (*Pinb-D1c*). The *far left lane* is a 1-kb ladder (Gibco BRL), then from *left to right*: Avle (*c*), Bastian (*b*), Brakar (*b*), Reno (*c*), Sport (*b*), Tjalve (*c*), Bjørke (*c*), Folke (*b*), Portal (*c*), Rudolf (*b*) and Terra (*c*). Each cultivar's *Pinb-D1* allele is shown in *parentheses*

was developed. This endonuclease has only one restriction site in the entire sequence of puroindoline b, at the site of the proline 60 mutation (as indicated in Fig. 1). We therefore expect *Pvu*II to cut PCR-amplified puroindoline b only from genotypes not having the proline 60 mutation, and failure to cut at the restriction site can therefore be taken as evidence for the presence of this mutation (Fig. 2). The uniqueness of this single nucleotide mutation in puroindoline b was confirmed by sequencing 2 additional genotypes indicated to have the proline 60 mutation by the restriction analysis (data not shown). The *Pinb-D1c* allele was present in three spring and three winter wheat cultivars currently grown in Norway (Table 1) and almost one-third of the hard wheat germplasm (89 of 295, Table 2). Interestingly, six out of seven Scandinavian and Finnish landraces included in this study had this mutation (Table 3).

Identification of a third hardness mutation in puroindoline b

After the above described screening for *Pina-D1b*, *Pinb-D1b* and *Pinb-D1c* alleles, three hard winter wheats turned out to have none of these known mutations. These were Bercy (Netherlands), Mjølner and Sleipner (both Sweden). DNA sequencing revealed that all three had a third mutation in puroindoline b, characterized as involving a tryptophan to arginine substitution at position 44. This new allele was denoted *Pinb-D1d* and is shown in Fig. 1. Also for these lines, complete sequencing of puroindoline a proved that no mutations were present (wild-type, *Pina-D1a*) (data not shown).

Table 3 Scandinavian and Finnish landraces present among the 328 germplasm lines. The Swedish landrace Dala was the only one expressing the *Pinb-D1b* allele. All the others had the *Pinb-D1c* allele. See Fig. 2 for description of the hardness alleles of puroindoline b

Landrace	Nordic Gene Bank Accession Number	Origin	Hardness Index ± SD	*Pinb-D1* allele
Monola ME1301	43	Finland	70 ± 22	*c*
Sarkalahti ME0101	120	Finland	72 ± 18	*c*
Kr. Finset	21 218	Norway	76 ± 17	*c*
Landvårkveite	2 129	Norway	73 ± 16	*c*
Lantvete från Dalarna	6 673	Sweden	70 ± 19	*c*
Lantvete från Halland	6 674	Sweden	76 ± 19	*c*
Dala	9 708	Sweden	74 ± 17	*b*

