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# Biochemical characterization of lines descended from 8x triticale × 4x triticale cross

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**Summary.** Fifty-two progenies originating from a cross between 8x and 4x triticale were submitted to cytogenetic analyses and to various electrophoretic studies [high-molecular-weight (HMW) glutelins, HMW secalins, $\gamma$-secalins, $\alpha$- and $\omega$-gliadins, $\beta$-amylases] to identify new genetic structures, more specifically the input of the D genome in a genetic context other than the wheat one. Markers of the rye genome (HMW and $\omega$-secalins) were identified in all of the triticale lines, but they originated either from the 4x or from the 8x parent, or from both. Chromosomes *4A*, *1B*, and *2R*, present in both parents, showed the same banding patterns in all progenies. Chromosomes *1R* and *5R*, present in both parents, showed heterogeneous labelling. The expression of chromosomes *6A*, *1D*, and *4D*, present in the 8x parent only, was more complex with a possible involvement of a regulatory system. Several hexaploid progenies had introgressed part of the D genome, suggesting that crossing 8x and 4x triticale was a practicable approach for transferring D chromosomes into hexaploid triticale.

**Key words:** Triticale – Interspecific hybridization – Genome rearrangement – Biochemical markers – Electrophoresis

## Introduction

Triticale (*Triticosecale* Wittmack) is produced by combining the genomes of wheat (*Triticum* sp.) and rye (*Secale*). All the cultivars that have been released until now are hexaploid ($2n = 6x = 42$), and most of them have the genomic structure AA BB RR. These cultivars lack the D genome, which could provide valuable genetic information concerning various traits (disease resistance, dwarfism, gluten quality). D chromosomes can theoretically be substitued or translocated with either R, A, and/or B chromosomes. D/R substitutions were first obtained because they could be easily achieved by crossing 6x triticale and bread wheat (Bernard and Bernard 1978). Some results were of practical interest as far as photoperiod insensitivity, plant height, or earliness were concerned (Skovmand et al. 1985). However, the number of single or multiple substitutions or translocations appeared to be limited (Gustafson et al. 1985), as is the pairing between R and D genomes; it is, therefore, difficult to suggest a generalized introgression between these two genomes.

Substitutions between chromosomes of the D and A (or B) genome are probably better accepted, but their production is difficult since it implies the availability of tetraploid triticales. If so, it is possible: (i) either to cross them with the diploid donor of the D genome (*Aegilops squarrosa* = *Triticum tauschii*), giving rise to amphiploid structures like (AB) (AB) DD RR, which contain the complete D genome (Bernard and Bernard 1987); (ii) or to cross tetraploid triticales with a polyploid species bearing the D genome, the most desirable being probably the octoploid triticale (Bernard et al. 1985a; Lukaszewski et al. 1987). From the $F_1$ progeny [formula: AB (AB) D RR], a large number of genotypes can be derived with various chromosome compositions.

Segregation can lead to 6x forms where some D chromosomes are substituted for A or B chromosomes and possess a "double-mixogenome" (A/B/D) with 14 chromosomes instead of 21. Moreover, chromosomes probably exist that are not of the parental type but result from homoeologous recombination.

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The objective of this study was to examine the possibilities of several biochemical markers (prolamins, glutelins, and β-amylases) whose genetic control and homoeology relationships are relatively well established in Triticeae, for identifying elements of these new genetic structures.

