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ORIGINAL PAPER

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Chromosome mapping and identification of amphiphilic proteins of hexaploid wheat kernels

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Abstract Amphiphilic proteomic analysis was carried out on the ITMI (International Triticae Mapping Population) population resulting from a cross between "Synthetic", i.e.: "W7984" and "Opata". Out of a total of 446 spots, 170 were specific to either of the two parents, and 276 were common to both. Preliminary analysis, which was performed on 80 progenies (Amiour et al. 2002a), was completed here using a total of 101 selfed lines. Seventy two Loci of amphiphilic spots placed at LOD = 5 were conclusively assigned to15 chromosomes. Some spots mapped during the first analysis were eliminated because of the significant distortion segregation observed in the second analysis. Group-1 chromosomes had by far the greatest number of mapped spots (51). Using the Quantitative Trait Loci (QTLs) approach, analysis of the quantitative variation of each spot revealed that 96 spots out of the 170 specific ones showed at least one Protein Quantity Locus (PQL). These PQLs were distributed throughout the genome. With Matrix Laser Desorption Ionisation Time Of Flight (MALDI-TOF) spectrometry and Database interrogation, a total of 93 specific and 41 common spots were identified. This enabled us to show that the majority of these proteins are associated with membranes and/or play a role in plant defence against external invasions. Using multiple-regression analysis, other amphiphilic proteins, in addition to puroindolines, were shown to be involved in variation in kernel hardness in the ITMI population.

Keywords Wheat · Endosperm proteins · Amphiphilic proteins · Chromosome mapping · Grain hardness

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Introduction

The interface between the protein matrix and starch granules of bread-wheat endosperm has a great importance to explain the fundamental mechanism by which the endosperm texture of bread wheat is expressed. Indeed, at this interface—and particularly on the surface of soft bread-wheat starch granules—Greenwell and Shofield (1986) reported the presence of a type of protein that they named friabilin. The predominant components are puroindolines and "Grain Softness Proteins" (GSP). Puroindolines are the most recognised : they are very basic (pI=11), amphiphilic proteins. The amphiphilic proteins have both hydrophobic and hydrophilic sides and are soluble in the detergent Triton X-114 due to their affinity to lipids and their binding properties to membranes (Marion et al. 1994). Two puroindoline isoforms were characterised, Pin-a and Pin-b (Dubreil et al. 1997), with differing lipid-binding properties: Pin-a is able to bind closely to both wheat phospholipids and glycolipids, whereas Pin-b only interacts with negatively charged phospholipids. The difference between Pin-a and Pin-b was assumed to cause a difference in bread-kernel hardness, i.e. Pin-a was associated with softness and Pin–b with hardness. Recent studies on puroindolines were carried out using a proteomic approach with the aim of revealing their diversity (Branlard et al. 2003) and several isoforms were identified. Other studies have demonstrated that the explanation for the variation in kernel hardness is not as simple as we might suppose and that several mutations of Pin-a and particularly of Pin-b can affect this characteristic (for a review see Morris 2002). Moreover, Morris et al. (1994) have reported that friabilins may be a mixture of 10 to 20 distinct components, some of which are 'neutral' and others basic. These components could belong, like puroindolines, to the amphiphilic class, and may play an important role in the physico-chemical explanation of grain hardness. These proteins have been explored in a previous proteomic analysis, using an ITMI population (Amiour et al. 2002a) resulting from a cross between Synthetic

"W7984" and "Opata". The aim of the present complementary study was: (1) to determine more precisely the chromosomal location of the segregating spots, (2) to get additional data on their quantitative variation and information about their function, and (3) to study the relationship between the segregating spots and the variation in grain hardness in the ITMI population.

Materials and methods

Plant material

One hundred and one recombinant Inbred Lines (RILs) of the ITMI population (Amiour et al. 2002a) were used in our study. Plants were grown at the INRA Plant breeding station at Clermont Ferrand, France. Grains were harvested from bagged ears in order to prevent cross pollination. Wholemeal flour obtained with a Udy Cyclone mill was used for the extraction of amphiphilic proteins.

Methods

Extraction and protein quantitation

Due to their affinity to lipids, amphiphilic proteins were separated from the soluble proteins by phase-partitioning using a non-ionic detergent, i.e. Triton X-114. The upper phase which is rich of soluble proteins was discarded, but some soluble proteins may however occur at the interface of the two phases. Two hundred and fifty milligrams of amphiphilic proteins were extracted according to the protocol described by Marion et al. (1994) with the modification described in the first study (Amiour et al. 2002a): Tris buffer and Tris TX-114 were combined in the same extraction buffer. Protein quantitation was based on the protocol described by Amiour et al. (2002a) in which 250 μl of a first solution (urea 9 M and CHAPS 4%) was used to solubilize proteins. After quantitation, protein solubilisation was completed by a second solution containing CHAPS 4%, urea 6 M, thiourea 3 M, pharmalytes 3–10 and resolytes 6–11 1.25% and DTT 2.5%. Because wholemeal flour was used, some abundant amphiphilic proteins, in the aleurone layer or in the embryo, may occur in the amphiphilic extract.

2D gel-electrophoresis

The protocol of 2D gel-electrophoresis (pH 6–11) was performed according to Branlard et al. (2003) using 130×3 mm IPG gel-strips in order to reveal the majority of the amphiphilic proteins. For strip rehydration, a volume equivalent to 30 μg of each protein extract was added to the extraction solution, bringing the final volume to 250 μl. The running parameters of IEF were 300 V (1 h), 1,930 V (1 h 30 min), 1,420 V (19 h) making a total of 30 kVh. Staining procedure was according to the silver-staining kit (Amersham Pharmacia Biotech). From 3 to 6 replicates of 2D gel-electrophoresis were performed for each line to characterise, with satisfying reproducibility, the amphiphilic proteins. The gels were scanned and analysed using Melanie 3 software (Genbio, Geneva, Switzerland).

Amphiphilic Protein Loci (APL) mapping

Each segregating spot, being either absent (noted A) or present (noted B) in the progeny was used for mapping. The assignment of Amphiphilic Protein Loci (APLs) on their corresponding chromosomes was made using a framework map with Mapmaker/Exp 3.0 b (Lincoln et al. 1992). APLs were assigned to intervals between the anchor markers using the Mapmaker 'assign' command at LOD 3.0 and a recombination fraction of 0.35. After assignment, the APLs were placed at LOD 5.0. Segregation distortion was calculated using an in-house S+ program. Markers below the threshold of LOD 3.0 and above a recombination fraction of 0.35 were left unassigned.

Protein Quantity Loci (PQL) analysis

The protein quantity of each spot in a given line was obtained as the average (over the replicates) of the percentage of spot volume. These variates were submitted to Quantitative Trait Loci (QTL) analysis specifically termed Protein Quantity Loci (PQL) analysis. PQL analysis was performed for each segregating spot using Mapmaker/QTL 1.1b (Lander et al. 1987). A major PQL was considered significant when its LOD score was equal to or over 2.0.

Protein identification

For protein identification, first, analysis was carried out on spots excised from gels stained with Coomassie Brilliant Blue G 250. Samples were washed until destained in 100 μl of 50% acetonitrile/50 mM hydrogenocarbonate pH = 8 solution. Gel pieces were then dried under vacuum centrifugation for 30 min. After rehydration in 10 μl of 50-mM ammonium hydrogenocarbonate pH = 8 containing 0.5 μg of Promega porcine trypsin, samples were incubated overnight (16–18 h) at 37°C. Peptide fragments from digested proteins were then subjected to MALDI-TOF-MS (Applied Biosystems, Voyager DE super STR) for peptide finger-printing. This instrument is equipped with N_2 laser (337 nm, 3 Hz, 3 ns impulsion). Samples were irradiated in a matrix (α-cyano-4-hydroxycinnamic acid 3 mg/ml). Spectra were acquired in a reflectron mode using a mass range of 900 to 3,000 Da and a delay extraction time of 120 ns. Internal calibration was performed using trypsin peptide masses in a mass range of 500 to 5,000 Da.