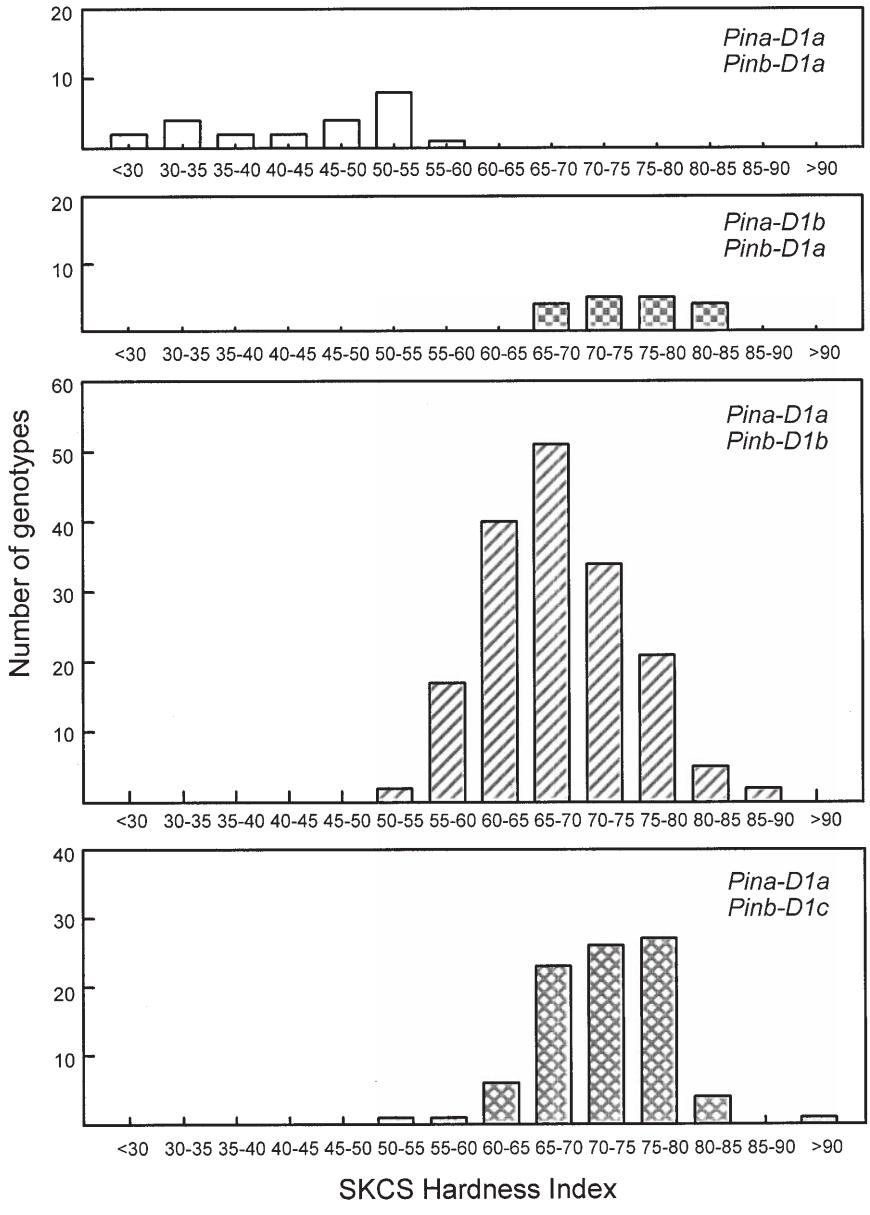

Fig. 3 SKCS hardness distribution for the four allelic combinations of puroindoline a and b present in 302 spring wheats grown under a common environment in Norway. *Pina-D1a* is the wild-type puroindoline a, and *Pina-D1b* the null-mutation. The different alleles of puroindoline b are described in Fig. 1. No recombination was detected between puroindoline a and b alleles

As can be seen in Table 1, all four alleles of puroindoline b are present among the currently grown wheat cultivars in Norway. In Table 2, the 328 hard germplasm lines are grouped by geographic origin and type of hardness mutation. It can be seen that both *Pinb-D1b* and *Pinb-D1c* are widely distributed throughout the world, whereas the *Pinb-D1d* allele is restricted to the 3 Northern European winter wheats described above (Table 1 and 2).

Mutations in puroindoline a or b have a profound effect on hardness

The dramatic effect of puroindoline mutations on wheat grain texture is clearly illustrated in Fig. 3, which shows the SKCS hardness distribution for each allelic combination of puroindoline a and b in the spring wheats analyzed. Data for the much smaller number of winter wheats exhibited the same pattern (data not shown). In all the lines investigated by PCR, restriction analysis or sequencing, no recombination was observed between the puroindoline a and b mutation types.

The mean SKCS Hardness Indexes for the four allelic combinations of puroindoline b shown in Fig. 3 were 45 (*Pina-D1a/Pinb-D1a*), 75 (*Pina-D1b/Pinb-D1a*), 68 (*Pina-D1a/Pinb-D1b*) and 72 (*Pina-D1a/Pinb-D1c*), respectively. Further research should be done to test whether the observed differences holds true or not.

Discussion

All hard wheat varieties included in this survey could be explained by a mutation in either puroindoline a or b (Tables 1 and 2, Fig. 3). Our data are therefore in good

