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Characterization of spelt (*Triticum spelta* L.) forms by gel electrophoretic analyses of seed storage proteins. I. The gliadins

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Abstract A comparison betweeen the electropherograms of the spelt and wheat cultivars showed specific differences in the gliadin band patterns which provided the possibility of a clear classification into spelt or wheat. A special nomenclature was developed to be able to improve the presentation of the gliadin band pattern of spelt, which is different from that of wheat. This nomenclature, however, has not yet been applied to other cereals. The gliadin band patterns were presented in a schematic form. As a parameter for comparison, idealized band patterns of both wheat and spelt were developed by comparing the proportions of the bands of all available types. When comparing the gliadin band patterns of the spelt cross-breeds with their corresponding parental generations, it was noted that the same parental bands were not always transmitted and that the cross-breeds showed differences in the intensity, mobility, occurrence, and the splitting of single bands. In general it can be said that the band pattern of the daughter generation – even in the examined F_5 and F_6 generations – is more similar to the band pattern of the mother than to that of the father, which proves a maternal effect.

Key words Spelt varities · *Triticum spelta* · Cross-breeds · Gliadins · Electrophoresis

Introduction

Over a long period of time the old grain-type spelt (*Triticum spelta* L.) has been almost entirely displaced by wheat which is easier to process and promised a higher yield than spelt which was more difficult to thresh. However, during the last few years there has been a"revival" of spelt, which shows a higher resistance to environmental influences than wheat (Kling 1991).

The distinction between wheat and spelt, however, is often difficult because they are closely related and certain spelt types contain high portions of wheat. The main factor of the differences in the nutritional value of grain flours is the variation in the share and type of the grain proteins, especially the main storage proteins, the prolamines, which comprise 80% of the total grain proteins (Payne et al. 1981/1982). These prolamines are subdivided into glutenins and gliadins.

In the present study we were interested to identify characteristic differences in the gliadin band pattern of spelt and wheat types, which should enable a clear distinction between these two cereal species. This would also be of great interest for the 'Bundessortenamt' (an institution of the German government) when classifying and endorsing new varieties and cross breeds. This paper describes a method which allows one to obtain single protein bands characteristic for either spelt or wheat, by splitting up the gliadin groups with the help of the acid-PAGE technique. Spelt, as well as wheat, is a hexaploid cereal species. Both contain three genomes, AABBDD, with seven chromosomes per genome. However, the genes controlling glutenins and gliadins are only located on six of the 21 chromosomes (Wrigley and Sheperd 1974; Payne 1987; Dach Kevitch et al. 1993; Metakovsky et al. 1993).

Materials and methods

Materials

The material utilized consists of 10–20 ears of each of the following hexaploid spelt and wheat cultivars:

German wheat varieties:	Basalt
	Farmer

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	Jubilar
	Kronjuwel
English wheat varieties:	Maris Bounty
	Maris Hustler
	Maris Mardler
	Maris Marksman
spelt varieties:	Albin
	Altgold Rotkorn
	Bauländer Spelz
	Fuggers Babenhauser
	'Goldir' (experimental line)
	Hercule
	Lueg
	Neuenegger Winterdinkel
	Oberkulmer Rotkorn
	Österreichischer Burgdorf
	Ostro
	von Rechbergs Brauner Winterspelz
	von Rechbergs Früher Winterspelz
	Rouquin
	Schwabenkorn
	Steiners Roter Tiroler
Crosses:	Albin × Goldir
	Maris Hustler × Ostro
	Maris Mardler × Ostro
	Maris Marksman × Ostro
	Österreichischer Burgdorf × Rouquin
	Ostro × Albin
	Ostro × Hercule
	Rouquin × Bauländer Spelz
	Steiners Roter Tiroler × Albin
	Steiners Roter Tiroler × Maris Bounty
	(Bauländer Spelz × Maris Bounty) ×
	v. Rechbergs Brauner Winterspelz
	(Goldir × Ostro) × Rouquin.

Gliadins were extracted from the flour of 20 caryopses/species with 25% 2-chlorethanol. After centrifugation (25 min, 12 000 rpm) a 50-µl extract of the remainder was pipetted into special Eppendorf refraction vessels.

Methods

The method using acid-PAGE was chosen because, compared to agarose or starch gels, it was possible with polyacrylamide to make thinner gels which enable a faster protein splitting and a clearer distinction of the protein bands. Moreover, there is a higher effective staining which improves the verification sensitivity. The polyacrylamid gels used measured 124 × 258 mm with a thickness of 0.5 mm and are separated into two functionally different sections, the collecting and the separating gel. This gel production technique is based on that of Westermeier (1990).

