Theor Appl Genet (1998) 97 : 1340–1346

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Characterization of spelt (*Triticum spelta* L.) forms by gel-electrophoretic analyses of seed storage proteins. III. Comparative analyses of spelt and Central European winter wheat (*Triticum aestivum* L.) cultivars by SDS-PAGE and acid-PAGE

Received: 27 April 1998 / Accepted: 26 May 1998

Abstract Seed storage proteins of a few selected spelt forms and crosses have already been electrophoretically analysed by SDS-PAGE and acid-PAGE and compared with a few winter wheat cultivars. In the analyses presented here further important Central European spelt varieties were included, as well as modern winter wheat cultivars which were chosen as standards. In this study gliadin and glutenin band patterns of modern Central European winter wheat cultivars were analysed, in particular for a comparison with spelt varieties. An improved differentiation within and between the two species was obtained.

Key words *Triticum spelta* · *Triticum aestivum* · SDS-PAGE · Acid-PAGE · Seed storage proteins

Introduction

An improved distinction between winter wheat and spelt cultivars, and especially a fast and reliable differentiation between the two species, is of a great importance for breeders, bakers, and for the registration of new cultivars. Pure spelt cultivars are also of increasing interest for consumers with different gluten allergies.

Spelt cultivars are often crossed with winter wheat varieties for an improvement of seed yield, lodging resistance, and the baking quality of spelt. These crosses make a differentiation between the two species even more difficult. Additionally, spelt and wheat flours are mixed in some cases for an improved baking quality. Pure spelt flours are, in general, rather suitable for pasta production.

Proven methods were chosen in spelt for a distinction between, and a comparison of, seed storage proteins. Over recent years we have developed and applied gel electrophoretic methods (acid-PAGE and SDS-PAGE) for the separation of seed storage proteins in spelt and bread wheat. We have also investigated a limited number of spelt varieties, progenies of crosses between spelt cultivars with a few wheat varieties and, for comparison purposes, with ancient and modern bread wheat cultivars. The results of these analyses of the band patterns of gliadins (Harsch et al. 1997) and glutenins (Radić et al. 1997) has served as a basis for the further investigations reported here. We have also analysed the gliadin patterns of hexa- and octo-ploid triticale forms and the corresponding wheat and rye cross parents (Günther 1996; Günther et al. 1996; Rozynek et al. 1998).

In the present study we have integrated an extensive number of spelt cultivars and, especially, modern winter wheat varieties. These investigations were carried out to identify characteristic differences in the gliadin and glutenin band patterns of spelt and bread wheat types, which allow clear distinctions within and between these two cereal species.

Communicated by H. F. Linskens

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Materials and methods

The spelt and wheat cultivars which were used for the electrophoretical analyses are listed in Table 1. Grains of these cultivars were milled to provide wholemeal flours for protein extraction studies.

Improved molecular-weight markers (by SIGMA) were chosen for the SDS-PAGE and reconstructed in a reducing sample buffer (RedProbP) with extra SDS (Radić et al. 1997).

Table 1 Spelt and wheat varieties

Type	Variety	Year
Spelt varieties	Albin, Hercule, Schwabenkorn	1994
	Albin, Altgold Rotkorn, Bauländer Spelz, Franckenkorn, Fuggers Babenhauser, Goldir, Hercule, Lueg, Neuenegger Weißkorn, Oberkulmer Rotkorn, Österreichisch Burgdorf, Ostro, Roter Kolbendinkel, Rouquin, Vöglers Dinkel, von Rechbergs Brauner Winterspelz, von Rechbergs Früher Winterspelz, Waggershauser Hohenheimer, Zuzger Winterdinkel	1995
	Steiners Roter Tiroler	1996
Wheat varieties	Jubilar	1990
	Basalt, Farmer, Kronjuwel	1993
	Caro, Orestis	1994
	Alidos, Agent, Apollo, Ares, Aron, Atlantis, Borenos, Gorbi, Herzog, Kanzler, Kontrast, Mikon, Monopol, Orestis, Ritmo	1995
English wheat varieties	Maris Bounty, Maris Huntsman, Maris Hustler, Maris Mardler, Maris Marksman	1995

The methods of the acid-PAGE and the SDS-PAGE techniques, including nomenclature, systematic illustration of the gliadin and glutenin band patterns in terms of densitograms and the evaluation of the densitograms using computer programmes, were described in the two above mentioned papers (1997) of our group. The 'acid-PAGE of gliadins' method was published by Harsch et al. (1997) and the 'SDS-PAGE of glutenins' method by Radić et al. (1997). Gradients of 13–20% total acrylamide concentration were used in the present investigations.