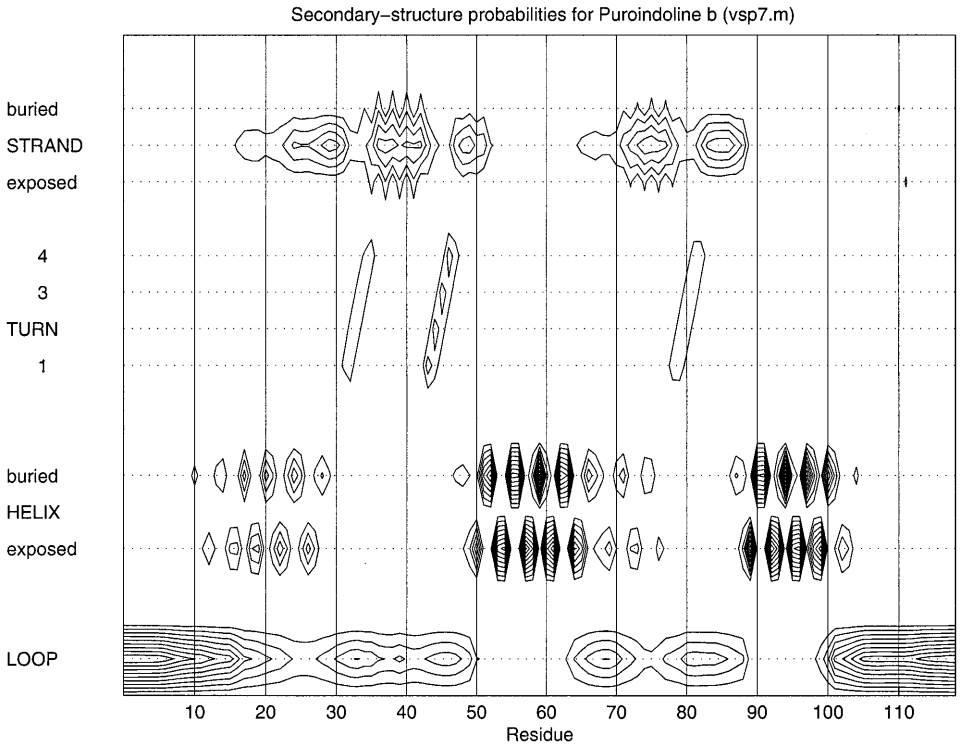

Fig. 4 Contour plot of secondary structure probabilities for puroindoline b, as computed by the Protein Sequence Analysis server at "http://bmerc-www.bu.edu/psa/". The analysis is based on probabilistic discrete state-space models and optimal filtering and smoothing algorithms as described by Stultz et al. (1993). The four rows denoted "TURN 1, (2), 3, 4" denote four-residue tight turns. The probabilities of each residue being in each of the structural states are depicted using contour lines of constant probability in increments of 0.1. Areas surrounded by many contour lines are regions of high probability, while areas outside of the contours have low probabilities of less than 0.1. The tryptophan-rich domain consists of residue 39–45 and is predicted to be in a beta-sheet conformation, as reported by Giroux and Morris (1997)

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                          3           4           5           6           7
                          5           5           5           5           5
Puroindoline b      FPVTWPT-KWWKGGCEHEVREKCCKQLS-QIAPQCRCDSIRRVIQGRL
Puroindoline a      FPVTWRWWKWWKGGC-QELLGECCSRLG-QMPPQCRCNIIQGSIQGDL
Tryptophanin 3B3    FPITWP-WKWWKGGCE-EVRNECCQLLG-QMPWECRCDAIWRSIQHEL
Tryptophanin 3B3T   IPITWP-WKWWKGGCESEVRSQCCMELNIQIAPHCRCKAIWRAVQGEL
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Fig. 5 Part of the wild-type "soft" puroindoline b sequence (residues 35–80), aligned with puroindoline a and two recently published oat "tryptophanins" (Tanchak et al. 1998). Residues corresponding to the three known mutation sites in puroindoline b (*shaded*) are conserved in all four proteins, indicating that these may be crucial for protein function

agreement with earlier results published by Giroux and Morris (1997, 1998) and provide support that puroindoline a and b comprise the molecular basis of the *Hardness (Ha)* locus.

The *Ha* locus is considered to be the major genetic factor controlling hardness, but there is no doubt other genetic factors contribute as well (see for instance Baker 1977; Baker and Sutherland 1991; Bebyakin 1982; Campbell et al. 1999). In this study, varieties with soft puroindoline a and b alleles (*Pina-D1a/Pinb-D1a*) had hardness indexes ranging from 20 to 58, and likewise the genotypes with hardness mutations in puroindoline a or b ranged from 50 to 91 in the SKCS Hardness Index. Obviously, this within-class variation is unlikely due to environment alone, and some other genetic factors must be considered.

The three-dimensional structure of puroindolines has yet to be assessed experimentally, but Marion et al. (1994) found a relatively good alignment with a wheat lipid transfer protein (LTP), except for the region corresponding to the tryptophan-rich domain. The structure of LTP has been determined by multidimensional ^{1}H NMR (nuclear magnetic resonance), and using it as a template, they concluded that the puroindoline structure most likely consists of a bundle of four helices, linked together by flexible loops. In their model the tryptophan-rich domain would be included in the loop between helix one and two, and probably stabilized by a disulphide bond. Giroux and Morris (1997) predicted the tryptophan-rich domain to be in a beta-sheet conformation. These data are also supported by the secondary structure prediction shown in Fig. 4, obtained by using the probabilistic discrete state-space modeling algoritm described by Stultz et al. (1993).