## Materials and methods

*Biochemical and cytological analyses*

These were performed on the following: (i) An octoploid triticale line (T 206), obtained by Y. Cauderon from a cross between the bread wheat (*Triticum aestivum* L. em Thell) "FEC 28" and the self-fertile rye line no. 51. (ii) A tetraploid triticale line (no. 532 $ 10), progeny of a cross between the hexaploid triticale line (T 532) and the self-fertile rye line no. 571. T 532 originated from a cross between the 8 x triticale line T 206 and the 6 x triticale line T 847 (from Jenkins). This cross gave rise to $F_1$ plants where homoeologous pairing was observed (Bernard and Saigne 1977). This particular 4x triticale line has been checked by chromosome C-banding as having the following chromosome composition: *1B 2A 3B 4A 5B 6B 7A*. Translocations, if any, were not detectable. (iii) Fifty-two progenies (harvested on $F_5$ or $F_6$ plants) originating by single-seed descent from the cross T 206 × no. 532 $ 10, made in 1979. In fact, the original $F_2$ population contained 250 plants. At each generation, a proportion of the population was lost because of sterility phenomena or because of the very poor quality of the seeds, some of which were unable to germinate. The chromosome evolution of this material was described previously (Bernard and Bernard 1985). Biochemical analysis was carried out on grains harvested from 42 + 2 chromosomes plants from the $F_5$ and $F_6$ generations, in order to identify some of the constituent chromosomes. According to the availability in seeds, 2–5 kernels per plant were analysed by each method.

*Polyacrylamide gel electrophoresis in the presence of SDS (SDS-PAGE)*

Proteins were extracted and reduced from single kernels by treatment with TRIS-SDS-mercaptoethanol buffer, according to Payne and Corfield (1979). The extraction procedure was expected to solubilize all protein species in the grain [soluble proteins, prolamins, low-molecular-weight (LMW) and high-molecular-weight (HMW) glutelin subunits].

Proteins were electrophoresed in vertical SDS-PAGE slabs in a discontinuous, pH 6.8/8.8, TRIS-borate-sodium dodecyl sulfate (SDS) buffer system (Payne and Corfield 1979) at a gel concentration of 13% (Autran and Berrier 1984) using a Desaga electrophoresis aparatus. Gels were fixed in 12% TCA and stained overnight with Coomassie Blue.

Wheat HMW glutenin subunits were identified in SDS-PAGE patterns and numbered according to the nomenclature for the major standard wheat subunits, based on genetic control, and described by Payne and Lawrence (1983). Specific rye HMW glutelin (HMW secalin) subunits were numbered as R1, R2, R3, and R4 according to increasing mobility.

*Polyacrylamide gel electrophoresis (PAGE)*

Gliadin proteins were extracted from single kernels and electrophoresed in 6% polyacrylamide gels, aluminium lactate buffer (pH 3.2), according to Bushuk and Zillman (1978). For the identification of β-amylase components, proteins were extracted overnight from individual kernels with 130 µl of 0.5 *M* sodium chloride, 0.2 *M* mercaptoethanol, pH 7.0 buffer. Electrophoresis was carried out for 3.5 h at 8 V/cm in polyacrylamide gels containing: 7.5% acrylamide, 0.2% methylene-bis-acrylamide, 0.3% dimethylaminopropionitrile, 0.1% ammonium persulfate in aluminium lactate buffer (pH 3.2).

The detection of β-amylase activity on gels was performed according to a previously described method (Joudrier 1974): gels were sliced longitudinally and incubated for 30 min at 40 °C in sodium acetate, pH 4.5 buffer, containing 1% starch. After incubation, the gel was washed with water and a solution containing 1.3% iodine and 3.0% potassium iodate was added. Zones showing any β-amylase activity were observed immediately.

## Results

Prior to a detailed examination of the results, some analytical difficulties inherent to the peculiarity of this material must be reported. Several poorly shaped or very shrivelled grains made it difficult to extract protein fractions and to get satisfactory electrophoresis separations. Also, for SDS-PAGE of total proteins (but not in β-amylase zymograms), several lines (nos. 95, 231, 240, 261, 304, and 391) yielded significantly different patterns when several generations or when several kernels were compared. Accordingly, we shall only refer to the major groups which could be identified by reproducible patterns.

