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Gel electrophoretic investigations of prolamins in eu- and alloplasmatic octoploid primary triticale forms

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Abstract Unreduced prolamin fractions of eu- and alloplasmatic octoploid triticale forms were investigated by means of gel electrophoresis. The electrophoretic separation of the prolamin fractions was carried out by using one-dimensional, horizontal, native acidic polyacrylamide gel electrophoresis (APAGE) and gradient gels. A comparison of the electropherogram patterns of triticale forms with those of the genome donors showed that most of the parent prolamins can be found again in the corresponding triticale. However, the bands of the triticale forms exhibited lower intensities than those of the genome donors. On average the gliadin bands of the triticale forms showed equal reductions in intensities, thereby preserving the relationships of the band intensities in most cases. On the other hand, secalin bands which came from the rye parent showed varying reductions in intensities. A comparative analysis of the band patterns revealed that differences could often be found between triticale forms with the same genomic constitution. Often it concerned differences in intensity. In some cases the bands showed changes in mobility or a splitting up into two sub-bands. Starting with the plant material presented here we developed a new nomenclature of the prolamin band patterns of the triticale forms.

Key words Triticale · Gliadins · Electrophoresis · Prolamin · Octoploids

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Introduction

As expected gliadins and glutenins of wheat as well as secalins and glutelins of rye can be found in triticale forms. In hexaploid wheat, the genes of the seed storage proteins are located on chromosomes 1A, 1B and 1D and 6A, 6B and 6D (Payne et al. 1982). In diploid rye, the corresponding genes are located on chromosomes 1R and 2R (Lawrence and Shepherd 1981; Shewry et al. 1984). Each of these chromosomes possesses numerous prolamin genes which are closely adjoined and are normally transmitted en bloc. The single genes within a bloc are very similar to one another and presumably originated through amplification and successive mutation from a first gene. Numerous allele types can be found for every gene of the seed storage proteins. These allele types differ in the number of coding components as well as in their electrophoretic mobility, stainability and molecular weight (Sozinov and Poperelya 1980; Metakovsky 1991; Metakovsky et al. 1994).

In the study described here unreduced prolamin fractions of octoploid eu- and alloplasmatic primary triticale forms showing the AABBDDRR genomic constitution were electrophoretically analyzed. A method of horizontal native acidic polyacrylamide gel electrophoreses (APAGE) using gradient gels was applied (Westermeier 1990; Harsch et al. 1997). In order to be able to draw conclusions about possible genomic and cytoplasmic interactions we analyzed different primary eu- and alloplasmatic triticale forms.

Materials and methods

Plant material

The investigated primary eu- and alloplasmatic triticale forms were produced by crossing hexaploid wheat (*Triticum aestivum*) cv 'Jubilar' and four different rye inbred lines, L283, L290, L301 and L304. Four hexaploid wheat species, *Triticum spelta*, *T. macha*, *T.*

compactum and *T. sphaerococcum*, and four tetraploid wheat forms, *Triticum durum* 'Arnaud', *T. turgidum*, *T. dicoccum* and *T. dicoccoides*, were used as cytoplasmic donors. For the investigations 34 different triticale forms were available, providing up to maximum of four progenies each, but as a rule only two progenies. All other cross combinations showed no seed set, so that the corresponding triticale forms were not available. In our investigations plants described as progeny were those derived from a single seed. In total, the prolamins of 71 flour samples were extracted and gel electrophoretically analyzed. Samples of single seeds were included in the investigations if conspicuous variations in prolamin expression were found.

As a rule 20 caryopses were used for the production of flour samples and for the analysis of single grains, respectively.

Electrophoresis

The one-dimensional horizontal acidic polyacrylamide gel electrophoresis was used for separation of the prolamins using gradient gels as described previously (Harsch et al. 1997).