Table 1 List of the solutions needed for gel production. The expressions "super heavy", "heavy" and "light" solution refer to the glycerine content. The APS solution is added only just a short time before the pouring procedure starts to prevent a too early polymerisation

Item	Solution 1 super heavy	Solution 2 heavy	Solution 3 light
Acrylamid-SI A	–	3.0 ml	5.2 ml
Acrylamid-SI B	0.8 ml	–	–
Buffer	1.3 ml	1.4 ml	1.5 ml
Glycerol (87%)	2.2 ml	1.7 ml	–
Aqua bidist.	0.7 ml	0.9 ml	0.3 ml
TEMED[a]	3.3 µl	4.7 µl	4.7 µl
APS[b]	7.0 µl	6.2 µl	4.0 µl
Final volume	5.0 ml	7.0 ml	7.0 ml

[a] TEMED: N, N, N, N'-Tetramethylendiamin
[b] APS: Ammoniumpersulfate 98%

Three different solutions are necessary for making the gels; i.e. a "super heavy", a "heavy" and a "light" solution (Table 1). First the collecting gel, i.e. the super heavy solution, is poured into the gel form. To produce a gradient, the separation gel is prepared by a gradient mixer which steadily mixes the light solution with the heavy solution. The separation gel is now poured into the gel form onto the collecting gel. Electrophoresis was started after application of the protein extract on the finished gels and was performed in two phases at 500 V and 900 V for 6 h. Afterwards the gels were fixed and stained with Giemsa for 1 h. Then the gel was de-stained in 10% acetic acid for 2 h. For storage the gel was put in 10% glycerol overnight.

Nomenclature

A special nomenclature has been developed to show the differences of the band pattern between the electropherograms of wheat and spelt. This new nomenclature is based on that developed by Woychick et al. (1961) for gliadin band patterns in starch gels.

As in Woychick's nomenclature the band patterns are classified into groups. These groups, however, are not called α, β, γ and ω, but rather A, B, C and Z. In addition after the first visual analyses it was evident that it would be useful to introduce another group called "D". The demarcations between the groups are formed by marked bands which, due to their position within the gel, make it possible to compare their own classification with that of others. The bands within one group are numbered according to their migration speed within the gel. Thus, the band with the highest mobility is always given the number "1". In case of a change in the mobility of single proteins, an increased migration speed is marked with a plus (+) and a decreased migration speed with a minus (−) after the band number. Sometimes one component splits, thus revealing two or three bands instead of one. The subdivisions created in this way are given Roman numerals, e.g. I, II and III. The newly developed nomenclature is illustrated by a model in Fig. 1.

Schematic illustration of the gliadin band pattern in terms of the densitograms

A schematic gliadin band pattern has been developed to provide a clearer picture of the protein bands of the gel. For this purpose the numerical value of the absolute absorption and the peak levels of the densitograms were compared to each other, each peak representing one protein band. The colour intensity of a band, as well as the height

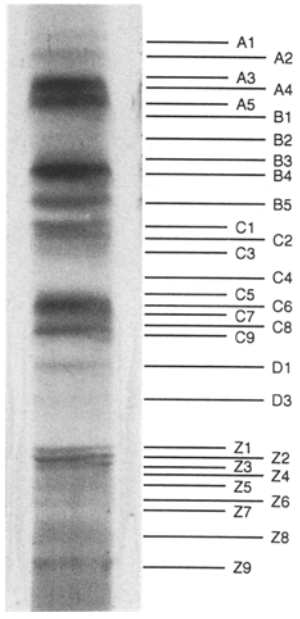

Fig. 1 Illustration of the new nomenclature using the band pattern of the spelt cultivar 'Fuggers Babenhauser'

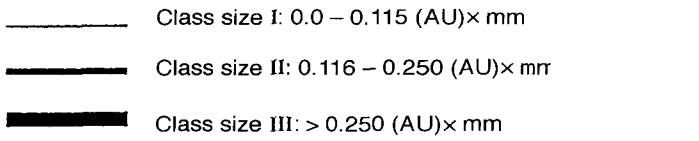

Fig. 2 Scheme of band classification. The class sizes result from the evaluation of the values of the peak levels

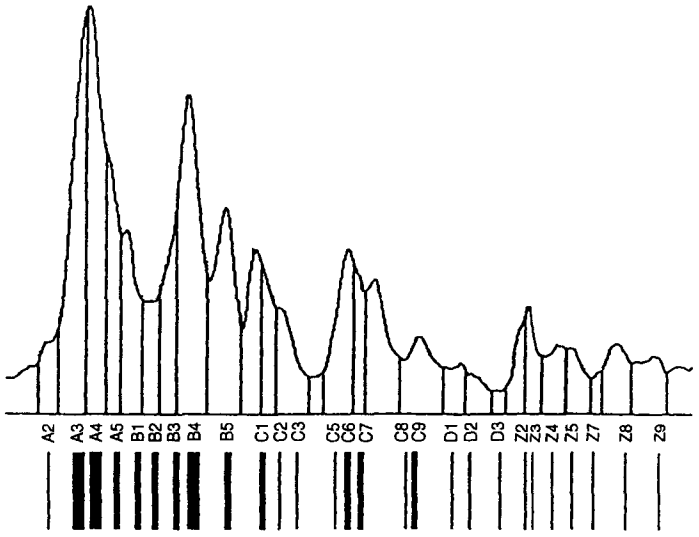

Fig. 3 Demonstration of the connection between the densitogram values and the protein band pattern

