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Characterization of spelt (*Triticum spelta* L.) forms by gel-electrophoretic analyses of seed storage proteins. II. The glutenins

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Abstract Seed proteins of 28 spelt cultivars (*Triticum spelta* L.), 16 cross combinations between spelt forms or between spelt and English winter wheat cultivars, and ten winter wheat varieties (*Triticum aestivum* L.) were analysed by SDS (sodium dodecyl sulphate)-PAGE (polyacrylamide-gel electrophoresis). Different wheat types were chosen for distinct purposes: five popular German wheat cultivars were used for a comparison of wheat and spelt protein band patterns, two of them are old varieties ('Kanzler' and 'Jubilar') and one is a modern wheat standard ('Orestis'); five English winter wheat varieties with short straw were used for the crosses with spelt cultivars to improve seed yield and especially the lodging resistance of spelt. The objectives of these studies were the adaptation of existing SDS-PAGE methods, which have been successfully applied in other crops, for the analysis of seed proteins in spelt, and the characterization and differentiation of spelt varieties from corresponding cross combinations with other spelt forms or with winter wheat cultivars using gel-electrophoretic methods (SDS-PAGE). Considerable differences in protein band patterns were found between spelt and winter wheat varieties, especially in three distinct lanes of the electropherogrammes where the molecular weights range from 40 to 49 , 53 to 62 and 74 to 115 kDa. Spelt cross combinations, and especially crosses between spelt and winter wheat cultivars, were easily distinguishable particularly after a preceding extraction in chlorethanol.

Key words *Triticum spelta* · SDS-PAGE · Glutenins

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Introduction

In the last three decades the cultivation of high-yielding winter wheat (*Triticum aestivum* L.) cultivars has led to considerable ecological problems. Because of this, the relevance of low input systems – such as the growing of *Triticum spelta* L. cultivars – has increased in Central Europe. These original allohexaploid *Triticum* forms can be grown with less effort, even at altitudes of about 800 m, over a short period and on heavy and shallow soils. For an improvement of lodging resistance, yield and the baking quality of spelt forms, crosses with *T. aestivum* cultivars have been made, especially with short English winter wheat varieties bearing 'rht'-genes. The resulting new spelt varieties possess wheat-typical characteristics. A differentiation between winter wheat and these spelt cultivars is difficult. In addition to desirable wheat traits, some undesired wheat traits have also been transferred into these spelt varieties. A few of the typical spelt traits can be restored to some extent by back-crosses, though electrophoresis patterns of these spelt forms demonstrate that they still include some typical wheat protein patterns even after a few back-cross generations.

Four distinct seed protein fractions of different solubility can be found in wheat (Osborne 1907) as well as in spelt: albumins soluble in water and dilute buffers; globulins not soluble in water but soluble in saline solutions; and the other two major protein groups of prolamines, which are soluble in hydrated ethanol (70–90%), and glutenins, which are soluble in dilute acids and dilute alkaline solutions. In the present project all SDS-soluble-proteins of each sample were analysed after a preceding extraction in chlorethanol to remove prolamines from the samples. Prolamines and glutenins also have a different effect on the baking quality of wheat and spelt flours. Depending on the quality and quantity of the protein fractions, doughs of different characteristics may be created, when special amounts and qualities of gliadins and glutenins combine with the starch fraction. Glutenins are responsible for dough strength and elasticity (Wall 1979). More proteins, which may combine with the starch fraction, can be found in spelt than in wheat, but they are of higher molecular weight and of inferior quality. Therefore, an integ-

ration of wheat protein genes or a change of the existing protein gene material in the spelt forms is necessary.

In previous projects, primary triticale gliadins (Günther et al. 1996) and spelt prolamines (Harsch et al. 1996) were analysed by standard PAGE. SDS-PAGE with sodium dodecyl sulphate as a solvent has some advantages in comparison with PAGE as a method for partitioning *Triticum* prolamines (Westermeier 1990): almost all proteins are soluble in SDS and the resulting protein SDS-complexes are all of high negative charge. The hydrogen bonds split up, and by reducing the sulphur bonds the proteins may be dissolved into low- and high-molecular-weight subunits. Consequently the electrophoretic separation of the protein subunits depends on only one parameter, the molecular weight. In the literature different procedures can be found for the characterization of wheat varieties by glutenin patterns using SDS-PAGE (Krause et al. 1988) or SDS-PAGE combined with the gel-electrophoretic method of isoelectric focussing (Holt et al. 1981). Results from gel-electrophoretic analyses of spelt cultivars have been published by only a few authors: 118 Spanish spelt landraces were analysed with SDS-PAGE by Rodriguez-Quijano et al. (1990), while Yakobashvili and Naskidashvili (1988) studied the polymorphism of HMW glutenins in some hexaploid wheat forms (including spelt) in Georgia/SSR, and spelt proteins in general were analysed by Belitz et al. (1989).