Unambiguous identification of protein components among different patterns and among different experiments was difficult, because equally apparent mobilities do not ensure identification of the proteins but can only be presumed. When possible, the bands were considered as groups of linked components, since the presumption is much more certain when based on groups of bands that occur simultaneously. When the proteins could not be clearly identified, however, an absence of the relevant genes cannot be inferred.

*Allelic variation of the biochemical markers among wheat and rye parents and chromosome relationships*

Figure 1 a shows the SDS-PAGE patterns of the total reduced proteins for the different parents used in this work. Brackets, in the range of increased mobilities, indicate the major groups of components that have been identified from previous reports on protein composition in rye and wheat (Field et al. 1982; Shewry et al. 1982, 1983 a, b; Singh and Shepherd 1984; Field and Shewry 1987): (i) in the wheat patterns: HMW glutenins, ω-gliadins, LMW glutenins, α-, β-, and γ-gliadins; (ii) in the rye patterns: HMW secalins, 75-K γ-secalins, ω-secalins, and 40-K γ-secalins.

Although chromosome control for individual subunits was not established for cultivars and lines used in this study, the very typical protein associations observed in the patterns made it possible to propose specific markers for the different genomes and even for some chromo-

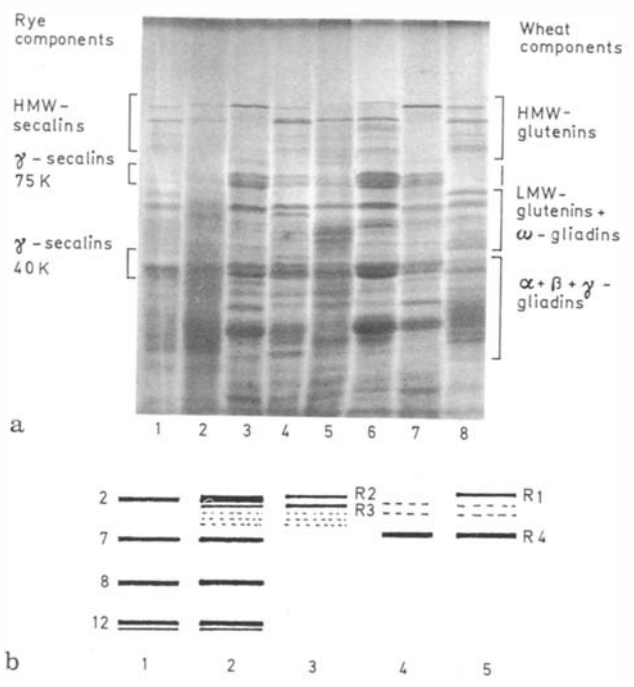


**Fig. 1.** **a** SDS-PAGE patterns of the total seed proteins contained in the following samples: *1* "FEC 28" wheat; *2* rye line no. 571; *3* rye line no. 51; *4* "Clercal" triticale; *5* triticale 4x line no. 532 \$ 10; *6* triticale 8x line no. T 206; *7* rye line no. 51; *8* "Etoile de Choisy" wheat. **b** Schemes of the high-molecular-weight secalin or glutelin patterns of the following samples: *1* "FEC 28" and "Etoile de Choisy" wheats; *2* triticale 8x line no. T 206; *3* rye line no. 51; *4* triticale 4x line no. 532 \$ 10; *5* rye line no. 571

some arms (Fig. 1 b). For instance, in wheats "FEC 28" and "Etoile de Choisy" (slot 1), a typical pattern of HMW glutenin subunits 2-7-8-12 was observed (Payne and Lawrence 1983; Branlard and Le Blanc 1984), with genes encoded by chromosome arms *1DL* (subunits nos. 2 and 12) and *1BL* (subunits nos. 7 and 8).

In the rye patterns, four major subunits were allocated to HMW secalins. They were referred to as R1-R4 in line 571 (slot 5) and as R2-R3 in line 51 (slot 3). These subunits are controlled by chromosome arm *1RL* (Lawrence and Shepherd 1981). On the other hand, two groups of bands were allocated to the 75-K and 40-K γ-secalins that are controlled by the chromosome arm *2RS* (Shewry et al. 1984). 75-K γ-Secalins comprise three components that we referred to as R5, R6, and R7 (Fig. 1 b).