Nomenclature of the prolamin bands

As a first step a practical nomenclature by which to describe the variations found in the band patterns was developed. The prolamin band patterns of wheat cv 'Jubilar' and the rye inbred lines L283, L290, L301 and L304 were used as a basis for the nomenclature. But this nomenclature can be also used for all of the other triticale forms analyzed. The classification of the band pattern into four different groups was carried out in the same manner as by Woychik et al. (1961) for gliadin band patterns in starch gels. The four groups were not marked as in the publication of Woychik et al. with α, β, γ and ω, but as A, B, C and Z. The reason for the change is as follows: the four areas of the gliadin band patterns were not identical in the investigations of both research groups. In our study prominent bands were selected which mark the corresponding border of an area. The bands within a single area were given continuous numbers. In every case the greatest possible mobility of a band was marked by the number 1. The letters "w" (for wheat) and "r" (for rye) were put in front to differentiate between gliadins and secalins. The band patterns of the genome donors were consecutively numbered (from beginning to end) using the above-mentioned principles. The designation of the triticale bands was performed on the basis of the genome band donors. For this purpose the marking of the parental prolamin bands was adopted and assigned to the homologous bands of the triticale forms. If an overlapping of gliadin and secalin bands occurred, both designations were given divided by an oblique stroke – for example, "wZ1/rZ2". Variations in band patterns were emphasized by labelling them with corresponding different indices as, for example, plus (+) or minus (−) signs for an increased or decreased mobility of bands. In case of splitting bands the subunits that appeared were additionally marked using Roman numbers, I, II and so on.

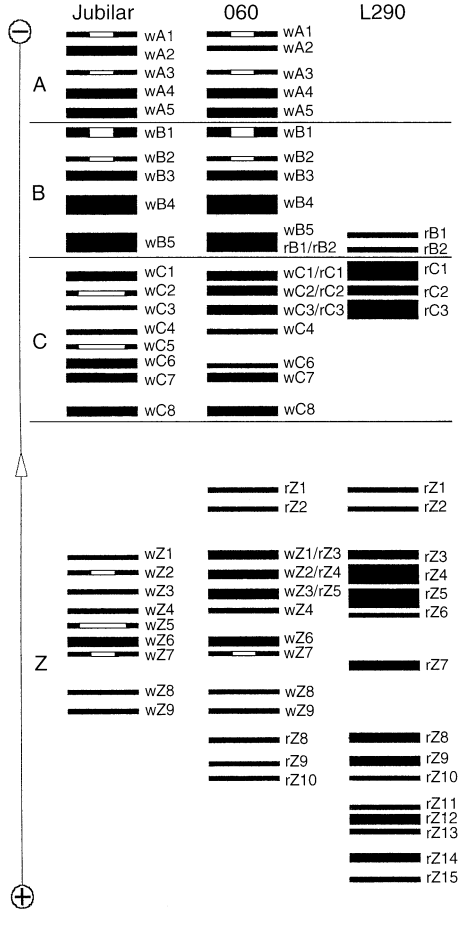

Fig. 1 Schematic representation of the prolamin band pattern of the euplasmatic triticale form 060 and its cross parents 'Jubilar' (wheat) and the rye inbred line L290

Schematic representation of the prolamin band patterns

Using densitometric analysis, we developed a schematic representation method of the prolamin band patterns. Consequently, the peak areas of the corresponding band regions were divided into three classes of intensity: 0.01–0.09 (AU × mm), 0.1–0.3 (AU × mm) and > 0.3 (AU × mm) (AU = absorption unit). On the basis of the corresponding class of intensity the bands were graphically presented as bars in varying widths. The result produced Fig. 1, which is an example of the triticale form 060 and the corresponding cross parents.

Results

Prolamin band pattern of the analyzed triticale forms

Our investigations of all cross combinations showed that most of the parental prolamin bands were also found in the triticale forms, but in a diminished intensity. Despite this, the relative intensities of the single gliadin bands derived from the wheat parent were preserved in the typical triticale band patterns. A quite different situation was ascertained in the secalin bands which came from the rye parent. The secalins of the C-region were much more affected with respect to a decrease of intensity than the ones of the upper Z-region. In the triticale forms the secalins of the lower Z-region were not even detectable. This type of expression of secalin bands was found in all triticale forms independent of the inbred rye line which was used as cross parent.