and percentage of the peak level in the entire densitogram, were taken as indices for the illustration of the various band widths. Three distinct bands widths were evident, thus making it possible to classify the gliadin bands into classes I–III. This classification was realised according to the scheme shown in Fig. 2. The unit involved is referred to as absorption unit (AU) × mm.

The band pattern shown in Fig. 3 was designed using the values listed in Table 2. The listed data of Fig. 3 and Table 2 are used as an example of the representation of the band diagrams. The densitometric data of all other analysed cultivars were collected by the research team of Hesemann and are available on request.

Production of an idealized spelt and wheat electropherogram

For the production of an "idealized spelt and wheat electropherogram" we attempted to develop a suitable method which allows a reliable comparison of the various types. For this purpose the single protein bands of all available spelt types were compared with each other. Thus the frequency of appearance of a single band in comparison to all spelt types was expressed as a percentage. In this way a typical band pattern of spelt was developed. This "idealized" spelt pattern was used both as a means of comparison and to establish relationships. The wheat types were treated in the same manner and an "idealized" wheat pattern was also developed (Fig. 4).

Results

German wheat varieties

The following old German wheat cultivars, 'Jubilar', 'Basalt', 'Kronjuwel', and 'Farmer', were examined because their gliadins show the typical protein band pattern of wheat. The aim was to show specific differences between the species of wheat and spelt in order to obtain a clear distinction between the two cereal species (Fig. 5).

In all four cultivars, the A1 protein band was always visible. Since this protein band is missing in the spelt electropherogram, it can be assumed that it is specific for wheat. Apart from that, sections A, B and C show no further species-characteristic differences but only type-characteristic ones. These sections can be used for the identification of types but not for the distinction of the wheat pattern from the spelt pattern. This does not apply to the D- and Z-sections. While the D-section is typically characteristic of spelt and does not appear at all, or else only very weakly, in the wheat electropherogram, the Z-section appears in both grain species. However, the Z1 protein band is clearly visible in the wheat electropherogram while it is missing in the spelt electropherogram. Therefore, this protein band can be used for the identification of a strong wheat content. All in all, it is evident that the appearance of protein bands A1 and Z1, as well as the absence of the D-section, can be taken as evidence for a strong wheat character.

Table 2 Values of the peak levels of the densitogram of Fig. 3

Band-No.	Locn.mm[a]	Height Au[b]	Area[c] AU × mm	Rel. ar. %[d]
A2	26.60	0.042	0.068	2.0
A3	28.80	0.185	0.335	9.8
A4	31.00	0.158	0.330	9.6
A5	31.80	0.116	0.162	4.7
B1	32.60	0.108	0.167	4.9
B2	34.20	0.069	0.098	2.9
B3	35.20	0.087	0.135	4.0
B4	37.00	0.188	0.352	10.3
B5	39.60	0.123	0.240	7.0
C1	41.40	0.096	0.128	3.8
C2	42.80	0.068	0.093	2.7
C3	43.60	0.061	0.114	3.4
C5	46.00	0.023	0.028	0.8
C6	48.00	0.099	0.165	4.8
C7	49.20	0.075	0.154	2.4
C8	50.20	0.081	0.082	4.5
C9	53.00	0.045	0.118	3.5
D1	56.00	0.031	0.052	1.5
D2	57.40	0.021	0.041	1.2
D3	58.60	0.015	0.018	0.5
Z2	59.80	0.033	0.062	1.8
Z3	61.00	0.062	0.079	2.3
Z4	63.00	0.043	0.078	2.3
Z5	64.40	0.039	0.064	1.9
Z7	65.80	0.022	0.024	0.7
Z8	67.60	0.043	0.088	2.6
Z9	70.40	0.035	0.096	2.8

[a] Locn.: location of the peak in the densitogram in mm
[b] Height: height of the peak in absorption units (AU)
[c] Area: volume of the peak in AU × mm
[d] Rel. ar.: relation of the peak area to the whole densitogram in %