Spelt cultivar 'Oberkulmer Rotkorn' and wheat cultivar 'Orestis' were chosen as standards for the SDS-PAGE. In the acid-PAGE the same spelt cultivar was chosen as standard, but wheat cultivar 'Orestis' was not suitable for this purpose. Therefore, in this case, the spelt cultivar was chosen as the sole standard.

For both methods, new and improved detection and evaluation programmes (by Pharmacia) were applied for band detection and for the determination of molecular weight by the SDS-PAGE.

Additionally, the nomenclature of the gliadin bands in the acid-PAGE, as described by Harsch et al. (1997), had to be extended.

Results

The glutenins

In the investigations presented the number of the spelt and wheat forms analysed was essentially increased in contrast to the initial investigation of our group, the results of which were previously published by Radić et al. (1997). In total we have analysed 21 different spelt and 25 distinct winter wheat cultivars. For demonstration purposes two examples were selected as standards to show the glutenin band patterns of the spelt variety 'Oberkulmer Rotkorn' in comparison to the wheat cultivar 'Orestis'. The band regions are subdivided into three areas with different molecular weights and a high variation of bands.

Area I (approximately 40.5–50.5 kDa):
(1) most wheat cultivars show three major bands of the wheat standard,
(2) 'Atlantis', 'Gorbi' and 'Herzog' have only two bands of 41.8 and 42.9 kDa which do not appear in the wheat standard,
(3) additionally 'Apollo', 'Atlantis', 'Gorbi', 'Herzog', 'Mikon' and 'Caro' possess a band of 44.6 kDa which also appears in the spelt standard,
(4) 'Borenos' and 'Contrast' do not look at all like the wheat standard, they even have four bands of completely different molecular weight,
(5) most spelt cultivars show spelt-typical bands in this area,
(6) except for 'Albin', 'Hercule', 'Goldir', 'Zuzger Winterdinkel', 'Neuenegger Weißkorn', 'Waggershauser Hohenheimer' and 'Vöglers Dinkel' they possess wheat-typical bands.

Area II (approximately 56.0–60.5 kDa):
(1) all wheat cultivars have two to four different bands,
(2) just one band of 56.0 kDa is exclusively wheat-typical but does not appear in 'Gorbi', 'Kontrast' and 'Mikon',
(3) the spelt cultivars 'Albin', 'Hercule', 'Goldir' and 'Neuenegger Weißkorn' show wheat-typical band patterns, all other cultivars look very spelt-typical.

Area III (approximately 69.0–112.1 kDa):
is not suitable for a distinction between the species, but may be helpful for a differentiation of the cultivars within one species.

In Fig. 1 the protein banding patterns of both standard varieties are shown without pre-extraction in chlorine ethanol. As previously mentioned, only the band patterns of area I and area II are suitable for the differentiation of wheat and spelt. Area III is characteristic for all the different cultivars, but is not suitable for a differentiation of the two species. In Table 2 the molecular weights of typical spelt and wheat bands (without pre-extraction) are listed. The spelt-typical