Numerous samples of the triticale forms showed prolamin band patterns which were different from the typical pattern of the corresponding cross combination.

In most cases deviations in the intensity of single prolamin bands were ascertained as well as deviations in the mobility and splitting of single bands. In one case an additional band was observable. The above-mentioned deviations were found in all four areas, the A-, B-, C- and Z-regions.

In total, 71 different triticale samples were investigated; 24 possessed one or more variations in band patterns.

Prolamin band pattern of the alloplasmatic triticale form 067

This triticale form was an extreme example of great heterogeneity between different descendants. The alloplasmatic triticale form 067 containing the cytoplasm of the tetraploid wheat form *Triticum turgidum* was produced as a cross between wheat variety 'Jubilar' and rye inbred line L290. The typical prolamin band pattern of the corresponding cross combination was found only in one of four descendants investigated; the electropherograms of the other three descendants showed variations in intensity and mobility and the splitting of bands.

Figure 2 shows the electropherograms of the four descendants in comparison with each other as well as the band patterns of corresponding genome donors. Deviations as those found in sample 067/4 of the typical band pattern are marked.

Deviations in intensity

The prolamin band patterns of descendants 067/1, 067/2 and 067/3 showed distinct variations in intensity.

1) Band pattern of 067/1. The bands wZ1/rZ3 and wZ3/rZ5 possessed diminished intensities in comparison with the corresponding genome donors; in the triticale form the band wZ2/rZ4 showed a greater intensity. On the other hand, the band pattern of paternal genome donor L290 demonstrated a continuous increase in intensity from band rZ3 to band rZ5.

2) Band pattern of 067/2. In this case the intensity of all bands was lower, but an extremely low intensity was found in bands wA2, wC6 and wC8.

3) Band pattern of 067/3. In the band pattern of this descendant a great increase in intensity for bands wZ1/rZ3, wZ2/rZ4 and wZ3/rZ5 was shown. These bands were produced by the overlapping of strong secalin and very weak gliadin bands and occurred at an intensity similar to that shown by the band of the paternal genome donor L290.

Variations in mobility

Band pattern of 067/1 and 067/3. The electropherograms of the triticale samples 067/1 and 067/3 showed distinct differences in mobility for band wA4 in comparison with the band pattern of the maternal genome donor 'Jubilar'. The bands of the triticale forms possessed reduced mobility.

Splitting of bands

Band pattern of 067/1. In this triticale grain sample a deviation of intensity was analyzed as well as a splitting of the gliadin band wB5, which came exclusively from the wheat parent. This result was clearly confirmed by densitometric measurements.