The second analysis was made on silver-stained spots (silver staining compatible with MALDI-TOF-MS) as described by Blum et al. (1987). Internal calibration for these spots had a mass range between 500 and 3,000 Da.

The peptide masses were then used to search in the SWISS-PROT and NCBI databases using http://129.85.19.192/profound_bin/WebProFound.exe software. The candidate proteins had their probability of occurrence equal or close to 1 (Zhang and Chait 2000) and, for the majority, a high score of confidence level (Z score > 1.65) (Tang et al. 2000).

Determination of kernel hardness

Grain was milled using a cyclotec lab mill (Tecator) for wholemeal production. Grain hardness of the 101 RILs was estimated by Near Infrared Reflectance (NIR-Percon Inframatic 8620) according to AACC Approved methods 39-70A (American Association of Cereal Chemists 1995).

Statistics

Stepwise multiple regression was performed in order to predict kernel hardness with amphiphilic proteins as explanatory variates. The REG procedure of SAS was used to perform multiple regression.

Results

2D-gel image analysis of a mixture of the two parents of the ITMI population revealed 446 spots ranging from 13 to 60 kDa and a pI between 6 and 11. Among these, 59

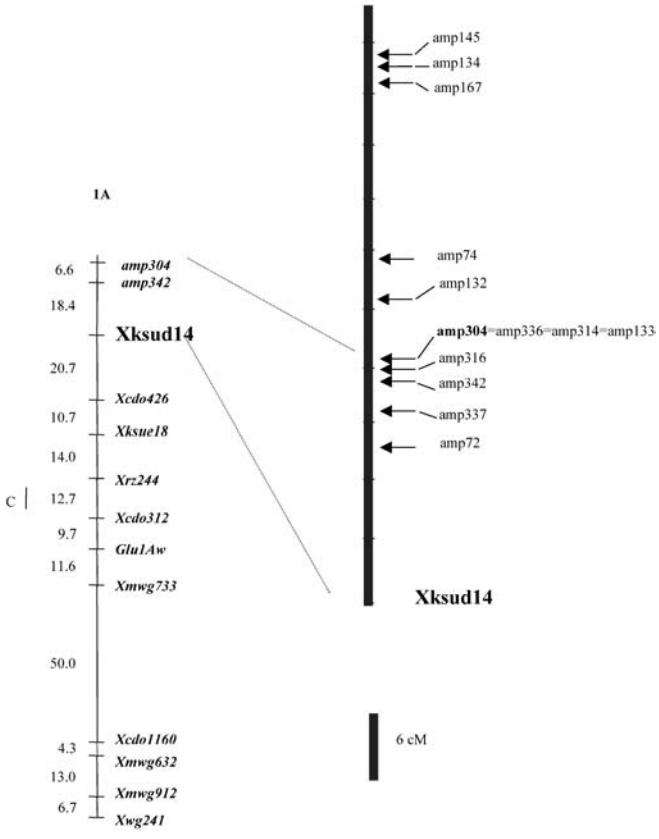


Fig. 1 APLs mapped on chromosome 1A of the ITMI population. = : spots of identical segregating profile

were specific to "Opata" and 111 specific to Synthetic "W7984", giving a total of 170 differential spots.

Allelic and isoform characterisation

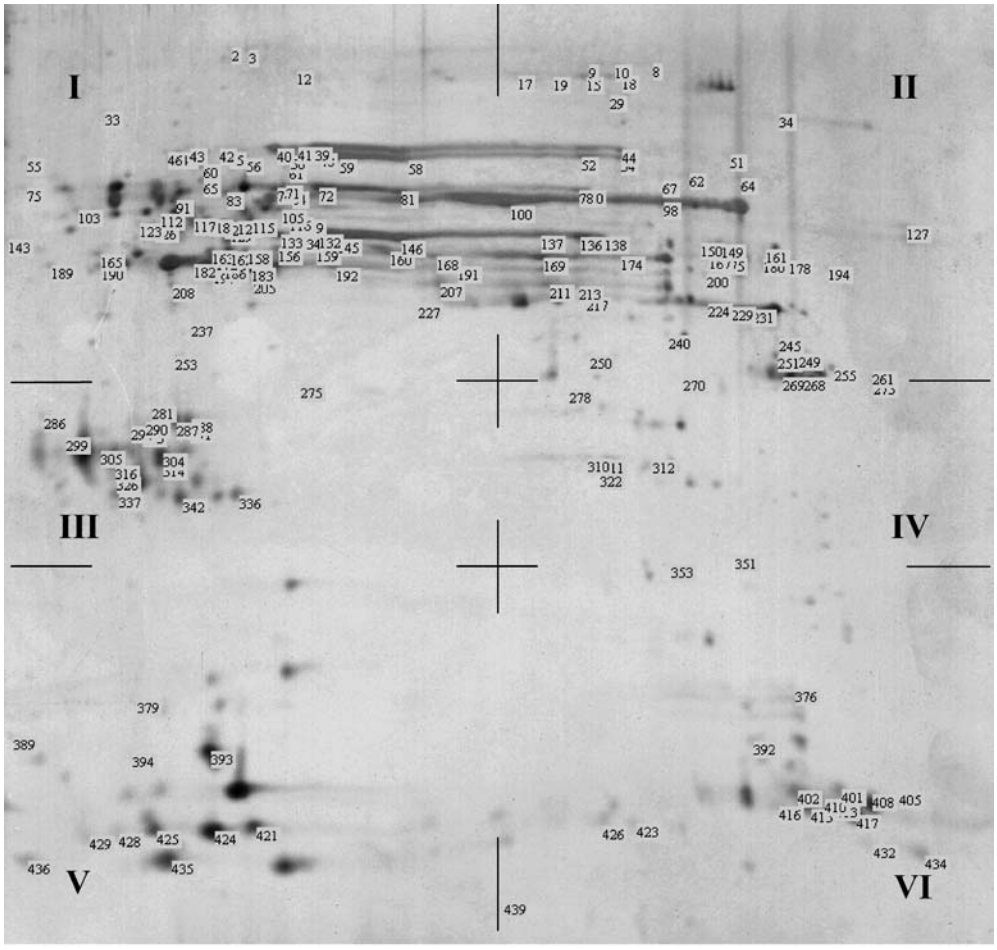
Chromosome 1A

Nineteen spots were assigned to chromosome 1A, 13 at the distal extremity of the short arm, five at the centromere zone and one at the distal extremity of the long arm, and a total of 14 spots were identified. After assignment, spots at the short arm were placed at LOD = 5 (Fig. 1). The genetic distances showed the proximity of genes coding for these proteins of which four mapped at the same position (amp133, amp304, amp314 and amp336) and very close to amp316 and amp342. Identification showed two main types of proteins: (1) Glutathione-S-transferase (GST) for amp304, amp316 and amp342; and (2) Ras-related-protein for amp336 and amp314. These spots belong to the same zone of the gel: zone III (Fig. 2). The four spots (amp133, amp304, amp336 and amp314) inherited together are specific to the "Synthetic" parent. These spots are either allele products of the same gene or closely dependant gene products. The latter case was confirmed with the identification of two other spots as GSTs (amp316 and amp342) located very close to the first group. Considering the role of the two main proteins, it will be noted that plant GST has agronomic and technological potential as it is involved in herbicide de-toxification (Sheehan et al. 2001), and also in protein polymerisation by allowing the formation of disulphide bridges (Taylor et al. 1994). Rabs are proteins associated with membranes belonging to a small GTPase family required for membrane fusion (Mesa et al. 2001). To-date, 40 different Rabs have been identified each specifically associated with a particular organite or metabolic pathway. All the other spots apart from amp134, which was identified as a soluble cytosolic protein (alcohol dehydrogenase), were identified as proteins associated with membranes and involved in defence response or cell division (Table 1). Because of their link to membranes, these proteins—in addition to puroindolines—, may contribute to grain-hardness variation.