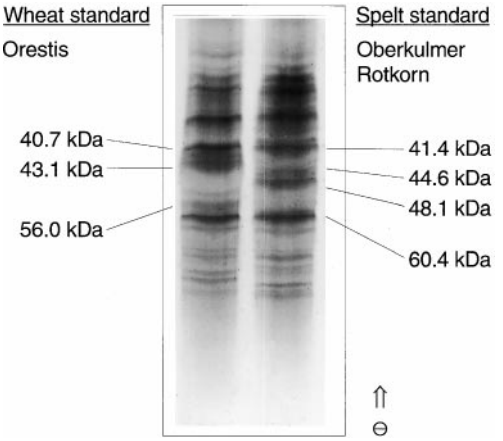

Fig.1 Banding patterns of all SDS-soluble proteins of the wheat standard 'Orestis' and of the spelt standard 'Oberkulmer Rotkorn' without pre-extraction in chlorine ethanol (SDS–PAGE, gel gradient 13–20%)

Table 2 Wheat- and spelt-typical protein bands of all SDS soluble proteins

Band type	Area I	Area II
Wheat-typical bands	40.7 and 43.1 kDa	56.0 kDa
Spelt-typical bands	41.4, 44.6 and 48.1 kDa	60.4 kDa

band patterns of the investigated spelt and wheat forms using the pre-extraction method are shown in Table 3. A summary of the results is listed in Table 4, where we have classified the spelt varieties according to their strong or weak spelt or wheat characters using the corresponding analysed band patterns.

Table 3 Wheat- and spelt-typical protein bands of all SDS soluble proteins with the pre-extraction method

Band type	Area I	Area II
Wheat-typical bands	43.9 and 45 kDa	59.5 kDa
Spelt-typical bands	45.7 and 47.5 kDa	60.5 kDa

The gliadins

The gliadin composition of the same material was analysed using the same densitometrical methods for evaluation of the band patterns as was described by our group in the paper of Harsch et al. (1997). Table 5 shows the band patterns of the tested wheat varieties, which were also found in the standard spelt cultivar 'Oberkulmer Rotkorn'. The winter wheat standard 'Orestis', which was used in the SDS-PAGE, showed many bands that also appear in the spelt standard or that are not only typical for wheat, and was therefore not suitable as a standard in the acid-PAGE. For an illustration of typical bands a few wheat and spelt cultivars were selected as shown in Fig. 2. Table 6 shows bands exclusively of the wheat forms, which were not found in the spelt standard. The summary of the results, which are demonstrated in Tables 5 and 6, reveals that all wheat varieties can be differentiated by their band patterns. Bands D1 and D2 are spelt-typical

Table 4 Classification of spelt varieties according to their strong or weak spelt or wheat characters

Spelt cultivars	SDS soluble proteins without pre-extraction	SDS soluble proteins with pre-extraction
Spelt cultivars with typical spelt character	Altgold Rotkorn Bauländer Spelz Franckenkorn Fuggers Babenhauser Lueg Österreichisch Burgdorf Ostro Roter Kolbendinkel Rouquin Schwabenkorn v. R. Brauner Winterspelz v. R. Früher Winterspelz	Altgold Rotkorn Bauländer Spelz Fuggers Babenhauser Ostro Roter Kolbendinkel Rouquin Schwabenkorn v. R. Brauner Winterdinkel v. R. Früher Winterdinkel
Spelt cultivars with weak wheat character	Vöglers Dinkel Waggershauser Hohenheimer Zuzger Winterdinkel	Franckenkorn Lueg Österreichisch Burgdorf Zuzger Winterdinkel
Spelt cultivars with strong wheat character	Albin Goldir Hercule Neuenegger Weißkorn	Albin Goldir Hercule Neuenegger Weißkorn Vöglers Dinkel Waggershauser Hohenheimer

Table 5 Wheat protein bands which also occur in the spelt standard 'Oberkulmer Rotkorn'. (1) Oberkulmer Rotkorn (spelt standard), (2) Gorbi, (3) Caro, (4) Kanzler, (5) Ares, (6) Aron, (7) Kontrast, (8) Borenos, (9) Alidos, (10) Agent, (11) Monopol, (12) Ritmo, (13) Orestis, (14) Mikon, (15) Herzog, (16) Apollo, (17) Atlantis (different classes of band intensities; 0 = low, 3 = high)