A difficulty arose, however, from the very close proximity of some particular bands in the patterns. Subunit R1 (rye 571) and subunit R2 (rye 51) are extremely close to subunit no. 2 from wheat, making it impossible to know which one was present in triticale patterns or which genome (D for the subunit no. 2 or R for subunits R1/R2) was involved. The same was true for 40-K γ-secalins that co-migrate with some wheat γ-gliadins. Accordingly, the only markers considered from SDS-PAGE patterns were: (i) two HMW glutelin subunits from rye – R3 and R4 (*1RL*); (ii) three HMW glutenin subunits from wheat – nos. 7 and 8 (*1BL*) and no. 12 (*1DL*); (iii) three 75-K γ-secalins – R5, R6, R7 (*2RS*).

In addition, the gliadin proteins were analysed in acid PAGE (Fig. 3), to permit an identification of chromosome arms *6AS* and *1DS* from the occurrence of specific groups of α-gliadins and slow ω-gliadins, respectively.

Concerning β-amylase zymograms, among the six different types, I to VI (Fig. 2), that were identified according to the presence of components $C_1$, $C_2$, and $C_3$ (Joudrier 1974; Joudrier and Cauderon 1976; Bernard et al. 1977), cv "FEC 28" came under type II (presence of components $C_1 + C_3$) and the rye lines under type VI (presence of $C_2$-like components). From the analysis of nulli-tetrasomic, ditelosomic, and addition lines obtained for the cv "Chinese Spring," the chromosome location of the genes involved in the synthesis of some of the β-amylase components has been previously determined: components $C_1$ are controlled by genes on the beta-arm of chromosome *4A*, and components $C_3$ are controlled by genes on the long arm of chromosome *4D* (Joudrier and Cauderon 1976; Artemova 1982). On the other hand, the use of wheat-rye addition lines made it possible to determine that rye $C_2$-like components are under the control of chromosome *5R* (Bernard et al. 1977).

*Electrophoresis patterns of parental triticale lines*

Figure 1 (slots 5 and 6) shows the SDS-PAGE protein patterns of the two parental triticale lines. The pattern of the octoploid triticale T 206 was qualitatively very similar to those of the combined fractions of the two parents: presence of the HMW subunit group 2-7-8-12 from the wheat, HMW subunit R3 and γ-secalin R6 from the rye. This result corroborates the fact that the protein pattern of a triticale line is generally the sum of those of its wheat and rye parents, a finding previously reported by Chen and Bushuk (1970), Orth et al. (1974), Preston et al. (1975), Lupano and Anon (1985), and Lei and Reeck (1986). On the other hand, the pattern of the tetraploid triticale clearly contained bands from rye 571, such as subunit R4 and the 75-K γ-secalins, and very faint HMW glutelin bands 7 and 8 (B genome). No specific marker for the A genome was detected. Surprisingly, the octoploid and tetraploid triticales contained β-amylase components $C_1 + C_3$ and $C_1 + C_2$, respectively. This result tended to demonstrate that the $C_2$-like rye components observed in lines 51 and 571 were expressed in the tetraploid triticale, along with $C_1$ components (*4A*-coded), but were not present or not expressed in the octoploid triticale T 206.