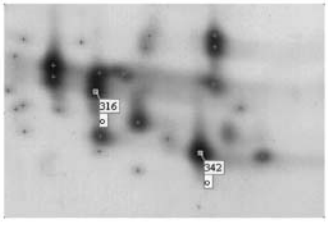
Chromosome 1B

From the 19 spots assigned at the distal extremity of 1BS (Fig. 3), only two (amp44 and amp54) did not map on loci due to their conflicting position. A cluster of four loci was found at a distance of 1.5 cM: amp41 was located at the first locus; amp288, amp291 and amp39 at the second; amp45, amp40 and amp162 at the third, and amp56 and amp48 at the last. Out of the 19 APLs, 14 were identified, for example amp39, which corresponded to a tyrosine decarboxylase, amp40, which corresponded to a mixture of an "isocitrate dehydrogenase" and an "embryonic abundant protein group-3 precursor", amp45 to a "lysophospholipase isologue", amp50 to a "aspartate carbamoyl transferase-3 precursor", amp56 to a protein bound to the guanine "guanine nucleotide-binding protein" and amp162 to a "carbonic anhydrase, chloroplast precursor". The last protein occurs at the chloroplast stroma; it catalyses carbon hydration in bicarbonate and may be associated with the Calvin enzymatic complex located at the thylakoid membranes (Jebanathirajah and Coleman 1998).

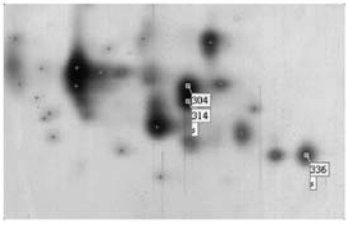
Several isoforms of the isocitrate dehydrogenase exist at cytoplasmic, mitochondrial and chloroplastic locations. They are involved in the assimilation of ammonia by plants (Galvez et al. 1998). The precursor of the embryonic protein is induced by drought stress (Moons et al. 1995). Phospholipases are linked to many cellular processes such as hormonal signals during stress, membrane synthesis and lipid degradation (Wang 2000). Likewise a gene coding for a phospholipase involved in plant defence was previously identified (*Pdl*) in nullitetrasomic lines of the wheat cultivar "Chinese spring" located at chromosome 1B (Li et al. 1999). The guanine nucleotide-binding protein (amp56) is associated with a cytoplasmic membrane and transmits signals from transmembrane receptors to the cell. In turn, these signals produce a cascade of reactions and changes in cell metabolism (Hong et al. 1990). Tyrosine decarboxylase



a : gel of a mixture of the two ITMI populations parents



b : zone III " Opata " gel



c : zone III " Synthetic " gel

Fig. 2 (**a**) Gel image of a 50:50 mixture of "Synthetic" and "Opata" segmented in six zones (I to VI). Identity number of the 170 segregating and few common spots is shown. (**b**) Zone III in "Opata gel". (**c**) Zone III in "Synthetic" gel

serves as a precursor to an important class of plant defence response-metabolites. Amides induced by this enzyme are usually found as integral cell-wall components and form a physical barrier against pathogens (Facchini et al. 1999).

Chromosome 1D

Among the 12 spots assigned to the short arm of chromosome 1D, nine spots were placed at LOD = 5 (Fig. 4) and 11 were identified. Two were involved in flavonoid metabolism: amp120 corresponded to a "Chalcone synthase" and amp183 to a "dihydroflavono-4-reductase". They are associated with membranes and located at two different loci at a distance of 8 cM from each other. Amp190 was identified as a putative P-50 cytochrome. Genes coding for a "chalcone synthase" (Chs) and a "flavonol-methyl-transferase" (Fmt) have already been identified on the same chromosomal arm (Li et al. 1999). In addition a protein marker (amp189) was identified as agamous like the MADS box protein which is a probable transcription factor active in inflorescence development and floral organogenesis. Moreover, a "Ras" protein (amp253), a peroxidase (amp116) and a cystein proteinase precursor (amp182) were identified. Amp115 corresponded to a transcription factor OBF4, which was reported to play a role in the plant pathogen response (Büttner and Singh 1997).