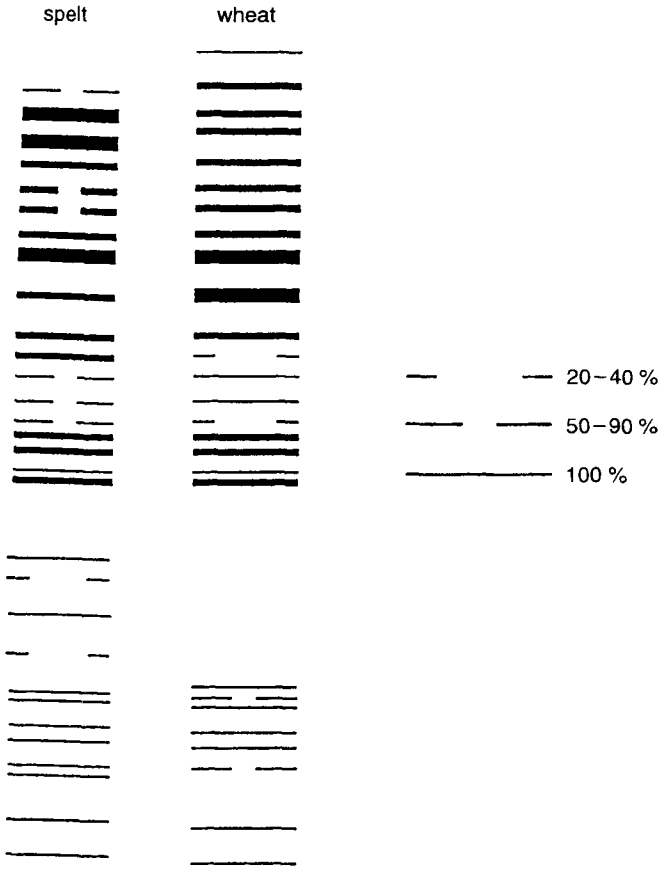

Fig. 4 Diagram of the idealized patterns of spelt and wheat. Next to the diagram is the classification of the three band widths corresponding to the three classes of the frequency of appearance of a band as a percentage

English wheat varieties

English types differ from German wheat varieties not only in phenotype (small size, due to a dwarf gene) but also in the gliadin band pattern (Fig. 6). In terms of the previously explained classifications, a strong wheat content can be noted in the English types examined. In contrast to the German wheat types, these English types show also spelt characteristics. Since the English types show spelt characteristics as well as wheat characteristics, they cannot be clearly classified into wheat or spelt as is possible with the German types examined.

Cultivars with very strong spelt characteristics

The band patterns shown in Fig. 7 belong to the two Swiss spelt cultivars 'Ostro' and 'Oberkulmer Rotkorn', to the German cultivar 'Schwabenkorn', and to the Belgian cultivar 'Rouquin'. The strong differences to wheat that these patterns show in the A, D and Z sections are indicators for the high spelt content of these types (Fig. 7).

The electropherograms of the types mentioned above are very close to that of the idealized spelt. In contrast to the wheat pattern, the A1 protein band is missing in all these types. Bands A3 and A4 belongs to class III, while band 5 also shows a strong appearance and is located in the region between class II and class III. The D-section of the spelt electropherogram can also be seen very clearly in these types, whereas it is missing in the wheat pattern. A further aspect of the distinction between these spelt types from wheat is the absence of band Z1 and the marked appearance of bands Z2 and Z3, which although very close together can nevertheless be seen as two distinct bands. Thus the absence of bands A1 and Z1, the strong appearance of bands A3, A4 and A5, as well as the existence of the D-section, can be considered as characteristic for the spelt pattern.

Cultivars with strong wheat characteristics

A clear classification into spelt or wheat is not possible for the cultivars 'Fuggers Babenhauser', 'Bauländer Spelz', 'v Rechbergs Brauner Winterspelz' and ' Altgold Rotkorn' because these types contain characteristic wheat bands as well as spelt bands (Fig. 8). The A and Z sections show mainly the typical wheat characteristics. 'Fuggers Babenhauser', 'Bauländer Spelz' and 'Altgold Rotkorn' contain the typical wheat band A1 and, except for 'Altgold Rotkorn', also the wheat band Z1. A typical characteristic for spelt in all four cultivars is the existence of the D-section. These four cultivars show a relatively high wheat content in the gliadin band pattern. Nevertheless, they also contain some typical spelt characteristics, thus creating problems for a clear classification. Even so, the evaluation of the gliadin band patterns showed clear differences between the four cereals.

In a comparison of all wheat and spelt forms the following results were obtained. Characteristic for wheat is the existence of bands A1 and Z1, whereas the entire D-section is missing. Typical spelt characteristics are the extremely strong bands A3, A4 and A5, the clearly visible D-section, as well as the missing bands A1 and Z1. In Table 3 all examined spelt varieties are listed according to their gliadin band pattern (Table 3).