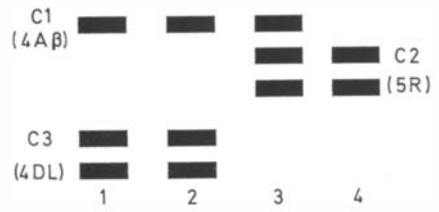


**Fig. 2.** Scheme of the $\beta$-amylase zymograms of the following samples: *1* "FEC 28" wheat; *2* triticale 8x T 206; *3* triticale 4x line no. 532 \$ 10; *4* rye line no. 51. The chromosome control of the $C_1$, $C_2$, and $C_3$ components is indicated

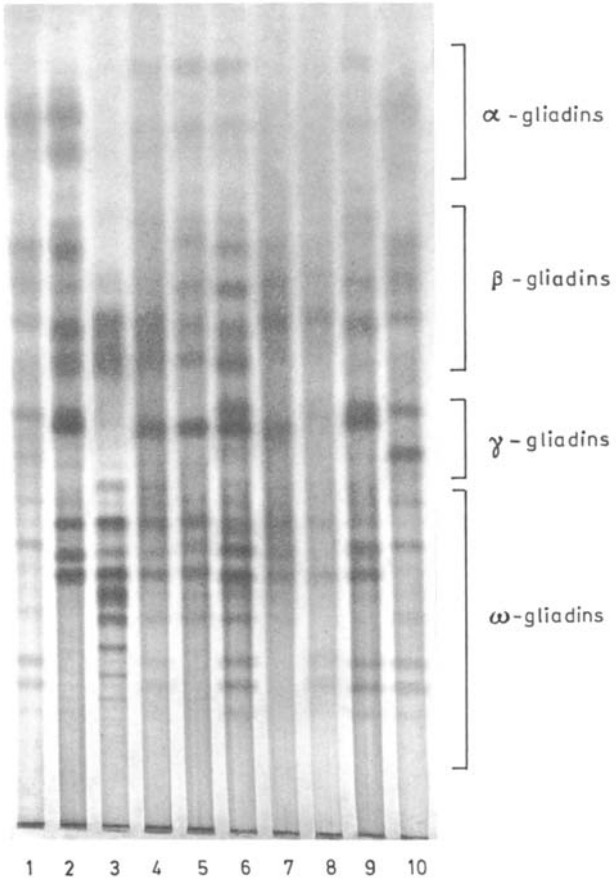


**Fig. 3.** PAGE patterns of gliadins contained in the following samples: *1* "Etoile de Choisy" wheat; *2* "Clercal" triticale; *3* rye line no. 51; *4* triticale T 249; *5* triticale T 254; *6* triticale T 68; *7* triticale T 206; *8* triticale T 217; *9* triticale T 231; *10* "Etoile de Choisy" wheat

*Electrophoresis patterns of 8x by 4x triticale lines*

*"A" genome.* The "A" genome is generally difficult to identify from HMW subunits in SDS-PAGE patterns because most wheat varieties (including the wheat parent "FEC 28" used in this cross) have a null allele at locus *Glu-A1*. Accordingly, no characteristic *1A*-coded glutelin band could be used in this work. However, gliadin patterns (Fig. 3) contain two major $\alpha$-gliadin bands that have been reported in many cultivars in the world as "A-gliadin" type, which is controlled by chromosome arm *6AS* (Kasarda et al. 1976). Since cv "FEC 28" clearly belongs to this type, the presence of an "A-gliadin" type has made possible an identification of chromosome arm *6AS* in the different triticale lines.