Table 1 Specific spots identified by peptide mass fingerprinting

Spot ID	MTL/ PQL[a]	Protein identity	pI/MW		SC (%)[b]	Accession	Species
			Theor	Obs			
amp10	1A/-	Glycine hydroxymethyltransferase-like protein	8.3/51.24	8.9/56	41	NP194978	*Arabidopsis taliana*
amp33	-/1A	Glucose-1-phosphate adenyltransferase small subunit precursor	5.7/54.02	5.8/51	68	P15280	*Oryza sativa*
amp34*		Putative exonuclease	9.2/41.64	10.01/55	22	AAD13713	*Arabidopsis thaliana*
amp39*	1B/1B	Tyrosine decarboxylase	7.9/49.70	7.01/49	15	AAA33859	*Petroselinum crispum*
amp40	1B/1B	Isocytrate dehydrogenase	6.2/47.53	6.9/46	39	S42892	*Nicotiana tabacum*
		Embryonic abundant protein group-3 precursor	6.7/51.17		36	S41428	*Soybean*
amp41*	1B/1B	Transfactor-like	6.7/45.01	6.90/48	16	NP187053	*Arabidopsis thaliana*
amp45	1B/1B	Actin 104	5.6/37.23	6.5/45	40	P93375	*Nicotiana tabacum*
		Lisophospholipase isolog, putative	7.2/43.85		53	NP177867	*Arabidopsis thaliana*
amp47	1B/1B	26S protease regulatory subunit a homolog	5.9/49.87	6.1/45	62	P46466	*Oryza sativa*
amp48*	1B/1B	Chalcone synthase 1	6.7/43.43	7.04/47	22	Q9ZS41	*Daucus carota*
amp50	1B/1B	Aspartate carbamoyltransferase 3 precursor	6.4 /45.06	6.9/45	68	Q43064	*Pisum sativum*
amp51	6A/6A	Putative serine/threonine kinase similar to NAK	9.6/47.22	9.70/47	24	AAK92662	*Oryza sativa*
amp54	1B/1B	Ferredoxin-NADP reductase	9.1/42.4	8.4/46	44	O23877	*Oryza sativa*
		Putative receptor-like kinase Xa21 binding-protein 3	9.3/49.6		37	BAB63825	*Oryza sativa*
amp56	1B/1B	Guanine nucleotide binding protein alpha-1 subunit	6/45.16	6.6/45	45	P18064	*Arabidopsis thaliana*
amp60	-/1B	Alpha-amylase isozyme 3A precursor	6.8/48.85	6.2/44	57	P27932	*Oryza sativa*
amp61*		Putative O-deacetylbaccatin III-10–0-acetyltransferase	6.6/46.54	6.84/45	14	AAL83353	*Oryza sativa*
amp64*		Lipoxygenase	9.6/42.33	9.77/43	24	AAL69951	*Oryza sativa*
amp65	-/6B	Mitogen-activated kinase. Homolog 2	6.2/43.96	6.2/41	37	Q39022	*Arabidopsis thaliana*
		Chalcone synthase 1	6.2/43.23		49	P24826	*Soybean*
amp72*	1A/1A	Cell-division protein PtsZ chloroplast homolog	6.9/45.86	7.03/42	10	NP200339	*Arabidopsis thaliana*
amp80*	-/1A	Zeta-carotene desaturase precursor	9.1/48.16	8.78/42	14	AAG14399	*Oryza sativa*
amp83	-/1A	Putative aldo/keto reductase	5.8/37.26	6.5/40	34	AAD39334	*Arabidopsis thaliana*
amp91	6D/6D	Putative retrotransposable elements TNP2 protein	5.6/41.85	6.1/40	62	BAB16340	*S. Oryza sativa*
		Alternative repiratory patway oxidase (similarity)	6.9/40.45		80	T51615	*Arabidopsis thaliana*
amp105	-/6B	DNA-Directed RNA polymerase alpha chain	7/39.28	6.8/38	37	P93962	*Psathyrostachys rupestris*
amp112*	6D/6D	Stearoyl-ACP desaturase I	6.1/44.7	5.97/39	10	CAC80359	*Helianthus annuus*
amp115*	1D/1D	Transcription factor OBF4	6.7/42.17	6.61/38	20	S48121	*Arabidopsis thaliana*
amp116*	1D/1D	Peroxidase, putative	6.1/38.34	6.84/38	14	NP175380	*Arabidopsis thaliana*
amp118*	6D/6D	Hypothetical protein	6.7/42.60	6.34/38	9	T01717	*Arabidopsis thaliana*
amp119*	6A/6A	DP-2 transcription factor—like	7.1/32.96	6.92/38	28	NP195867	*Arabidopsis thaliana*
amp120	1D/1D	Chalcone synthase C2	6.3/43.91	6/39	50	P24825	Zea mays
		Hypothetical protein	5.6/43.87		32	NP193641	*Arabidopsis thaliana*
amp121	6A/6A	Cell-division control protein homolog 1	6.5/34.37	6.4/37	55	P29618	*Oryza* sativa
amp123*	1D/1D	Putrescine N-methyltransferase	5.9/35.13	5.86/38	15	BAA74543	*Nicotiana sylvestris*
amp126	6A/6A	Chorismate mutase precursor	5.9/38.4	5.9/37	57	P42738	*Arabidopsis thaliana*
		Oxygen evolving enhancer protein 1	6.3/35.3		68	O49079	*Fritilaria agrestis*
amp132*	1A/1A	12-Oxophytodienoate reductase (OPR2)	7.1/42.08	7.03/37	20	NP177795	*Arabidopsis thaliana*
amp133*	1A/1A	Hypothetical protein	7.0/44.11	6.78/37	22	AAFO2848	*Arabidopsis thaliana*
amp134	1A/1A	Alcohol dehydrogenase	6.3/42.33	6.91/37	17	P00333	*Zea mays*
amp143*	-/1D	Putative apospory-associated protein C	5.1/38.25	5.20/36	20	AAL87174	*Oryza sativa*
		Replication protein A 30kDa	5.6/30.16		15	BAB40535	*Oryza sativa*
amp156*	6D/-	Allergen Amb a I.2 precursor	6.6/44.89	6.77/36	15	B53240	*Common ragweed*
amp158*	1B/1B	Amino-cyclopropane carboxylic acid oxidase	6.0/37.21	6.57/36	12	AAA33273	*Dianthus caryophyllus*
amp162	1B/1B	Carbonic anhydrase, chloroplast precursor	6.4/35.2	6.5/35	45	P27141	*Nicotiana tabacum*
amp163		Putative chloroplast RNA binding protein precursor	5.5/33.98	6.3/36	51	NP181084	*Arabidopsis thaliana*
		Aldose reductase	6.5/36.31		55	P23901	*Hordeum vulgare*

Table 1 (continued)

Spot ID	MTL/ PQL[a]	Protein identity	pI/MW		SC (%)[b]	Accession	Species
			Theor	Obs			
amp165*	1B/1B	Alcohol dehydrogenase	5.6/34.43	5.67/36	20	AAG42523	*Hordeum vulgare*
amp178*	1A/-	Maturase K	10.0/34.24	10.06/35	20	AAK94648	*Talinum caffrum*
amp182*	1D/1D	Putative cysteine proteinase precursor	6.8/41.48	6.21/35	16	AAL49820	*Arabidopsis thaliana*
amp183	1D/1D	Dihydroflavonol-4-reductase	6/41.44	6.7/35	32	P51103	*Callistephus chinensis*
amp186*		Putative protein	7.2/39.09	6.45/35	14	CAC39063	*Oryza sativa*
amp189*	1D/-	Agamous-like MADs box-protein AGL homolog	6.6/30.01	5.42/35	29	Q04067	*Spina alba*
amp190	1D/1D	ESTs AU056036, C72753, AU056035, similar to putative cytochrome P-450	6.7/46.98	5.7/35	58	BAA83370	*Oryza sativa*
amp191*		Homeobox-leucine zipper protein HAT9	7.8/30.80	7.93/35	13	P46603	*Arabidopsis thaliana*
amp192*	1B/-	Peroxidase	7.1/37.71	7.14/35	23	CAA6696	*Arabidopsis thaliana*
amp194*	1A/-	ADP,ATP carrier ptotein (ADP/ATP translocase)	9.9/36.98	10.30/35	27	P31692	*Chlorella kessleri*
amp197*	1D/-	Hypothetical protein~similar to *Oryza sativa* chromosome 1	6.4/32.24	6.39/35	28	BAB68010	*Oryza sativa*
amp205*	1D/-	Unknown protein	6.0/30.93	6.61/34	24	AC078829	*Oryza sativa*
amp207*		Transfactor, putative	8.0/32.52	7.82/34	17	NP 565209	*Arabidopsis thaliana*
amp208*	1D/-	Alcohol dehydrogenase	5.6/34.46	6.03/34	20	AAG42522	*Hordeum vulgare*
amp224*	4A/6B	Protein kinase, putative	9.2/34.22	9.56/33	16	NP173814	*Arabidopsis thaliana*
amp227*	1B/-	Putative wall-associated protein kinase	7.1/36.17	7.68/33	10	AAL91604	*Oryza sativa*
amp237*		Dioscorin A	6.1/31.10	6.18/32	22	AAF60191	*Dioscorea alata*
amp240*		Maturase K	9.7/34.07	9.31/32	13	AAK94563	*Dillenia philippinensis*
		Ribosome-inactivating protein (rRNA N-glycosidase)	9.3/31.32		16	P21326	*Mirabilis jalapa*
amp245*		Teosinte-branched-like-protein	9.7/38.59	10/32	24	AAG45501	*Populus balsamifera*
amp249*		DIP1 protein	10.7/31.19	10.12/31	12	CAC01083	*Arabidopsis thaliana*
amp250*	1A/-	Annexin-like protein	7.8/36.76		25	NP196584	*Arabidopsis thaliana*
amp251*		Expressed protein	9.7/26.95	9.9/31	28	NP198768	*Arabidopsis thaliana*
amp253*	1D/-	Ras related protein	6.5/29.68	6.04/31	18	P28188	*Arabidopsis thaliana*
amp255*		Putative N-myristoyl transferase	9.8/30.26	10.34/31	20	NP566012	*Arabidopsis thaliana*
amp261*	7A/-	ADP,ATP carrier ptotein (ADP/ATP translocase)	9.9/36.98	10.57/30	29	P31692	*Chlorella kesleri*
amp268*	5B/-	Maturase	10.2/34.55	10.15/30	12	BAA89104	*Pinus aristata*
amp269*	5B/-	Putative chloroplast RNA helicase VDL' isoform 6	9.6/25.70	10.03/30	21	AAG38498	*Nicotiana tabacum*
amp270*	3D/3D	Unknown protein	9.9/27.19	9.5/30	21	NP174871	*Arabidopsis thaliana*
amp273*	7A/-	Hypothetical protein, putative	10.8/27.02	10.58/30	22	BAA99383	*Oryza sativa*
amp286*		Putative MYB family transcription factor	5.7/33.95	5.38/29	23	NP179910	*Arabidopsis thaliana*
		Putative hydrolase	6.3/28.63		10	AAD15390	*Arabidopsis thaliana*
amp287*	1B/1B	Vacuolar ATP synthase subunit-E	6/26.47	6.05/28	24	Q39258	*Arabidopsis thaliana*
amp288*	1B/1B	NADH dehydrogenase	6.3/20.81	6.18/29	29	NP054992	*Spinacia oleracea*
amp294*		Chlorophyll a/b binding protein	5.3/28.96	5.82/28	25	AAD27877	*Vigna radiata*
amp295*	1B/-	Vacuolar ATP synthase subunit	6.0/26.47	5.88/28	35	Q39258	*Arabidopsis thaliana*
amp304	1A/1A	Glutathione S-transferase II	5.8/24.6	6/26	39	AAC64007	*Oryza sativa*
		Vacuolar ATPsynthase subunit-E	6/26.5		38	Q39258	*Arabidopsis thaliana*
amp305*		ATP synthase beta-subunit	5.8/26.33	5.56/27	39	BAA75575	*Astrephomene gubernaculifera*
amp314	1A/1A	Mitochondrial heat shock 22 kDa precursor	6.5/23.6	6/26	71	Q96331	*Arabidopsis thaliana*
		Ras-related protein rab-2-A	7/23.4		56	P49103	Zea mays
amp316	1A/1A	Glutathione S-transferase ERD13	5.5/24.2	5.8/25	83	P42761	Arabidopsis thaliana
amp326*		(S)-Acetone-CyanohydrinLyase	5.2/29.74	5.75/25	25	P52704	*Hevea brasiliensis*
amp336	1A/1A	Ras-related protein rab-2-A	7/23.4	6.5/24	87	P49103	*Zea mays*
amp337*	1A/1A	RISBZ4	5/29.79	5.76/24	17	BAB39174	*Oryza sativa*
amp342	1A/1A	Glutathione S-transferase	5.9/24.69	6/24	61	Q04522	*Silene vulgaris*
amp353*		Ribulose biphosphate carboxylase small chain C precursor	9.4/20.05	9.32/22	17	P18567	*Oryza sativa*
amp379*	3A/-	Glutathione peroxidaseNt-SubC08	6.3/19.08	5.85/18	31	BAB16430	*Nicotiana tabacum*
amp391		Grain softness protein 1	9.2/19.24	10.3/17	35	AAG09277	*Aegilops tauschii*
amp393		Auxin-induced protein	6.8/21.83	6.4/17	76	P13088	*Glycine max*
amp394*		P-type Ca2+-ATPase	5.8/17.95	5.83/17	19	BAB39363	*Vallisneria gigantea*
		Cellulose synthase A1	5.8/23.90		22	AAD33798	*Gossypium hirsutum*
amp404		40S ribosomal protein S16	10.9/17.9	10/16	77	RS16	*Orysa sativa*
amp408*		Puroindoline-B precursor	9.9/17.83	10.57/16	22	Q10464	*Triticum aestivum*
		Puroindoline b	9.7/17.67		18	CAC33505	*Aegilops speltoides*