More recently Federmann et al. (1991) identified wheat seed storage protein components after mixing spelt with wheat flours. To-date there has been no characterization of Central European spelt cultivars and their cross combinations. Therefore, in the present study all SDS-soluble seed storage proteins were analysed in spelt varieties, spelt cross combinations, crosses between spelt and English winter wheat cultivars and different winter wheat cultivars including the English varieties of the cross combinations. Due to the large amounts of SDS-soluble seed proteins, and in order to remove ethanol-soluble proteins (prolamines in particular), a preceding extraction in hydrated chlorethanol was necessary.

Materials and methods

Plant material

In a previous project, PAGE prolamine patterns of spelt cultivars grown in 1993, wheat cultivars and of some of their crosses – including crosses with English wheat varieties with 'rht'-genes – have already been analysed (Harsch et al. 1997). The same plant material was analysed in the present paper in corresponding investigations using SDS-PAGE. Therefore, flour samples were milled from 20 seeds which came from ten random ears. Additionally, in this programme ten of the most important spelt varieties were grown again in 1994 and analysed, as were two new cross combinations (generation F_7) and a modern wheat cultivar, as a standard for a comparison, using SDS-PAGE (Table 1). The variety 'Orestis' was chosen as the wheat standard, because its SDS-PAGE protein patterns were similar to the protein banding patterns of older wheat cultivars like 'Kanzler' and 'Jubilar'. Cross combinations between typical spelt varieties are of poor lodging resistance and are low yielding, while crosses between spelt cultivars with typical wheat patterns – improved in lodging resistance and higher yield – lack typical spelt characteristics. Therefore, a cross combination between a typical spelt cultivar ('Ostro') and another spelt variety ('Rouquin') with some wheat typical traits (for pedigree see Table 2), and the reciprocal cross combination, were chosen for additional investigations. The flour of 20 kernels were milled out of 25 random ears for the electrophoretical analysis.

Gel-electrophoretic method of SDS-PAGE

All solutions, electrode and sample buffers were mixed as described in Schickle et al. (1989). The flour samples were dissolved and reduced in the reducing sample buffer (RedProbP), modified by adding 20 mg of SDS per ml of sample buffer. Per 25 mg of flour, 200 µl of reducing sample buffer was added. Then the samples were heated for 1 min (80–90°C) and after cooling off 4 µl of DTT (dithiothreitol) solution (250 mg per 0.5 ml H_2O) was added. Finally the samples were centrifuged. To remove ethanol-soluble proteins from each sample, a previous extraction in 150 µl of 2-chlorethanol (25%) per 25 mg of flour had also been made overnight. Marker proteins were reconstructed in 1 ml of the reducing SDS sample buffer, as described by the producer. Gel production and treatment are described in Westermeier (1990) and Schickle et al. (1989). A gradient of 10–20 or 13–20% T was chosen and 400 mg of glycine and 12 g of urea per 400 ml were added to the re-hydrating buffer. Electrode buffers as described in Schickle et al. were used in a different rarefaction of 30 ml of electrode buffer (conc.) per 1 l of water. An electrophoresis duration of 3 h was chosen, which is equivalent to an integral of 90–110 mAh (for one gel), with a maximum voltage of 900 V applied for protein separation. The staining and fixing of the gels was accomplished in Coomassie Brilliant Blue R 250 (in 10% acetic acid solution; 0.25 g per 500 ml) for 1 h, and all further treatment was done as described in Schickle et al. (1989). Afterwards the gels were wrapped in cellophane and dried. Further detailed information on the above applied methods are provided in the investigations of Harsch (1995) and Harsch et al. (1997).