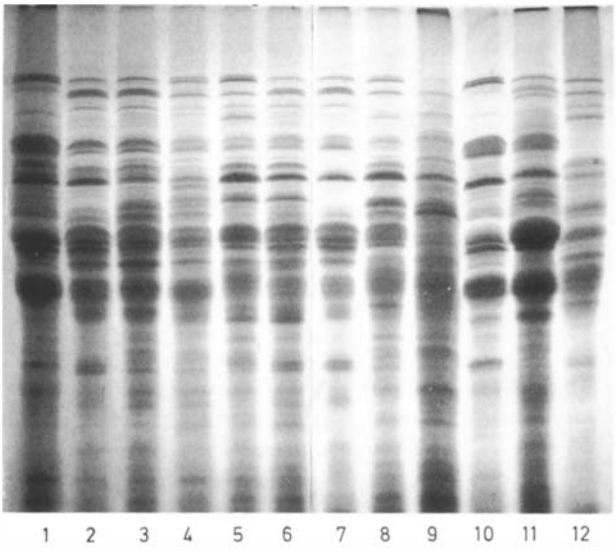


**Fig. 4.** SDS-PAGE patterns of the total seed proteins contained in the following lines of the T 8x × T 4x progeny: *1* rye line no. 51; *2* triticale T 29; *3* triticale T 30; *4* triticale T 52; *5* triticale T 76; *6* triticale T 77; *7* triticale T 216; *8* triticale T 217; *9* triticale 4x line no. 532 \$ 10; *10* rye line no. 51; *11* rye line no. 571; *12* "Etoile de Choisy" wheat

For instance, in lines 52, 68, 76, 81, 93, 107, 109, 169, 171, 186, 199, 206, 237, 262, 263, 268, 303, and 505, locus *Gli-A2* was certainly present and expressed. In several other lines (36, 118, 217, 233, 249, 254, 261, 282, 508, 514), however, the "A-gliadin" characteristic group could not be clearly identified, and the traces of proteins observed in the $\alpha$-gliadin region did not permit us to determine whether we dealt with other minor $\alpha$-gliadin components originating from wheat or rye or with weakly expressed *6A*-coded proteins. On the other hand, no complementary information could be obtained from $\beta$-amylase patterns, since $C_1$ (*4A*-coded) components were observed in both triticale parents and in all the progenies.

*"B" genome.* The "B" genome could be clearly marked by the two HMW wheat subunits 7 and 8 coded at locus *Glu-B1*. These subunits were observed in most of the triticale lines (Fig. 4), but with different intensities compared with the wheat parent: subunit 8 was often weaker and even present as traces in some lines. The absence of the whole B-coded group was noticed only in two grains (out of three) of line 301 and in one grain (out of two of line 95. It can be concluded that locus *Glu-B1* of chromosome *1B* (which has been identified in both T 8x and T 4x parents) is generally present in the progeny of the T 8x by T 4x cross.

*"D" genome.* The "D" genome could theoretically be identified by the two HMW wheat subunits 2 and 12 coded at locus *Glu-D1* on chromosome arm *1DL*. However, subunit no. 2 was extremely difficult to separate from the HMW subunits R1 and R2 originating from rye lines 571 and 51, respectively. Consequently, subunit no. 12 could only be used as a reliable marker for chromosome *1D*. This subunit was clearly identified in 31 triticale lines, but was not detected at all in 11 lines (nos. 53, 70 106, 110, 118, 186, 233, 240, 262, 305, 505). In two lines (nos. 232 and 237), no conclusion could be drawn because of some heterogeneity in the presence of subunit no. 12 among the patterns from different grains of the same generation. In eight other lines, only traces of this subunit could be noticed. This results seemed to indicate that locus *Glu-D1* was generally present, but weakly expressed.

The presence of the slowest $\omega$-gliadin bands in PAGE patterns (especially the triplet bands with mobilities 12-15-18, whose genes occur at locus *Gli-D1*) was also investigated (Fig. 3). This triplet was observed in 12 triticale lines only and was clearly absent in the 40 others. In these 12 lines, the HMW subunit no. 12 was always present. Therefore, in the 19 other lines having subunit no. 12 (marker for the chromosome arm *1DL*), the $\omega$-gliadins (markers for chromosome arm *1DS*, were either absent or not expressed.

Further information was obtained upon examination of $\beta$-amylase zymograms: 12 lines out of the 52 analyzed contain the $C_3$ components that are markers for the chromosome arm *4DL*. No association was noted, however, between the presence of chromosome arms *4DL* and *1DL* or *1DS*.