Table 1 (continued)

Spot ID	MTL/ PQL[a]	Protein identity	pI/MW		SC (%)[b]	Accession	Species
			Theor	Obs			
amp421		17.8 kDa class-1 small-heat-shock protein	5.6/17.8	6.7/14	72	AAD30453	*Lycopersicon esculentum*
amp424	3B/3B	Cysteine proteinase inhibitor II	6.1/12.1	6.4/14	54	P20907	*Oryza sativa*
amp425		Cystein proteinase inhibitor II	6.1/12.1	6/14	79	P20907	*Oryza sativa*
amp428*	3B/3B	50S Ribosomal protein L12, chloroplast precursor	5.3/16.30	5.76/14	23	O22386	*Oryza sativa*
amp429*	3B/3B	Hypothetical protein	5.7/14.20	5.6/14	30	NP194938	*Medicago sativa*
*amp432**		Puroindoline-b	9.7/17.77	10.6/16	18	CAB89542	*Triticum monococcum*
*amp434**		Puroindoline-a precursor	9.7/17.42	10.9/16.1	24	P33432	*Triticum monococcum*
amp435		Alpha amylase inhibitor 0.28	6.2/14.36	6.1/13	82	P01083	*Triticum aestivum*
amp436*	6B/6B	Alpha-amylase inhibitor	6.2/14.36	5.30/13	37	PO1083	*Triticum aestivum*

Underlined ID correspond to common identified spots mentioned in the text. Specific puroindolines are not shown (see Branlard et al. 2003)

[a] Only major PQLs showed

[b] SC: Sequence coverage, Theor: Theoretical, Obs: Observed

[*] Spots identified with a mass calibration range of 500 to 3,000 Da which explained their low sequence-coverage percentage

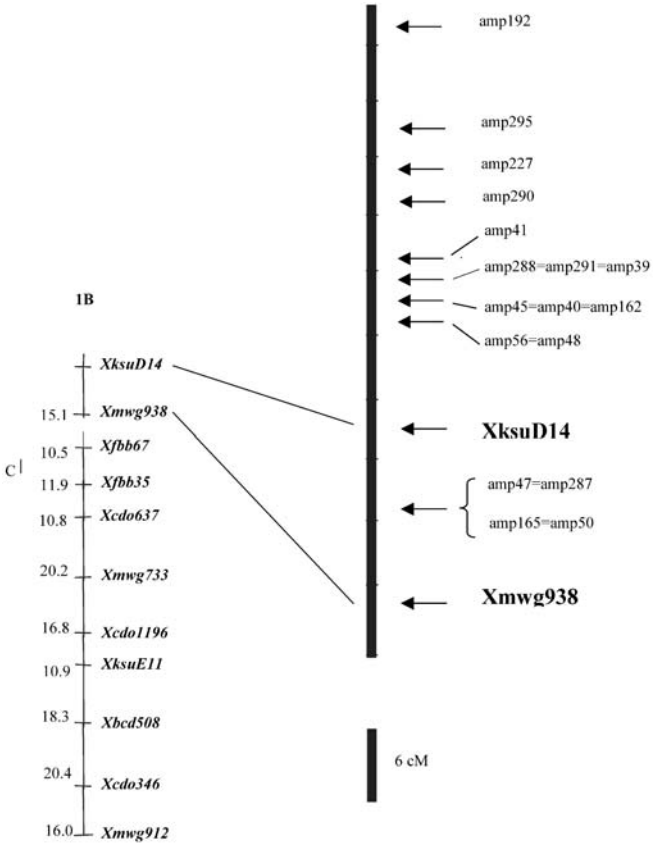


Fig. 3 Main APLs mapped on chromosome 1B. Bracketed spots were located at less than 3 cM between them; = : spots of identical segregating profile in the ITMI progeny