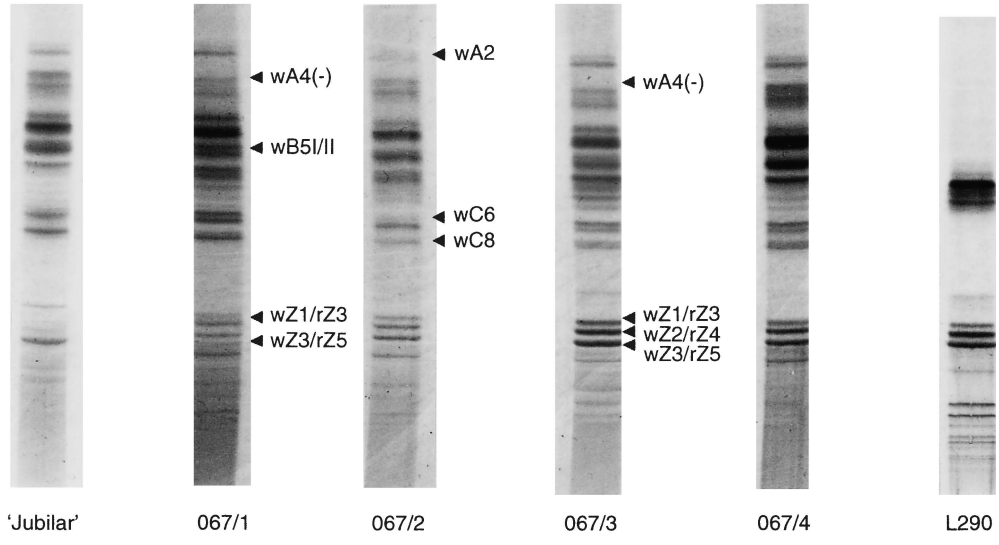

Fig. 2 Electropherograms of the four descendants of all alloplasmatic triticale form 067 and its corresponding genome donors 'Jubilar' and L290. Deviations of the typical band pattern are indicated by *arrowheads*

Prolamin band pattern of the alloplasmatic triticale form 063

The results of grain sample 063 are additionally informative. One of its triticale descendants showed a distinct deviation in its prolamin band pattern. Figure 3 is an electropherogram of the corresponding cross parents, 'Jubilar' and the rye inbred line L290, in comparison with the two available descendants, 063/1 and 063/2, of cross 063. Descendant 063/2 clearly shows the extreme increase in intensity in band wC4 and the simultaneous absence of band wC6. The corresponding densitometric intensity data of grain samples 063/1, 063/2 and 'Jubilar' are presented in Table 1.

The band wC4 shows similar values, 0.07 and 0.06 AU × mm, in the maternal genome donor 'Jubilar' and descendant 063/1. However, the densitometric value (0.25) of descendant 063/2 was much higher than that of 063/1. Band wC6 could not be proven densitometrically to be present in the pattern of 063/2. The grain samples of 'Jubilar' and 063/1 show values of 0.13 and 0.07 AU × mm, respectively. The deviation between these two values still lies within the normal range. It was ascertained that the gliadin bands of the triticale form which came from the wheat parent occurred only up to a 50% reduced intensity.

In descendant 063/2 single grain samples were investigated, but all 20 investigated samples showed the same result with respect to the bands of wC4 and wC6.

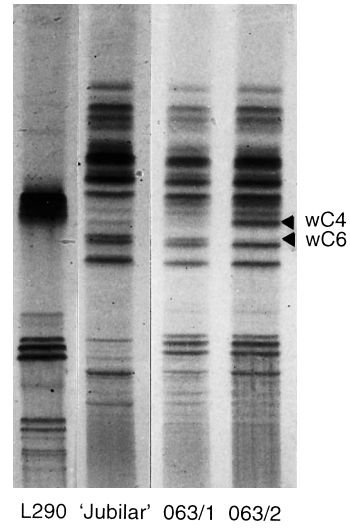

Fig. 3 Electropherograms of the alloplasmatic triticale form 062 and its corresponding genome donors. Sample 063/2 shows an increase in intensity of band WC4 and the absence of band WC6

Table 1 Values of the peak levels of the densitogram of triticale samples 063/1, 063/2 and the maternal genome donor 'Jubilar'. Sample 063/2 shows a distinct deviation of its prolamine band pattern

Gliadin bands	Values of peak levels in AU × mm[a]		
	Genotypes 063/1	063/2	Jubilar
wA2	0.06	0.08	0.15
wA4	0.15	0.18	0.23
wA5	0.12	0.17	0.21
wB1	0.10	0.13	0.17
wB4	0.41	0.53	0.56
wC4	0.06	0.25	0.07
wC6	0.07	0.00	0.13
wC7	0.13	0.17	0.18
wC8	0.15	0.19	0.23

[a] AU × mm = absorption units

Discussion

Nomenclature

Classification of the gliadins in wheat has been carried out by several research groups (Jones et al. 1959; Woychik et al. 1961; Autran and Bourdet 1975; Bushuk and Zillmann 1978; Metakovsky and Novoselskaya 1991; Redaelli et al. 1994). A classification has also been published for secalins in rye, but not in detail. A completely different principle was used in the case of the gliadins in wheat (Field et al. 1982; Shewry et al. 1983).