Table 1 A survey of wheat and spelt cultivars and their cross combinations which were cultivated in 1993 and 1994

Type of line or cross combination	Year of cultivation	Names of the cultivars or cross combinations
Winter wheat cultivars	1993	Kanzler, Jubilar, Maris Marksman, Maris Mardler, Maris Bounty, Maris Hustler
	1994	Orestis
Spelt cultivars	1993	Bauländer Spelz, Schwabenkorn, Rouquin, Fuggers Babenhauser, Albin, Hercule, Lueg, Ostro, Oberkulmer Rotkorn, Goldir (experimental line), Oesterreichisch Burgdorf, Steiners Roter Tiroler, Altgold Rotkorn
	1994	Ostro, Rouquin, Schwabenkorn, Bauländer Spelz, Fuggers Babenhauser, Oberkulmer Rotkorn, Altgold Rotkorn, Albin, Hercule, Lueg
Cross combinations	1993	Ostro × Hercule (F_6), Hercule × Ostro (F_6), Ostro × Albin (F_6), Maris Hustler × Ostro (F_6), Ostro × Maris Marksman, Maris Mardler × Ostro, Steiners Roter Tiroler × Albin, Albin × Goldir, Rouquin × Bauländer Spelz (F_5)
	1994	Ostro × Rouquin (F_7), Rouquin × Ostro (F_7)

Table 2 Pedigree and origins of the spelt cultivars Oberkulmer Rotkorn, Ostro, Schwabenkorn, Rouquin, Lueg, Albin and Hercule

Spelt line	Origin	Pedigree[a]
Oberkulmer Rotkorn	CH	Selection from Swiss land variety (1)
Ostro	CH	'Oberkulmer Rotkorn' × 'Steiners Roter Tiroler' (1)
Schwabenkorn	D	Selection from 'Roter Tiroler' (Austrian origin) (3)
Rouquin	B	('Lignée 24' × 'Ardenne') × 'Altgold' (2), (4), (5)
Lueg	CH	'Ostro' × 'Uniplanta 77–62' (1)
Albin	B	'von Rechbergs Früher Winterspelz' × 'Ardenne' (2), (4)
Hercule	B	'von Rechbergs Brauner Winterspelz' × 'Ardenne' (2), (4)

[a] (1): 'Uniplanta 77–62': a winter wheat line, Siedler et al. (1994); (2): A. Dekeyser; personal communication; (3): Dr. Kling, personal communication; (4): 'Ardenne' = 'Virtus' (Swedish winter wheat) × 'Lignée 24' (Belgian spelt race), A. Dekeyser, personal communication; (5): 'Altgold' = 'Oberkulmer Rotkorn' × 'Sandmeier', Siedler et al. (1994)

Results

Spelt cultivars and cross combinations between spelt varieties and spelt forms with wheat were analysed by the gel-electrophoretic banding patterns of seed proteins. Standards with typical spelt and wheat characteristics were used for the purpose of comparison within the complete material. The spelt variety 'Oberkulmer Rotkorn' was chosen as the spelt standard, with the wheat cultivar 'Orestis' as the wheat standard. SDS-protein-markers were used to determine the molecular weights of the protein bands analysed. They showed that most of the proteins of interest are of medium and high molecular weight (MMW/HMW) and range from about 30 to 115 kDa. The protein band patterns of all SDS-soluble proteins, and of the remaining proteins, after a preceding extraction of the wheat and spelt flours in chlorethanol (glutenins in particular), are shown in Figs. 1 and 2.

Wheat and spelt standards

Winter wheat cultivar 'Orestis' and spelt line 'Oberkulmer Rotkorn' were chosen as standards for a comparison of the different varieties and crossing combinations. Major differences can be detected by analysing the seed protein patterns of all SDS-soluble proteins (Fig. 1). Especially in three distinct parts of the lanes, where the molecular weight ranges from 40 to 49, 53 to 62 and 74 to 115 kDa, bands of different intensities and molecular weights can be found. In the wheat standard wheat-typical bands of 53, 56, 57, 59, 73, 84, 97 and 104 kDa were present. The wheat standard lacked the 30- and 40-kDa bands, which appear in important spelt cultivars. Typical spelt bands were detected in the spelt standard with molecular weights of 40, 45, 49, 60, 62, 82, 88, 95, 101, 108 and 109 kDa. Bands of 31, 32, 41, 44, 79 kDa appear in both standards, but are of different intensities. Less seed protein bands of high molecular weight (over 60 kDa) were detected in wheat cultivars, and the cross combinations with wheat forms, than in the spelt cultivars. In general the HMW bands of wheat forms and cross combinations with wheat were of quite weak intensities. After a preceding extraction in chlorethanol (Fig. 2) wheat-typical bands can be found with molecular weights of 44, 45, 57, 100, 106 and 112 kDa, while typical spelt seed protein subunits are of 48, 50, 60, 62, 66, 85, 92, 105, 110 and 113 kDa. The