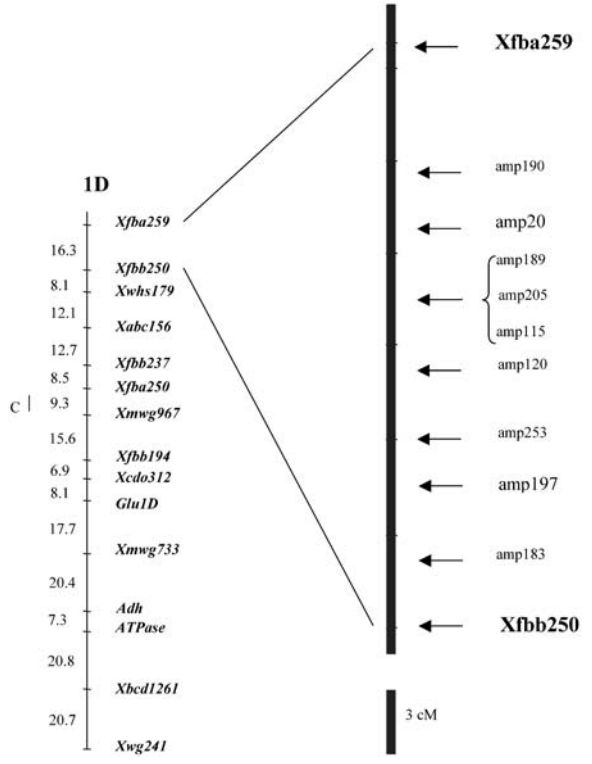


Fig. 4 Main APLs mapped on chromosome 1D. Bracketed spots were located at less than 2 cM between them

Chromosome 3A

Two APLs were mapped on chromosome 3A: amp379 at the distal extremity of the short arm identified as a glutathione peroxidase, and amp423 at the centromere region, identified as a protein of the ubiquinol cytochrome-c reductase complex. Glutathione peroxidase is found in stressed tissues, and protects cells and enzymes from oxidative damage. In wheat, there are many peroxidase genes located on numerous chromosomes. On chromosome 3A, three genes are known to be located on the long arm, *Per-A3a*, *Per-A3b* and *Per-A3c* (McIntoch et al. 1998). The ubiquinol cytochrome-c reductase complex protein is a mitochondrial inner-membrane component and forms part of the mitochondrial respiratory pathway.

Chromosome 3B

Three spots were assigned at the centromere region of chromosome 3B in a cluster of two loci. The first one belonged to amp424, which was identified as a cysteine proteinase inhibitor, and the second to amp428 and amp429, which are specific to the "Synthetic" parent and were identified respectively as the 50 S ribosomal protein L12 chloroplast precursor and ferredoxin-thioredoxin reductase. These two proteins are located in the chloroplast. Among the common spots belonging to the same zone V of the gel (Fig. 2), amp425 also corresponded to a cystein proteinase inhibitor, amp435 to an alpha-amylase inhibitor, amp421 to a heat-shock protein and amp393 to a protein induced by auxin. It has been reported that some protease inhibitors were induced by auxin (Casaretto and Corcuera 1995). Protease inhibitors form a significant group of proteins involved in plant defence against external aggressions. They are widely present in plants, often in their storage bodies. They can be induced by a wound caused by insect attacks and thus inhibit insect-larvae digestion enzymes and microbial proteases. They can also be induced by fungal infection. Their cellular location is not yet known, some are associated with protein bodies, but location in the cytoplasm cannot be excluded (Richardson 1977). It has been reported that genes coding for some protease inhibitors were identified on the long arms of group-5 chromosomes of the cultivar "Chinese spring" (*Ti-A2, Ti-B2* and *Ti-D2* for the gene of trypsin inhibitor) and only on the long arm of the 5D (*Ti-D2c*) in the "Synthetic" variety. In rye and barley, these genes are in Group 3 (respectively *Ti-R1* and *Ti-H1*). Other genes coding for the inhibitors of subtilisins and alpha-amylases were respectively identified on the short arms of group-1 chromosomes (*Si-B2* on 1BS and *Si-D2* on 1DS) and on the long arms of group-2 chromosomes (*Isa-A1* on 2AL, *Isa-B1* on 2BL and *Isa-D1* on 2DL) (McIntosh et al. 1998). This shows that these protease inhibitors can be located at many different loci in the genome.

Chromosome 3D

On chromosome 3D, only one spot was assigned: amp270, deduced from DNA sequencing of *Arabidopsis thaliana*, was a hypothetical protein.

Chromosomes of group 4

Chromosomes 4A and 4B displayed respectively four (amp224, amp154, amp149 and amp67) spots and one mapped (amp46) spot. Only one spot, amp224, was identified as a protein kinase.

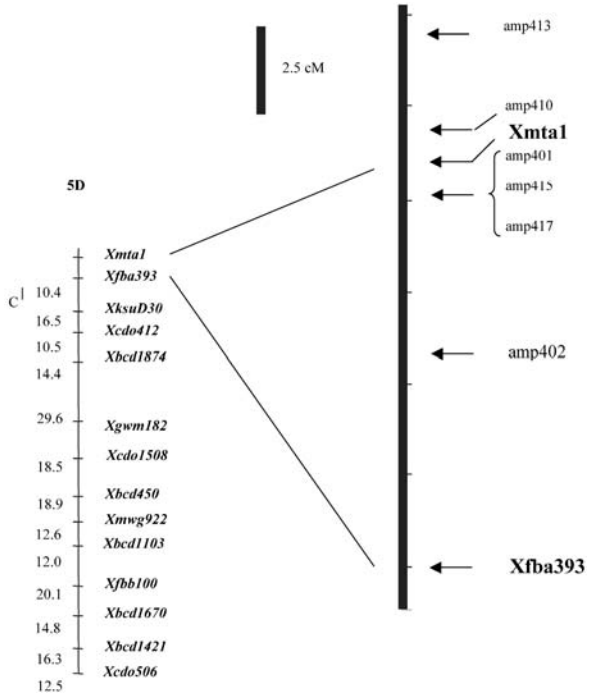


Fig. 5 The six APLs mapped on chromosome 5D. Bracketed spots were located at less than 1.5 cM between them

Chromosome 5B

On chromosome 5B, two spots were assigned: amp268, identified as a maturase and amp269, identified as a chloroplast RNA helicase.

Chromosome 5D

The major gene involved in hardness (*Ha*) is located on the short arm of chromosome 5D. Genes of both puroindoline isoforms, Pin-a and Pin-b, have been seen to be tightly linked to this major gene (Sourdille et al. 1996). Mapmaker software positioned six spot loci at a distance of 10 cM (between amp402 and amp413) around the puroindoline *Xmta10* marker (Fig. 5). In the first analysis (Amiour et al. 2002a) five spots were mapped on the 5DS, of which four were identified as puroindolines (amp402, amp410, amp413 and amp417). In this study, an additional spot was mapped on 5DS (amp401) and identified as a Pin-a. Spot positioning on chromosome 5D showed as many loci as proteins. Three spots (amp401, amp415 and amp417) were tightly linked. Using mass spectrometry (MALDI-TOF) amp401, amp402 and amp410 were identified as Pin-a; amp413 and amp417 as Pin-b; and amp415 as a dehydrin (Rab-17) (Branlard et al. 2003). Amp 413 also showed similarities with a chloroplast ribosomal protein. In zone VI of the gel (Fig. 6), three spots of Pin-a were almost aligned, indicating possible post-translation modifications of the same protein resulting from variations in protein charge.