Sequence alignment (Fig. 5) shows that the residues corresponding to the three known hardness mutation sites in puroindoline b are conserved in both puroindoline a and two recently published puroindolines from oat, named "tryptophanins" (Tanchak 1998). Considering this together with the structural information discussed above, all three mutations in puroindoline b may be explained as a loss of function. The glycine to serine change at position 46 (*Pinb-D1b*) occurs in a loop between the tryptophan-rich domain and the second helix, and the reduced flexibility introduced by the serine residue may, as

proposed by Giroux and Morris (1997), alter the lipid binding abilities of the tryptophan-rich domain. This is probably also the case with the mutation at position 44 (*Pinb-D1d*), which actually occurs within the tryptophan-rich domain, substituting the last tryptophan residue with the positively charged arginine. The leucine to proline change at position 60 (*Pinb-D1c*) occurs in a region highly predicted to be in an alpha helix conformation, and the rigidity introduced by the proline residue would most likely alter the helix conformation. Proline is well-known as an alpha helix breaker due to its irregular side chain constraints and sterics: the side chain will be forced into the space occupied by the helix backbone, and the methyl group at the position normally occupied by an amide proton will disrupt the hydrogen bonding network and sterics of the helix (Piela et al. 1987; Yun et al. 1991).

The way puroindolines act to affect the endosperm texture of wheat is still unknown, but the earlier reported structural similarity with lipid transfer protein (Marion et al. 1994), the tryptophan-rich domain and the association with the surface of the starch granule all indicate an interaction of puroindolines with polar lipids. This is also supported by the findings of Greenblatt et al. (1995) that two classes of bound polar lipids exhibit the same pattern of occurrence as friabilin, i.e. abundant on the surface of water-washed soft wheat starch, but scarce on hard. We may therefore anticipate that the possible structural alterations imposed by the three known mutations in puroindoline b may affect grain hardness through reduced affinity for bound polar lipids.

The lipid binding ability of puroindolines has also been assessed experimentally, indicating that low concentrations of puroindolines can overcome the foam destabilization effect of certain lipids (Wilde et al. 1993). Dubreil et al. (1997) further showed that puroindoline a binds phospholipids and glycolipids tightly, whereas puroindoline b only binds negatively charged phospholipids and forms loose lipoprotein complexes with glycolipids.

One possible role of the puroindolines in determining hardness in wheat could be through stabilization of the amyloplast lipid bilayer membrane during dessication of the grain. Evidence for this comes from experiments with freeze-drying of wheat grains at different developmental stages, showing that freeze-drying of immature grains from hard wheat resulted in soft endosperm, as opposed to the hard endosperm that resulted when grains of the same developmental stage were allowed to dry slowly at room temperature or 40°C (Bechtel et al. 1996).

During maturation of the wheat grain, the developing starch granules are surrounded by the amyloplast membrane. If the hypothesis of the membrane-stabilizing effect of puroindolines holds true, it can explain the differences in binding strength between the starch granules and protein matrix in soft and hard wheat. During dehydration of the endosperm, the puroindolines would prevent the amyloplast membrane from collapsing totally, and thus make the starch granules be separated from protein matrix with a thin layer of membrane remnants. In hard wheats, however, the mutated puroindolines would not be able to stabilize the membrane during maturation of the grain, causing a more direct contact and tighter binding of the starch granules to the protein matrix. The higher levels of friabilin (puroindoline a and b) bound to water-washed starch in soft wheats can easily be explained by the proposed hypothesis as being remnants of the puroindoline-stabilized amyloplast membrane.

Since the failure to express puroindoline a has a nearly similar effect on hardness as a mutation in puroindoline b (Fig. 3), both puroindoline a and b must act together to form soft endosperm. However, experiments designed to detect any 30-kDa heterodimer of puroindoline a and b have been unsuccessful (Giroux and Morris 1998). Since puroindoline a and b have different tryptophan-rich domains (WRWWKWWK and WPTKWWK, respectively) and bind lipids differently, as reported by Dubreil et al. (1997), they probably serve different roles in stabilizing the amyloplast membrane. Further research is needed to gain a better understanding of the molecular basis for how the puroindolines act to confer soft endosperm and to test whether the proposed membrane-stabilizing hypothesis holds true.

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