In our study the gel electrophoretic method was applied, and the plant material, triticale, did not enable us to apply the nomenclature used by the above-mentioned authors. It is known, however, that triticale represents a cross product between wheat and rye. Therefore, a nomenclature had to be developed which takes into consideration both types of bands, the gliadin bands of wheat and the secalin bands of rye. We consequently grouped the bands in four different regions, and the single bands were then consecutively numbered. The gliadin bands were additionally marked with "w" (for wheat); the secalin bands with "r" (for rye). In this way, the differentiation between gliadin and secalin bands in the band patterns of triticale forms was preserved. Clearly discernible as well was the type of super bands produced by an overlapping of gliadin and secalin bands. Deviations in the normal band pattern were marked by additional indices, particularly variations in mobility and splitting of bands. The development of this new nomenclature has made a clear analysis of band patterns in the cross parents possible. This new nomenclature method can also be effectively used in other cereals as spelt (Harsch et al. 1997; Radic et al. 1997) and can generally be applied in gel electrophoretic investigations of wheat, rye, triticale and spelt forms.

Prolamin band patterns of primary octoploid triticale forms

Most of the parental bands were also present in the crossed forms upon a comparison of the band patterns of the triticale forms with the band patterns of the genome donors. In contrast to the gliadin bands of the triticale forms which showed decreases in intensity in nearly all cases of the same relationship, the proportions of intensity were not conserved in the secalin bands. Secalin bands which showed very strong intensities in rye were represented as extremely weak ones in triticale forms. The very weak expression of the secalins could be explained by the hypothesis that wheat and rye parental genomes may interact with each other in the octoploid triticale forms. It may be, however, that the higher dose of the wheat genome can suppress the expression of distinct secalin genes which are coded by the rye genome. Intensity variations in gliadin bands found in the investigated octoploid triticale forms could have been caused by various factors. On the one hand, genes can presumably be inactivated by the interactions of both parental genomes in combination with the influence of distinct alien cytoplasms. On the other hand it is also conceivable that promotor sequences may mutate, initiating a change in the transcription rate. The high frequency of aneuploids, which were especially present in the octoploid triticale forms, also has to be kept in mind. The total loss of single bands could be explained by the elimination of chromosomes possessing gene loci.

The grain samples 063/2, 067/1 and 067/3 showed changes of mobility in single bands. This phenomenon could presumably be initiated by mutation events, which could have produced a variation in the protein structure and changed the migration velocity.

The splitting of band wB5 into two components could presumably be initiated in the same manner as mentioned above. Under normal circumstances the band consists of two protein components which overlap each other and are coded by different genes. The splitting could be produced by switching off the first gene and switching on the second one which controls a very similar protein with only slightly deviating mobility.

The following conclusion can be drawn: that different wheat-rye genome combinations affect the variability of the prolamin band patterns to a different extent. Outstanding is that grain samples of the cross combination 'Jubilar' × L304 showed very homogenous prolamin band patterns among themselves. On the other hand, differences were often found in the expression of single gliadin and secalin bands in grain samples of the cross combination 'Jubliar' × L290. These differences could presumably be put down to different combination abilities of the rye inbred lines L304 and L290 with the wheat cultivar 'Jubilar'.

The results of agronomical investigations have demonstrated that distinct differences exist between the four rye inbred lines L283, L290, L301 and L304 with regard to the combination suitability of these lines for production of triticale forms (Brandenstein et al. 1992). Various research groups have established that alien cytoplasm in different cereals, e.g. wheat and rye, can have a big influence on the expression of agronomical characteristics such as growth height, date of heading or thousand seed weight (Hsam and Larter 1974; Tsunewaki et al. 1984; Hainer 1991; Brandenstein et al. 1992). The influence of foreign cytoplasms on the expression of the prolamin bands could not be proven in the investigated triticale forms. But up to now the number of analyzed triticale forms have been very limited. Therefore, the possibility is suggested that distinct alien cytoplasms in cooperation with distinct genome combinations have some influence on the expression of prolamins.

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