Cultivar / Band no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
A2.II	0	2	2	2	2						2			2	2	2	2
A3.I	3											2	0			2	
A4	3										0			0	1	0	
A5	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1
B1	1	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0
B3.0	0	0	0	0	0	0	0	0	0	0					1		0
B4.I	3	3	3	3	3	3	3	3	3	2	3		2	3	3	2	3
B4.II	0		2		1	2	2	2	2	2	3	3		2	1		2
B5.I	0								0		2	1	1	0			
B5.II	2	2	2	2	2	2	3	2	2	1	2	1	1	3	3	2	3
C1	2	2	2	2	2	2	2	2	1	1	2	1	1	2	3	3	3
C2	2								1	0					?	?	
C6	1	2	2	1	1	0	1	1	1	1	1	2	2	1	1	1	2
C7.I	2	2	2	0	1	1	2	2	2	2	1	2	2	1	2	2	2
C7.II	1					0	1										
C8	0	1	1	0	2	1	1	2	1	1	2	2	2	2			
C9.I	2	2	2	1	2	2	2	2	2	2	2	2	2	2			
D1	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
D2	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Z1	1	0		0	0	0			0	0	0	0	0				
Z2	1						1	1					0	0			
Z3	0	0		0	1		0		1	1					2	2	2
Z4	0				0	0	0		1	0			0		0	0	
Z5	0	1	1	1	1	1	1	0	1	1	1	1	1	1			
Z8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Z9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

bands, which we have found only in spelt forms. All the tested spelt cultivars could be differentiated from each other. Corresponding to the nomenclature of Harsch et al. (1997) the following five areas were analysed.

Area A:
(1) band A1 appears exclusively in wheat cultivars (often combined with the appearance of band A2.1 or A2.II) but not in spelt,
(2) also A3.II appears more often in wheat forms,
(3) band A5 is rather spelt-typical.

Area B:
(1) most wheat cultivars have major (B2.I), B2.II and B4.I bands,
(2) the bands B1, B4.I, B5.I and B5.II appear in the spelt standard.

Area C:
(1) typical wheat bands are (C0), C3.0 and C3.II,
(2) all other bands of the C area occur in many of the different varieties of both species and are therefore not exclusively typical for either wheat or spelt.

Area D:
(1) 'Apollo', 'Herzog' and 'Atlantis' show D-bands (D3 and/or D4),
(2) D1 and D2 are extremely typical for spelt.

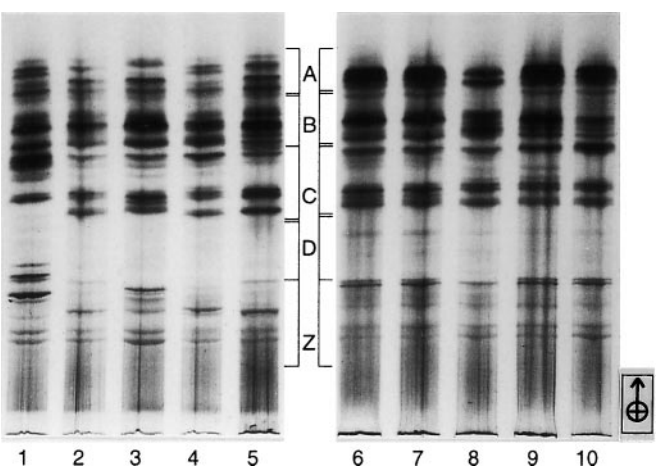

Fig. 2 Gliadin banding patterns of selected wheat (1–5) and spelt (7–10) varieties *1*: Herzog, *2*: Kanzler, *3*: Kontrast, *4*: Mikon, *5*: Monopol, *6*: Oberkulmer Rotkorn, *7*: Ostro, *8*: Bauländer Spelz, *9*: Schwabenkorn, *10*: Rouquin (acid–PAGE, gel gradient 13–20%)

Area Z:
(1) the bands Z5 and Z6 are rather wheat-typical,
(2) Z10 appears exclusively in four wheat cultivars.