*"R" genome.* One or both of the HMW secalin markers from rye (subunits R3 or R4) were present in all the triticale lines examined (with a possible exception of line 282). Subunit R3 alone was observed in 17 lines, whereas subunit R4 was observed in 24 lines. In three lines (240, 261, 301), the presence of both subunits was noted, indicating probable heterozygosity. In six lines, however, there was some heterogeneity of the patterns among grains from the same generation or among grains from different generations. In those cases, subunit R3 was present with R4 absent, or vice versa.

The other group of markers for the R genome that was investigated corresponded to the three very intense 75-K $\gamma$-secalins that are characteristics of all rye patterns (Shewry and Miflin 1984). These proteins, although homologues of wheat $\gamma$-gliadins (Kasarda et al. 1983; Kreis et al. 1985), are coded by genes located on the short arm of rye chromosome *2R* (Shewry et al. 1984). This group of markers for the rye genome was observed in all the triticale lines, suggesting an expression of the chromosome *2R* genes. However, some variation were noticed in the intensity of the intensity of these three bands: in 14 lines, a pattern with the R6 component alone or more intense than the other two was observed (Fig. 4, slot 8), instead of the typical pattern of the rye parents, which comprised three regularly spaced bands with similar intensities.

Concerning $\beta$-amylases markers, 22 lines contained the $C_2$ components, indicating the presence of chromosome *5R*, and suggesting a different system of expression in these lines, compared with the one of octoploid parent (T 206) in which the $C_2$ components were not expressed.

*Summary of the observed differences*

The attempts to identify markers for the different A, B, D, and R genome are synthesized in Table 1. Markers that displayed very little or no genetic variation between lines, such as HMW glutenin subunits 7 and 8 (*1BL*) or $\gamma$-secalin R6 (*2R*) were not reported. Question marks added imply that the results were not totally certain, as the components were observed in traces only.

For the following reasons, care must be taken when discussing the results (i) All information in this table is based on the identification of electrophoresis components with equal apparent mobilities (identity presumed). (ii) The presence of a protein marker suggests the presence of the relevant locus, but does not imply the presence of the whole chromosome or the whole genome. The markers used in this study do not characterize the whole chromosome but a part of it. For example, different loci of the same chromosome were separated, some lines having locus *Glu-D1* but not locus *Gli-D1*, or vice versa. (iii) The absence of a component may not signify an absence of the gene but perhaps only an absence of expression of the gene (or an expression at an undetectable level).

## Discussion and conclusion

Tetraploid triticale are usually derived from crosses between 6x triticale and rye (Bernard and Saigne 1977). Therefore, they usually possess a complete R genome, along with a set of seven wheat chromosomes called mixogenome (Bernard et al. 1985 b). The number of randomly extracted mixogenomes of intact chromosomes – excluding translocated ones – is thus 128. In the T8x × T4x $F_1$ hybrid, again, the two sets of 14 chromosmes contributed by both parents should pair and segregate regularly. The remaining chromosomes, including D chromosomes, are in a hemizygous condition and will be included at random in gametes and zygotes in the following generations. Here again, the theoretical number of chromosome combinations is 128.

The identification of each chromosome pair requires the availability of at least one marker per chromosome; the detection of homoeologous events, if any, requires that of one marker on each chromosome arm. Unfortu-

**Table 1.** Classification of the progenies originating from the cross triticale 8x by triticale 4x according to the presence (+) or the absence (−) of the various biochemical markers for wheat or rye chromosomes