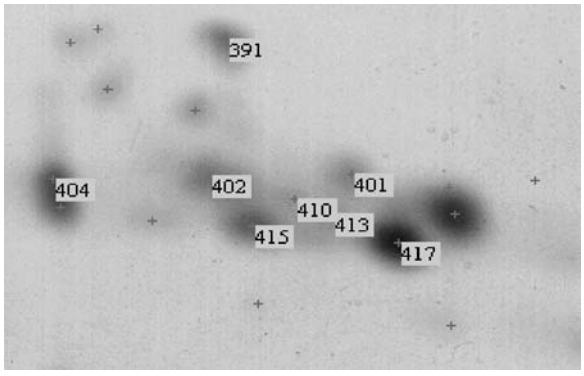


Fig. 6 Isoforms of puroindolines in zone VI of "Synthetic" gel

However, these isoforms are coded by different genes, each causing a post-transcriptional modification. For example, mutation could occur sporadically resulting in amino-acid substitution. This type of mutation has already been observed at Pin-b, for instance the Gly46/Ser mutation (Turnbull et al. 2000). Sequencing by tandem-mass spectrometry is required to obtain more precise information about these proteins, which will allow distinction between a post-transcription or post-translation modification. Moreover, the presence of a locus coding for a dehydrin (amp415) close to puroindoline loci, leads us to think that there are other genes around the major gene *Ha* that could influence hardness. In the same zone of the gel (zone VI, Fig. 2), pin-b (amp432) and pin-a (amp434) spots were identified. These spots were excised from the "Synthetic" parent. Other common spots such as amp404 and amp391 were respectively identified as a ribosomal protein and the grain-softness protein (GSP).

Chromosome 6A

On the chromosome arm 6AS, three spots were identified: amp 119 as a DP-2 transcription factor involved in the expression of cell division related genes; amp121 as a protein controlling cell division; and amp126, which showed similarities with a chorismate mutase and an oxygen-evolving enhancer protein. The two last were located in the chloroplast, and the first belongs to the metabolic pathway of aromatic amino-acid (Phe, Tyr and Try) biosynthesis. These amino acids are necessary for protein synthesis.

Chromosome 6D

Among the four spots assigned to the short arm of chromosome 6D, three were placed at LOD = 5: amp91, amp112 and amp118. Amp156 displayed a conflicting position. Amp91 was identified as a putative transposable element "TNP2 protein" or as an oxidase of the alternative respiratory pathway of Arabidopsis. This oxidase is located in the internal membrane of the mitochondrion. It is induced during the disturbance of the normal respiratory pathway (the pathway of the cytochrome). This disturbance can be caused by several different types of stress such as pathogen attacks (Maxwell et al. 1999). Amp112 was identified as a stearoyl-ACP desaturase (S-ACP DES). This enzyme plays an important role in regulating the overall level of desaturated fatty acids (FAs) in the cell. Reduced S-ACP DES leads to the induction of certain defence responses and to the inhibition of others. The fatty acid (FA)-derived signals are thought to modulate the crosstalk between different defence-signalling pathways (Kachroo et al. 2001).

Chromosomes of group 7

On the homoeologous group 7, chromosomes 7A and 7D displayed, respectively, two (amp261 and amp273) and one (amp17) mapped spots. Amp261 corresponded to ADP/ATP translocase and amp273 to an unknown protein deduced from DNA sequencing of *A. thaliana*.

PQL detection

The quantitative variation in the specific spots determined precisely through 3 to 6 replicates of 2D gels, was submitted to Mapmaker QTL in order to detect the chromosome location of their PQLs. Ninety six out of the 170 specific spots had at least one PQL (35 out of 88 specific spots presented PQLs in the first study). They were distributed throughout the genome. Group-1 chromosomes had the greatest number of PQLs. In group 2, where no APL was detected, 17 PQLs (six on 2A, nine on 2B and two on 2D) were observed. Other chromosomes presented more PQLs than APLs, e.g. chromosomes 3A, 3D, 5A and 7D.

In the first analysis (Amiour et al. 2002a), only one spot of puroindoline had its PQL on 5DS. In this study, eight PQLs were detected on the 5DS of which six were of puroindolines.

Among the 72 mapped spots, 66 (92%) had at least one PQL [only 13 APLs out of 64 (20%) had PQLs in the first analysis]. The highest PQL had a LOD score equal to 19 (amp342). Several others had LODs between 10 and 13. All the major PQLs were at the same location as the APLs, except for amp224. In the first study, only five spots out of 13 (38%) had their PQLs at the same chromosomal location as the APL. Increasing the number of the population (101 lines instead of 81) greatly helped to detect the loci controlling the quantitative variation of the specific spots with more precision.

Analysis of spot quantitative variation will also be useful to better-explain factors involved in variation in hardness. Indeed, if we consider the grain hardness of both parent populations ("Synthetic" and "Opata"), there is no apparent significant difference: "Synthetic" is of the "medium soft" type with, respectively, two values 43 and

42, while "Opata" is of the "medium hard" type with 49 and 65; these values were calculated in 1996 and 1999 (Igrejas et al. 2002). However, the hardness values measured in the same population varied between 16 (soft) and 100 (very hard) in the F7 generation in1996, and between 22 and 100 in the F9 generation in 1999. The presence of transgressive lines for this characteristic was probably due to the presence of positive and additional alleles in both parents, as suggested by Sourdille et al. (1996). Further, the puroindoline mapped loci belonging to "Synthetic". Because of locus linkages, few recombinations took place in the population and there were only two predominant profiles "Synthetic" or "Opata" in the progeny. Because a significant difference in variation in hardness was observed between lines, we cannot attribute this variation only to the number and to the diversity of puroindolines, it is likely that other proteins of the amphiphilic class are involved in this variation. This can be also explained by the variation in quantity of some proteins located on other chromosomes than 5DS. This is supported by other studies that have reported the involvement of other chromosomes in variation in grain hardness: 3B, 7D (Doekes and Belderok 1976), 5A (Morrison et al. 1989; Sourdille et al. 1996), and 2A, 2D, 5B, 6D and 7A. All the spots determined in this study with PQLs on these chromosomes may be potential candidates to explain hardness variation. Among these, those having the highest number of PQLs are 2A, 6D and 7D.

To study the relationship between amphiphilic proteins and variation in grain hardness, a stepwise multiple regression was performed using only 49 spots as explanatory variates. Each spot showed a locus. A significant contribution was attributed to the following amphiphilic proteins: amp116, amp121, amp126, amp269, amp401 and amp413. Only two puroindolines amp401 (pin-a) and amp413 (pin-b) out of five were involved in the multiple regression.

When we performed a multiple regression without puroindolines, the following amphiphilic proteins were chosen as explanatory variates: amp115, amp116, amp120, amp121, amp126, amp167, amp227, amp269, amp342 and amp415, of which four were in the first regression. The effects of other spots were highlighted when puroindolines were absent (amp115, amp120, amp167, amp227, amp342 and amp415). The most significant spot was amp415, which was identified as a dehydrin located close to the puroindolines loci. Most of these proteins were associated with membranes and/or involved in plant defence (Table 1); they belonged to zone I of the gel (Fig. 2), and 6 spots out of the 10 were mapped on group-1 chromosomes. This group has already been shown to be involved in variation in kernel hardness (Amiour et al. 2002b). Indeed, grain hardness was also shown to be affected in wheat substitution lines, particularly by 1R(1A) and 1R(1D) substitutions where a decline in grain hardness was observed compared with the normal parent lines (not substituted). This grain softness could be due to the presence of genes involved in rye defence and located on the short arm of chromosome 1R, which is homoeologous to group-1 wheat chromosomes. A comparison between addition/substitution wheat lines with a proteomic analysis would be useful to characterise expression of the R genome and to highlight its contribution to variation in grain hardness.