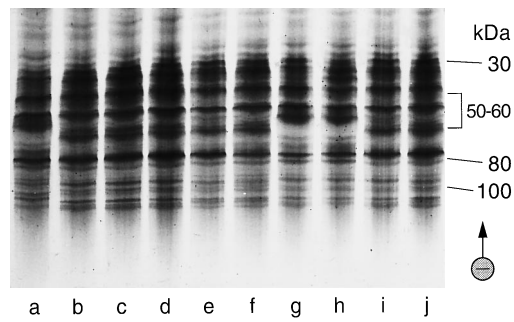

Fig. 1 SDS-soluble proteins (PA-Gel with a gradient of 13–20% T) of the following cultivars: *a* Orestis, *b* Oberkulmer Rotkorn, *c* Ostro, *d* Schwabenkorn, *e* Rouquin, *f* Lueg, *g* Albin, *h* Hercule, *i* Ostro × Rouquin and *j* Rouquin × Ostro

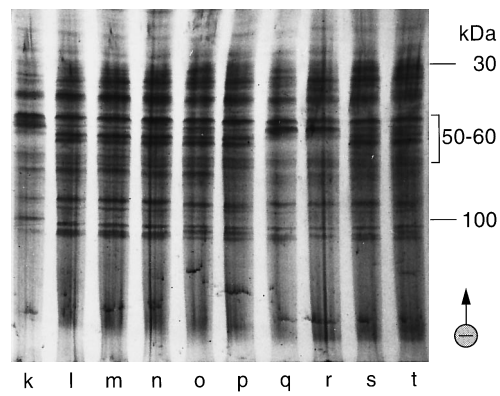

Fig. 2 SDS-soluble proteins after a preceding extraction in chlorethanol (PA-Gel with a gradient of 10–20% T) of the cultivars in Fig. 1: *k* Orestis, *l* Oberkulmer Rotkorn, *m* Ostro, *n* Schwabenkorn, *o* Rouquin, *p* Lueg, *q* Albin, *r* Hercule, *s* Ostro × Rouquin and *t* Rouquin × Ostro

42-, 45- and 62-kDa bands are found in both cultivars, but the 45-kDa band is of weaker intensity in spelt.

Wheat and spelt cultivars

All spelt cultivars, except 'Ostro', to a great extent show spelt-typical banding patterns combined with more or less wheat protein bands (Figs. 1 and 2) due to preceding crosses with wheat. Due to its close relationship (for pedigree see Table 2), the spelt cultivar 'Ostro' looks a lot like the spelt standard 'Oberkulmer Rotkorn' except for a more intense band of 45 kDa (Fig. 1) and intense bands of 34, 35 and 42 kDa (Fig. 2). 'Schwabenkorn' lacks the 40-kDa band, but has intense bands with molecular weights of 46 and 92 kDa. The Belgian spelt cultivar 'Rouquin' also lacks the 40-kDa band and has wheat-typical bands of 44 and 82 kDa. Over 75 kDa typical wheat protein band patterns can be seen, too, especially at 112 kDa (Fig. 2).

Previous crosses with wheat are even detectable after a few back-cross generations. The Swiss spelt cultivar 'Lueg', for example, was crossed with different wheat forms to improve yield and reduce plant height and lodging susceptibility and then systematically selected for morphological characteristics such as fragility of the rachis and tight spikelets (Winzeler et al. 1991). Though frequent back-crosses for spelt characteristics were made (Winzeler et al. 1991), the SDS-PAGE protein patterns still showed some typical wheat protein band patterns, especially of seed proteins with high molecular weights of over 75 kDa. The spelt-typical 82-kDa band was missing, but the typical spelt band of 40 kDa could be detected (Fig. 1). The Belgian spelt varieties 'Albin' and 'Hercule' show extremely typical wheat protein banding patterns at 41 and 44 kDa (Fig. 1) and were lacking the typical spelt 40-kDa band. Protein band patterns of about 50 kDa were similar to spelt, but of less intensity, which could also be seen after a preceding extraction in chlorethanol (Fig. 2). A detailed description of the band patterns of all spelt cultivars could not be given here, but obvious results of some other spelt varieties may be presented. In the spelt variety 'Oesterreichisch Burgdorf' a protein banding pattern, which is typical for spelt, was detected, similar to 'Oberkulmer Rotkorn' and 'Ostro'. In other cases, as in the spelt cultivars 'Goldir' (experimental line), 'Steiners Roter Tiroler' and 'Altgold Rotkorn', wheat bands were found to a great extent. In general, Swiss spelt varieties show protein banding patterns which are typical for spelt, while the band patterns of Belgian varieties demonstrate that these forms are cross products with wheat cultivars. With the exception of the cultivar 'Maris Mardler', short English wheat varieties, which were used in cross combinations with spelt cultivars for an improvement of seed yield and lodging resistance, were quite uniform in their protein banding patterns.