Cross-breeds

In the examined cross-breeds of the F_5 and F_6-generation, we attempted to find out which bands were passed on by which parent and whether single marked bands were transferred in all cases, irrespective of whether they were descended from the maternal or the paternal parent.

Regretably, it was not possible to prove that a certain parent always transfers the same marked bands to its daughter generation. The gliadin pattern of the cross-bred generation differs rather unspecifically. (Table 4).

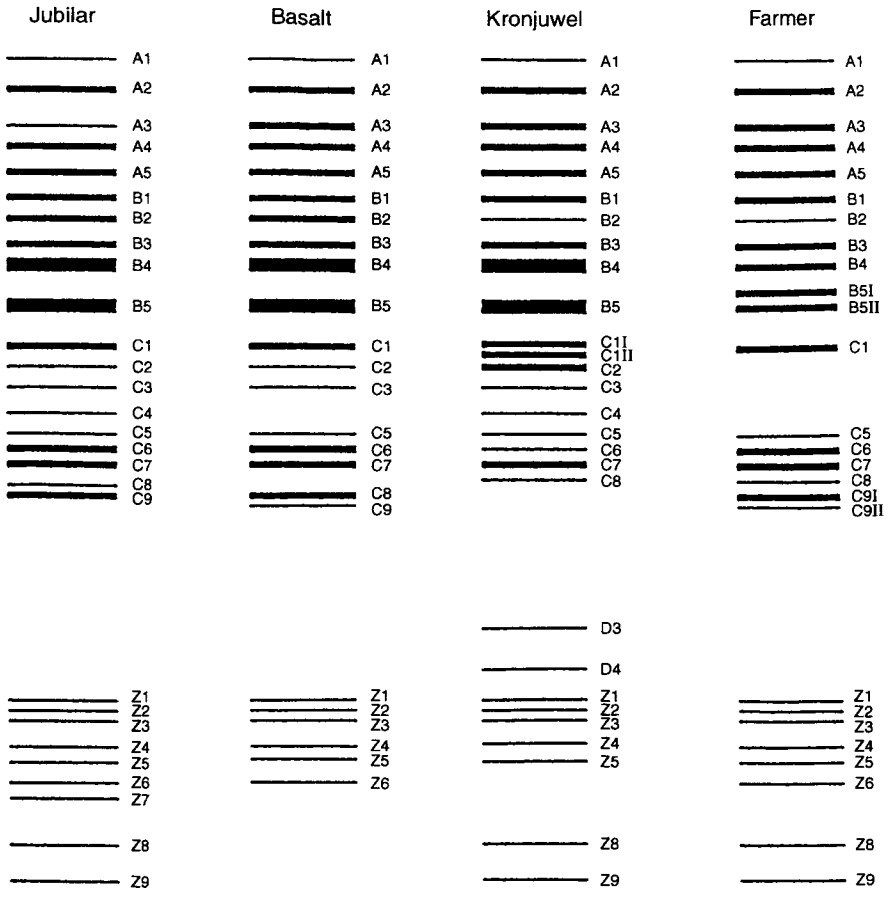

Fig. 5 Comparison of the gliadin band patterns of four German wheat varieties

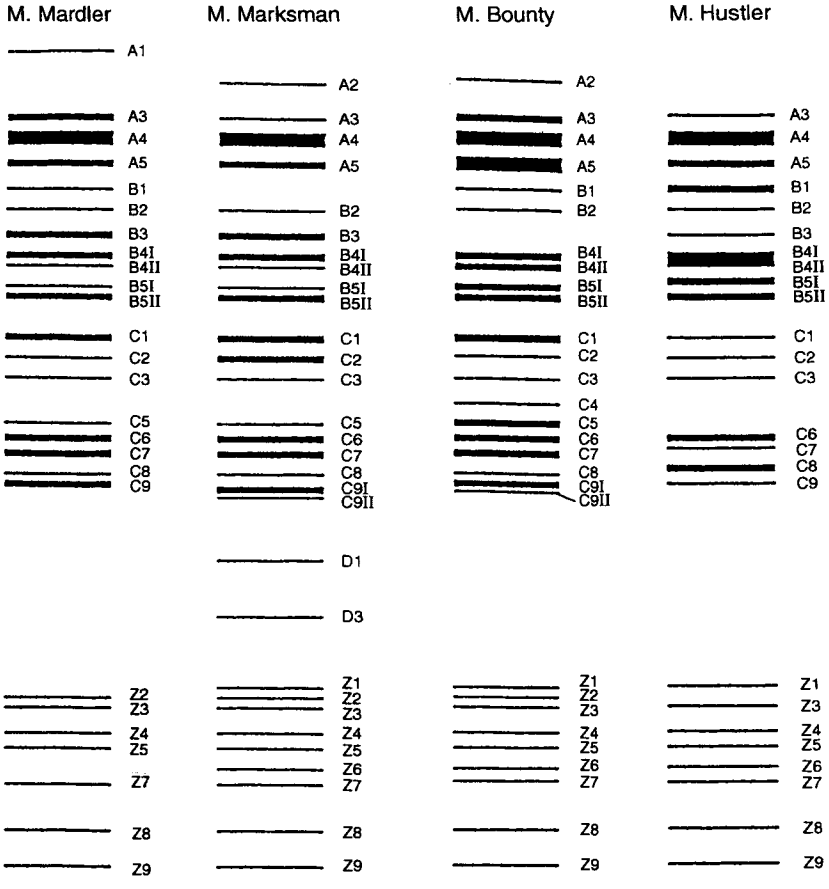

Fig. 6 Comparison of the gliadin band patterns of four English wheat cultivars

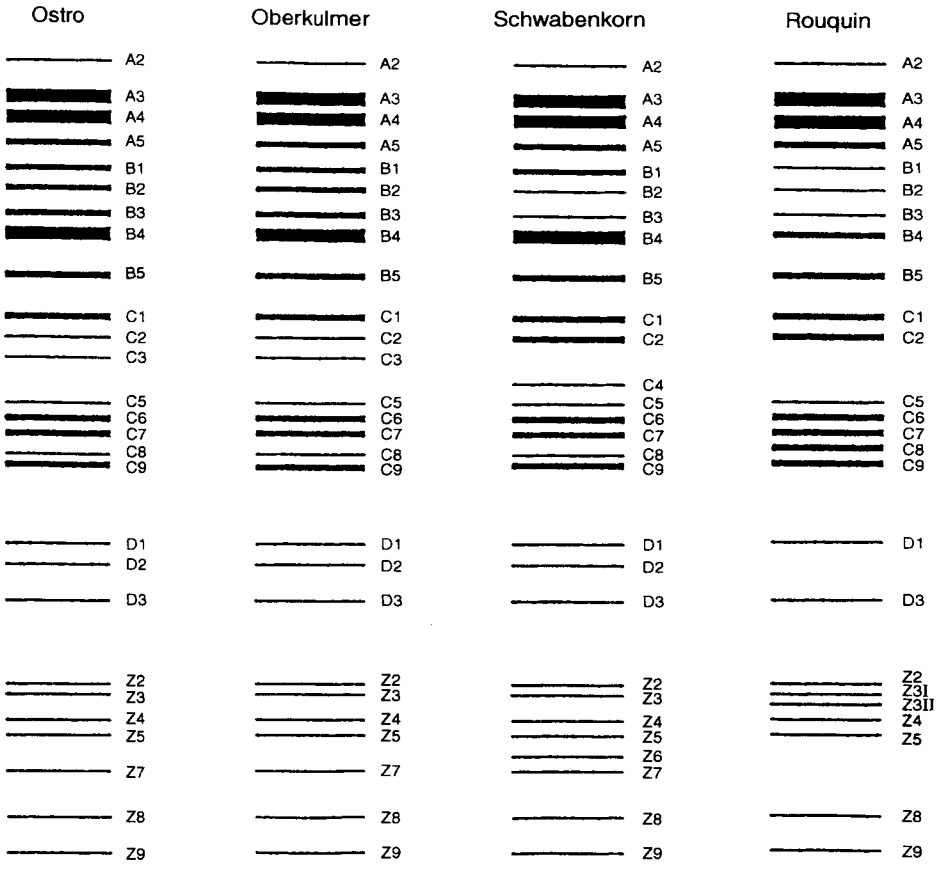

Fig. 7 Comparison of the gliadin band patterns of four spelt varieties with strong spelt characteristics