Conclusion

Multiple regression evidenced that, among the amphiphilic proteins, the amp415, which has been identified as a dehydrin, was significantly associated to grain hardness in the ITMI population. In addition to their effect on grain hardness, puroindolines are related structurally to several plant proteins that have anti-microbial properties such as thionins and nsLTPs. Some of these proteins have been shown to disrupt lipid bilayer membranes, leading to an anti-microbial activity. Likewise, in our study, statistical analysis revealed significant association between other amphiphilic proteins (such as peroxidase, chalcone synthase and the ribosome-inactivating protein, which are encoded by defence-response genes) and grain hardness. These proteins are mainly present in the "Synthetic" parent. This could be due to its inter-specific origin which adds diversity that is probably not yet described in the current genome of hexaploid wheat. The "Synthetic" parent had 111 specific spots against 59 for "Opata". The majority of these 170 spots showed remarkable similarity to proteins involved in plant defence. The numerous amphiphilic proteins found in the "Synthetic" wheat may result, for some of them, of genome interactions. Beside the fact that membrane associated proteins were frequently seen involved in plant defence, some of them could play an important role in linking the protein matrix to starch granules. The present results may also emphasise the interest of creating synthetic wheats for future wheat breeding programs.

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References

American Association of Cereal Chemists (1995) Approved methods of the AACC, 9th ed. method 39670A. The Association, St. Paul, Minnesota

Amiour N, Merlino M, Leroy P, Branlard G (2002a) Proteomic analysis of amphiphilic proteins of hexaploïd wheat kernels. Proteomics 2:632–641

Amiour N, Jahier J, Tanguy AM, Chiron H, Branlard G (2002b) Effect of 1R(1A), 1R(1B), 1R(1D) substitution on technological value of bread wheat. J Cereal Sci 35:149–160

Branlard G, Amiour N, Igrejas G, Gaborit T, Herbette S, Dardevet M, Marion D (2003) Diversity of puroindolines as revealed by two-dimensional electrophoresis. Proteomics 3:168–174

Büttner M, Singh KB (1997) *Arabidopsis thaliana* ethylene-responsive element binding protein (AtEBP), and ethylene-

inducible, GCC box DNA-binding protein interacts with an *ocs* element binding protein. Proc Natl Acad Sci USA 94:5961–5966

Blum H, Beir H, Gross HJ (1987) Improved silver staining of plant proteins, RNA and DNA in polyacrylamid gels. Electrophoresis 8:93–99

Casaretto JA, Corcuera LJ (1995) Plant proteinase inhibitors: a defensive response against insects. Biol Res 28:239–249

Doekes GJ, Belderok B (1976) Kernel hardness and baking quality of wheat—a genetic analysis using chromosome substitution lines. Euphytica 25:565–576

Dubreil L, Compoint JP, Marion D (1997) The interaction of puroindolines with wheat polar lipids determines their foaming properties. J Agric Food Chem 45:108–116

Facchini PJ, Yu M, Penzes-Yost C (1999) Decreased cell-wall digestibility in Canola transformed with chimeric tyrosine decarboxylase genes from Opium Poppy. Plant Physiol 120:653–663

Galvez S, Roche O, Bismuth E, Brown S, Gadal P, Hodges M (1998) Mitochondrial localization of a NADP-dependent (corrected) isocitrate dehydrogenase isoenzyme by using the green fluorescent protein as a marker. Proc Natl Acad Sci USA 95:7813–7818

Greenwell P, SchofieldJD (1986) A starch granule protein associated with endosperm softness in wheat. Cereal Chem 63:379–380

Hong MA, Yanofsky MF, Meyerowitz EM (1990) Molecular cloning and characterization of GPA1, a G protein α subunit gene from *Arabidopsis thaliana.* Proc Natl Acad Sci USA 87:3821–3825

Igrejas G, Leroy P, Charmet G, Gaborit T, Marion D, Branlard G (2002) Mapping QTLs for grain hardness and puroindoline content in wheat (*Triticum aestivum L.*). Theor Appl Genet 106:19–27

Jebanathirajah JA, Coleman JR (1998) Association of carbonic anhydrase with a Calvin cycle enzyme complex in *Nicotiana tabacum*. Planta 204:177–182

Kachroo P, Shanklui J, Shah J, Wittle EJ, Klessig DF (2001) A fatty acid desaturase modulates the activation of defense signaling pathways in plants. Proc Natl Acad Sci USA 98:9448–9453

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lincoln S, Daly M, Lander E (1992) Mapping genes controlling quantitative traits with MAPMAKER/QTL 1.1. Whitehead Institute Technical Report, Cambridge, Massachusetts

Li WL, Faris JD, Chittoor JM, Leach JE, Hulbert SH, Liu DJ, Chen PD, Gill BS (1999) Genomic mapping of defense response genes in wheat. Theor Appl Genet 98:226–233

Marion D, Nicolas Y, Popineau Y, Branlard G, Landry J (1994) A new and improved sequential extraction procedure of wheat proteins. In: Wheat kernel proteins. Viterbo, Italie, pp 197–199

Maxwell DP, Wang Y, McIntosh L (1999) The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells. Proc Natl Acad Sci USA 96:8271–8276

Mesa R, Salomon C, Roggero M, Stahl PD, Mayorga LS (2001) Rab22a affects the morphology and function of the endocytic pathway. J Cell Sci 114:4041–4049

McIntosh RA, Hart GE, Gale MD (1998) Catalogue of gene symbols for wheat. In: Slinkard AE (ed) IXth Int Wheat Genet Symposium, , August 2–7th 1998, Saskatoon, Canada, pp 1333–1500

Moons A, Bauw G, Prinsen E, Van Montagu M, Van der Straeten D (1995) Molecular and physiological responses to abscissic acid and salts in roots of salt-sensitive and salt-tolerant Indica rice varieties. Plant Physiol 107:177–186

Morris CF (2002) Puroindolines: the molecular genetic basis of wheat grain hardness. Plant Mol Biol 48:633–647

Morris CF, Greenblatt GA, Bettge AD, Malkawi HI (1994) Isolation and characterisation of multiple forms of friabilin. J Cereal Sci 21:167–174

Morrison WR, Law CN, Wylie LJ, Coventry AM, Seekings J (1989) The effect of group-5 chromosomes on the free polar lipids and breadmaking quality of wheat. J Cereal Sci 9:41–51

Richardson M (1977) The proteinase inhibitors of plants and microorganisms. Phytochemistry 16:159–169

Sheehan D, Meade G, Foley VM, Dowd CA (2001) Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. Biochem J 360:1–16

Sourdille P, Perretant MR, Charmet G, Leroy P, Gautier MF, Joudrier P, Nelson JC, Sorrells ME, Bernard M (1996) Linkage between RFLP markers and genes affecting kernel hardness in wheat. Theor Appl Genet 92:580–586

Tang C, Zhang W, Chait BT, Feyö D (2000) A method to evaluate the quality of databases search results, ABRF 2000, Bellevue, Washington

Taylor CA, Graveland A, Henderson MH, Kerkhoff MAT, Veerman SM (1994) Distribution of total and protein-bound glutathione in wheat flours and gluten. In: Wheat Kernel Proteins Molecular and Functional Aspects. Viterbo, Italie, pp 339–344

Turnbull KM, Gaborit T, Marion D, Rahman S (2000) Variation in puroindoline polypeptides in Australian wheat cultivars in relation to grain hardness. Aust J Plant Physiol 27:153–158

Wang X (2000) Multiple forms of phospholipase D in plants: the gene family, catalytic and regulatory properties, and cellular functions. Prog Lipid Res 39:109–49

Zhang W, Chait BT (2000) ProFound–an expert system for protein identification using mass spectrometric peptide-mapping information. Anal Chem 72:2482–2489