Slight differences were also found between the results of the gliadin band patterns in wheat and spelt forms

Table 6 Wheat protein bands which do not occur in the spelt standard 'Oberkulmuer Rotkorn' (different classes of band intensities; 0 = low, 3 = high)

Band no. / Wheat variety	A1	A3.II	B2.I	B2.II	C0	C3.II	C4	C5	D4	Z0	Z6	Z10
Gorbi	1	2	0	1		0					0	0
Caro	1	2	0	0		1	1					
Kanzler	1	2	0	0		0		0			0	
Ares	1	2		1		0					0	
Aron	1	2		1		0						0
Kontrast	1	2		2		0						
Borenos	1	2		2		0						0
Alidos	1	2		2		0					0	
Agent	1	2		0	0						0	
Monopol	1	2		2	0		0				0	
Ritmo	1?	3			0	0	2				0	
Orestis		3				0		0			0	
Mikon	1			1		0		0			0	
Herzog	1		1	0		0			2	1	0	
Apollo	1			2	0	0			2	1	0	
Atlantis	1	2	0	2		1			2	1		0

analysed by Harsch et al. (1997) and the present results, especially in area Z.

When we compare the investigations of the glutenin and gliadin band patterns of spelt cultivars in comparison to the wheat varieties, we can identify following relations: (1) the band patterns of seed storage proteins are appropriate for a differentiation between the two cereal species and within different spelt and wheat cultivars, and (2) the band patterns of the glutenins, in principle, allow a similar division of spelt varieties as the band patterns of the gliadins.

Discussion

Seed storage proteins are useful and reliable genetic markers of great variation since they are hardly influenced by environmental parameters. The fractionation of seed storage proteins by Osborne (1907) into albumins, globulins, and the two main fractions in wheat, gliadins and glutenins, is still in use, though with modifications. Early investigations proved that gliadins are monomers of 36–68 kDa (Galili and Feldman 1983a, b), subdivided into four groups (α, β, γ, ω) and consisting of subunits with a molecular weight of 16–50 kDa connected by intramolecular disulphide bonds (Bietz and Wall 1972). By contrast, glutenins are polymers of 50–2000 kDa (Payne 1987), subdivided into three groups (LMW, MMW, HMW; Payne and Corfield 1979) with subunits ranging from 20 to 100 kDa (Bietz and Wall 1972) and bound by inter- and intra-molecular disulphide bonds (Wall 1979). The location of seed storage protein genes in wheat was obtained by using different aneuploid lines (Galili and Feldman 1983a) and substitution lines (Payne et al. 1980; Galili and Feldman 1985) and led to the conclusions that gliadin-coding genes are located on the short arms of the chromosomes 1A, 1B, 1D (γ- and ω-gliadins) and on the short arms of the chromosomes 6A, 6B, 6D (α- and β-gliadins) (Baker and Bushuk 1978), while glutenins are coded on the long arm of homoeologous group-1 chromosomes (Payne et al. 1980; Shepherd 1988). In recent years a number of papers has been published giving details of the genetic structure of seed storage protein genes in wheat, especially in hexaploids but also in tetraploids. The results concerning the large number of alleles of distinct gliadin- and glutenin-loci, as well as the appearance of 'silent genes' and pseudogenes, will not be discussed here because as of yet, there is no information about the structure of seed storage protein genes in spelt.

Similarities in solubility, electrophoretic mobility (Bietz and Wall 1973; Payne and Corfield 1979) and the distribution of N-terminal amino-acid sequences (Bietz and Wall 1980) of α-/β-/γ-gliadins and LMW glutenins were established and led to discussions about the differentiation and nomenclature of gliadins and glutenins. Miflin and Shewry (1979) recommended that all wheat endosperm proteins should be called gliadins, while Field et al. (1982) named the two major storage protein groups 'aggregative gliadins' (= glutenin polymers) and 'non-aggregative gliadins' (= α-/β-/γ-/ω-gliadin monomers), due to the formation of inter- and intramolecular disulphide bonds, respectively. Two-dimensional fractionation (Brown et al. 1979; Jackson et al. 1983; Payne et al. 1985; Masci et al. 1991), finally allowed an improved differentiation of the different and distinctive LMW glutenins and α-/β-/γ-/ω-gliadins.