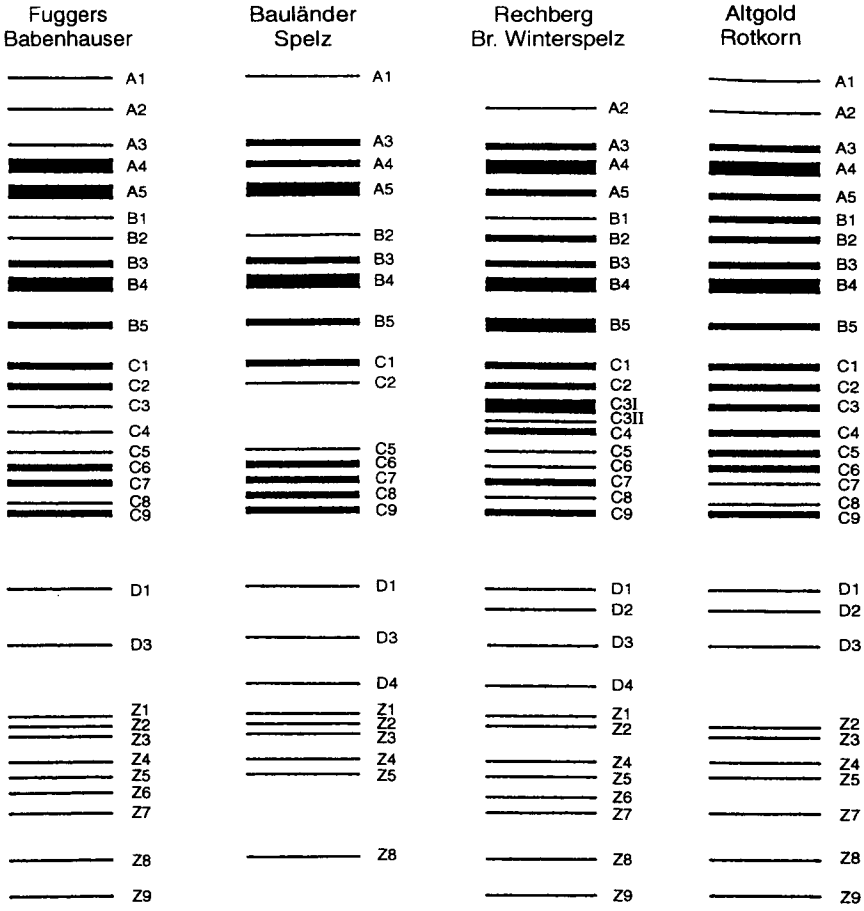

Fig. 8 Comparison of the gliadin band patterns of four spelt cultivars with strong wheat characteristics

Table 3 Survey of the spelt characteristics of the analysed cultivars

Cultivar	A-section	B-section	C-section	D-section	Z-section
Ostro	D[a]	Dw	D	D	D
Oberkulmer	D	Dw	D	D	D
Schwabenkorn	D	Dw	Dw	D	D
Rouquin	D	O[d]	dw	D	Dw
Österreichischer Burgdorf	Dw[b]	dw[e]	O	D	D
Neuenegger Winterdinkel	D	dw	dW	D	D
Lueg	D	dW	dw	D	D
Altgold Rotkorn	Dw	Dw	O	D	D
v. Rechbergs Brauner Winterspelz	D	dw	O	D	W[f]
Goldir	D	O	dW	D	W
Steiners Roter Tiroler	D	dW	O	D	W
v. Rechbergs Früher Winterdinkel	D	dW	dW	W	Dw
Fuggers Babenhauser	dW[c]	D	dw	D	W
Bauländer Spelz	dW	D	O	D	W
Albin	D	dw	dw	W	W
Hercule	Dw	dW	dw	D	W
Maris Marksman	D	dW	Dw	D	W
Maris Mardler	dW	dW	Dw	W	D
Maris Bounty	D	dW	dW	W	W
Maris Hustler	D	dw	dw	W	W

[a] D: strong spelt characteristics
[b] Dw: wheat bands exist, but spelt characteristics are stronger
[c] dW: spelt bands exist, but wheat characteristics are stronger
[d] O: the band pattern is very independent and cannot clearly be characterized as spelt or wheat
[e] dw: spelt bands as well as wheat bands exist, but a clear characterization is not possible
[f] W: strong wheat characteristics

Table 4 Survey of cross combinations and their deviations from the relevant parental generations

Cross combinations	Band sections with maternal effect	Band sections with missing of bands	Band sections with changes in the band intensity/mobility
Albin × Steiners Roter Tiroler	B1, C4, C6, D-section, Z6	B2, B3	B3/C8
Albin × Goldir	B4, B5, D-section, Z7		A3/C8
Ostro × Hercule			
Ostro × Maris Mardler	A2, A3, B1, B4, B5	B2	-/C8
Österreichischer Burgdorf × Rouquin	C3, C4	A2, B3, C7	A4, C4, C8/-
Maris Hustler × Ostro	B5, C2	B2	A5, C2/C8, Z1
Maris Hustler × Steiners Roter Tiroler	B1, B3, C2, C3, D-sections, Z3, Z7	B2, C7, Z1, Z5	B5, C4/-
Maris Marksman × Ostro	A3, B2, B4, C8		B3, C6, C7, C9/C8
Rouquin × Bauländer Spelz	A2, A3, A4, A5, C2, Z1	B2	B1/-
(Bauländer Spelz × Maris Bounty) × von Rechbergs Brauner Winterspelz		C5, C7, C8	B3, C3/C8
(Goldir × Ostro) × Rouquin			B5/C8, D2

Discussion

Protein electrophoresis offers a suitable method for the determination of specific forms because the examined proteins are direct structural products of gene-loci and therefore represent the current genome type. The protein band pattern of the electropherogram shows only genotypic variations, while environmental factors, such as location or cyclical influences, can be totally, or at

least to a large extent, excluded (Autran and Bourdet 1976; Günzel 1976; Baker and Bushuk 1978; Jones et al. 1959; Bushuk and Zillman 1979).