Cross combinations

Most analysed cross combinations indicate an inheritance of maternal traits due to maternal heredity and the formation of triploid endosperm. Even reciprocal crosses between spelt cultivars like 'Ostro' and 'Rouquin' have slightly different maternal protein banding patterns, often of more than 60 kDa (Fig. 1). But the spelt-typical 40-kDa band of 'Ostro' was also detected in the cross combination 'Rouquin' × 'Ostro'. This band did not appear in the reciprocal cross combination. The high intensity of the 60-kDa band was not expected in this cross combination.

Discussion

The desired differentiation of wheat and spelt was obtained by SDS-PAGE. Wheat gliadins and glutenins had already been determined and characterized. The original fractionation of wheat seed proteins was made by Osborne (1907). A slightly modified version is still in use today. Seed proteins are polymers, consisting of two or more subunits with different molecular weights, linked by inter- and intra-molecular hydrogen and di sulphide bonds. Glutenins of wheat are much more complex than gliadins. The subunits are linked by intermolecular disulphide bonds to polymers (Wall 1979). The reducing treatment used here was previously described by Schickle et al. (1989). A preceding extraction in chlorethanol removes not only gliadins, but also some glutenins of low and medium molecular weight. These gliadin and LMW/MMW glutenin subunits are of similar solubility, electrophoretic mobility, and to some extent are characterized by similar amino-acid sequences (Bietz and Wall 1972, 1973). A higher percentage of the LMW glutenin subunits become soluble in chlorethanol (Gupta and Shepherd 1990) when the temperature is raised, and are removed to some extent by the preceding extraction in chlorethanol.

The genetic control of HMW endosperm proteins in hexaploid wheat lines has already been analysed in PAGEs (Galili & Feldman 1983, 1985). Glutenins are coded by the genes on the long arm of the homoeologous group-1 chromosomes, while gliadins are coded by genes of the short arms of homoeologous chromosomes 1 and 6 (Payne et al. 1980; Shepherd 1988). Assignments of HMW glutenin and gliadin bands into groups and subgroups were described by Galili and Feldman (1983). The band designations and molecular weights prove that the glutenin subunits are coded by genes located on chromosomes 1A, 1B and 1D and have molecular weights ranging from 78 to 114 kDa. Also a specific combination of protein subunits has been found (Lawrence and Shepherd 1980, 1981).

There are also important differences between endosperm protein quality and quantity in wheat and spelt. Gliadins and LMW glutenins are responsible for dough viscosity, while HMW glutenins are responsible for dough elasticity and strength. The baking quality of spelt may be improved by mixing spelt with wheat flours or by cross combinations with wheat.

In our studies we found qualitative and quantitative differences of molecular weight, as well as in the intensity and number of bands, between wheat and spelt cultivars. Major differences are reported in wheat cross combinations and the inheritance of glutenin protein subunits (Lawrence and Shepherd 1981; Burnouf and Bouriquet 1983), and were detected here in cross combinations of wheat and spelt cultivars as well as in crosses within spelt varieties. Moreover, the inheritence of maternal traits, with a resulting expression of more maternal protein banding patterns in cross combinations, was detected due to the inheritance of maternal plasma genes and their regulatory effects on nuclear genes and also because of the formation of a triploid endosperm (Burnouf and Bouriquet 1983). In the cross combination presented as well as in its reciprocal cross, we found one paternal band of spelt cultivar 'Ostro' in the 'Rouquin' × 'Ostro' cross combination and one other band of increased intensity.

Cross combinations between spelt varieties and short English wheat cultivars (with 'rht'-genes for dwarfism) were made to improve both lodging resistance and the yield of spelt varieties. However, not only desired characteristics were detected in the cross combinations; there was also an inheritance of undesirable wheat-typical features, so back-crosses became necessary. In the Swiss spelt variety 'Lueg' (for pedigree see Table 2) back-crosses had already been made, but some wheat banding patterns were still detectable, even after a few back-cross generations.

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