Seed storage proteins of *Triticum aestivum* and *Triticum durum* forms have been studied and a large number of publications exists dealing with different aspects of general and applied genetics, food technology and human medicine, especially in connection with the coeliac disease phenomenon. But in spelt (*Triticum spelta*) the number of papers concerned with

genetic and biochemical investigations of the seed storage proteins is limited. The first publication dealing with gel electrophoresis of Central European spelt was that of Federmann et al. (1991). In this paper the authors have shown that *T. aestivum* components can be identified in flours consisting of different amounts of wheat and spelt. Our research group has published results on gel electrophoretic analyses especially of gliadins (Harsch et al. 1997) and glutenins (Radić et al. 1997) of Central European spelt cultivars and, for comparison, common wheat (*Triticum aestivum*) varieties. In both these papers the numbers of the varieties analysed was very limited.

In the present analyses the number of the tested varieties, especially of modern Central European wheat forms, was considerably expanded. We have found a greater variation of the band patterns, and it is possible in every case to identify typical gliadin and glutenin bands of spelt and wheat forms. Furthermore, a differentiation within and between spelt and wheat forms is possible and also gives clear results since we are able to provide a very detailed picture of the band patterns in many different spelt varieties in comparison to many different wheat varieties. In other papers a differentiation of distinct wheat varieties by their gliadin and glutenin band patterns was also successfully obtained: thus bread wheat (*Triticum aestivum*) varieties of different origins were analysed by acid-PAGE (Zillman and Bushuk 1979; Clements 1988; Metakovsky 1991a, b; Metakovsky et al. 1994; Vaccino and Metakovsky 1995; Johansson 1996) and SDS-PAGE (Payne et al. 1981; Field et al. 1982; Krause et al. 1988; Johansson 1996). Compared with the results of our analysed protein banding patterns there are similarities as well as differences, presumably due to different variety groups (origins), isolation methods, and the gel gradients employed by the different research groups. Compared to the large number of publications concerning seed storage proteins in tetra- and hexa-ploid wheat, the emphasis of our present investigation was on the analysis of Central European spelt varieties to provide an improved differentiation between the different spelt cultivars, and between a typical spelt and the popular hexaploid wheat cultivars. Therefore, wheat and spelt standards were defined, allowing a direct comparison of typical wheat and spelt characters, e.g. protein band patterns. Another publication about protein banding patterns is already in preparation as a review article which will present typical wheat and spelt standards allowing for a standardisation of banding patterns and, therefore, a comparison of the different results of other authors. In the planned standardisation the results of gel electrophoretical analyses of seed storage proteins in rye and triticale will also be included.

There is only partial knowledge – especially in spelt – about the genes which code for the corresponding proteins: the number of genes coding for storage proteins in wheat is very large, but essentially located only on a few chromosomes of homoeologous groups 1 and 6 where they are concentrated in tightly linked gene clusters (Lawrence and Shepherd 1981; Galili and Feldman 1984, D'Ovidio et al. 1996, 1997; Lafiandra et al. 1997). All the genes coding for glutenins or gliadins presumably originate from one progenitor gene. In the course of evolution, duplication and divergence of ancestral genes has led to a multigene family (Kasarda et al. 1976) and to many different groups of genes coding for the distinct seed storage proteins (Payne 1987). These genes are present as a large number of multiple alleles. Furthermore, the assumption can be made that there are 'null'-alleles (Pogna et al. 1995) and silent genes (Vaccino and Metakovsky 1995; D'Ovidio et al. 1996) in these gene regions. For example Anderson et al. (1984) described the nucleic acid sequence and chromosome assignment of a distinct wheat storage protein gene and putative control elements (TATA- and CAAT-box, secondary stem-loop structures), as well as a potential initiator codon for an open reading frame and a termination codon, were found. There was no indication of introns in the coding region, but concensus sequences characteristic for splice junctions were identified. Presumably the situation is similar in spelt, but further research work needs to be done.

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