Species characteristic differences in the gliadin band pattern of spelt and wheat

The densitometric analyses of the spelt and wheat gliadins showed characteristic differences in the band pattern of both grain species. Compared to spelt, the appearance of the gliadin bands A1 and Z1 as well as the absence of the D-section, a protein band group with relatively slow mobility, is characteristic for wheat. The extremely intense protein bands A3–A5, the clearly visible D-section, as well as the absence of the bands A1 and Z1, are all characteristic for spelt.

However, in case of the cultivar 'Rouquin' the strong similarity to spelt is rather surprising because, according to its pedigree, there should be a closer relationship to wheat. Therefore it is also necessary to examine other breeding factors such as glutenins, morphology, rheology, etc. in order to give a correct classification of spelt and wheat varieties.

Characteristic differences in the gliadin band pattern of different spelt cross-breeds

By examination of the spelt cross-breeds, differences occurred in the intensity, mobility, splitting and existence of a protein band compared to the parental generation. A majority of the cross-breeds showed more similarities with the maternal parent than with the paternal one. Seven cross-breeds lack protein bands which are present in the parental generation. The complete absence of single bands in the gliadin band pattern may be explained either by the absence of the corresponding gene loci on the chromosomes or by regulation effects between the genomes of both cross parents which lead to a reduction or absence of the gliadin gene products.

As early as 1978 Baker and Bushuk identified the chromosomes which carry the gliadin-coding gene loci in wheat. According to their findings the γ- and ω-gliadins are located on the short arm of chromosomes 1A, 1B and 1D, while genes for the α- and β-gliadins are located on the short arm of chromosomes 6A, 6B and 6D. These results were subsequently confirmed by several research groups (Branlard 1983; Galili and Feldman 1983; Jackson et al. 1983; Joppa et al. 1983; Metakovsky et al. 1984a, b; Payne et al. 1984; Galili and Feldman 1985; Mecham et al. 1985; Metakovsky et al. 1986; Payne 1987; Metakovsky 1991; Skerritt et al. 1991; Ciaffi et al. 1992; Redaelli et al. 1994). Due to the close relationship of spelt and wheat, conclusions could be drawn from these data about the arrangement of genes in wheat and spelt. As the ω-gliadins of wheat correspond to the Z-section of spelt and the γ-gliadins of wheat correspond to the C-section of spelt, it could be assumed that the corresponding gene loci of spelt are also located on chromosomes 1A, 1B and 1D. Additionally, it was possible to conclude that the gene locus of the additional D-section of spelt, which due to its mobility is located between the C-section and the Z-section, is present on chromosome 1. Moreover the α- and β-gliadins of wheat correspond to the A- and B-sections of spelt, so it can be assumed that the gene loci of spelt are also located on chromosomes 6A, 6B and 6D. In the event of the loss of a gene locus or the reduction or absence of gliadin bands as a consequence of regulation effects between the genomes of both cross parents, the corresponding gliadin would no longer be produced which would result in the absence of the corresponding band in the gliadin band pattern.

Additionally, five cross-breeds showed a splitting of single bands into two components compared to the parental generation. On the assumption that one protein band consists of two overlapping protein components which are encoded by different genes, the splitting of one gene could be explained by the switching off of one gene and the switching on at the same time of another gene, which encodes a similar protein with minimal deviation in mobility. Ten cross-breeds show differences in the intensity of single bands compared to the parental generation. Specific protein band-groups in the gliadin electropherogram were described for the first time by Wrigley and Sheperd in 1974. The protein bands are controlled by gene loci, whereas each gene locus has its neighbouring gliadin genes. Each gene locus and its neighbouring gliadin genes are passed on as a block. These blocks are so similar to each other that they supposedly originate from the same (single) gene by means of amplification and mutation. It can be assumed that these blocks are also inherited together (Mecham et al. 1978; Metakovsky et al. 1988; Metakovsky 1991; Metakovsky and Baboev 1992). Several allelic forms (multiple alleles) of each block exist (Sozinov and Poperelya 1980; Metakovsky et al. 1984a, b). Probably one gene locus contains several gene copies which all take part in forming the same protein. According to the number of the transcribed gene copies, the proportion of the different gliadin components varies, which has its expression in a stronger or weaker band width. An altered band mobility, as was the case in four cross-breeds, could be explained by a difference in width. The altered band mobility observed in these four cross-breeds, could also be explained by the existence of multiple alleles. Moreover, mutations could occur which change the protein structure and so lead to an altered protein mobility.

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