| Line | Marker: | α-gliad. | HMW glut. no. 12 | β-amylase $C_3$ | ω-gliad. | HMW-secal. $R_3$ | HMW-secal. $R_4$ | γ-secal. $R_{5-7}$ | β-amylase $C_2$ |
|---|---|---|---|---|---|---|---|---|---|
| | Chromosome: | $6A_s$ | $1D_L$ | $4D_L$ | $1D_s$ | 1R | 1R | 2R | 5R |
| FEC 28 wheat | | + | + | + | + | − | − | − | − |
| Rye 571 | | − | − | − | − | − | + | + | + |
| Rye 51 | | − | − | − | − | + | − | + | + |
| 532 $ 10 (4x) | | − | − | − | − | − | + | + | + |
| T 206 (8x) | | + | + | + | − | + | − | − | − |
| 6 | | − | + | − | − | − | + | − | − |
| 25 | | − | tr. | − | − | − | + | + | − |
| 29 | | − | + | − | − | − | + | + | − |
| 30 | | − | + | + | − | − | + | + | − |
| 36 | | tr. | + | − | + | + | − | + | − |
| 52 | | + | + | − | − | + | − | + | − |
| 53 | | − | − | − | − | − | + | − | + |
| 68 | | + | + | − | − | − | + | + | − |
| 70 | | − | − | + | − | + | − | + | + |
| 76 | | + | + | + | + | + | − | + | − |
| 77 | | − | + | + | − | − | + | + | − |
| 79 | | − | + | tr. | − | − | + | + | tr. |
| 81 | | + | + | − | − | + | − | + | − |
| 86 | | − | tr. | tr. | − | − | + | + | tr. |
| 93 | | + | + | + | + | + | − | + | − |
| 95 | | − | + | tr. | + | − | H | + | tr. |
| 97 | | − | + | − | + | + | − | − | − |
| 106 | | − | − | tr. | − | − | + | − | tr. |
| 107 | | + | + | + | − | − | + | − | + |
| 109 | | + | + | tr. | − | − | + | − | tr. |
| 110 | | − | − | − | − | − | + | + | + |
| 113 | | − | + | − | − | − | + | + | − |
| 118 | | tr. | − | tr. | − | − | + | + | tr. |
| 169 | | + | tr. | + | − | − | + | + | − |
| 171 | | + | + | − | − | − | + | + | − |
| 186 | | + | − | − | − | − | + | + | + |
| 199 | | + | + | + | − | − | + | + | − |
| 201 | | + | + | + | − | + | − | − | − |
| 216 | | − | tr. | − | − | − | + | + | − |
| 217 | | tr. | + | + | + | + | − | − | − |
| 231 | | − | + | − | + | H | H | − | − |
| 232 | | − | H | − | − | − | + | + | − |
| 233 | | tr. | − | − | tr. | tr. | tr. | tr. | + |
| 237 | | + | H | − | − | − | + | + | + |
| 240 | | − | − | − | − | H | + | + | + |
| 249 | | tr. | + | − | + | + | − | − | + |
| 252 | | − | + | − | + | + | − | − | + |
| 254 | | tr. | + | − | − | + | − | + | + |
| 261 | | tr. | + | + | − | H | + | + | + |
| 262 | | + | − | − | − | + | − | + | + |
| 263 | | + | + | − | − | − | + | + | + |
| 268 | | + | + | − | + | + | − | + | + |
| 282 | | tr. | tr. | − | + | − | tr. | + | − |
| 301 | | − | + | − | + | H | H | + | + |
| 303 | | + | + | + | − | − | + | − | − |
| 304 | | − | + | − | − | H | − | − | + |
| 305 | | − | − | − | − | − | + | + | + |
| 311 | | − | tr. | − | − | − | + | + | + |
| 505 | | + | − | − | − | H | + | + | + |
| 508 | | tr. | tr. | + | − | − | + | + | + |
| 514 | | tr. | + | − | − | + | − | + | + |

H – Indicates a heterogeneity among the patterns from different grains of the same generation
tr. – Indicates that the component was present in traces only

nately, this is not the case with the biochemical markers in wheat; in this particular study, we were able to label eight chromosomes only and, among them, one on both arms. The reason for this is that the answers based on various biochemical markers are quite different, depending on the initial situation and the events which have occurred during the evolution of that material.

Three chromosomes are present in both parents and show the same biochemical pattern: *4A* (labelled by β-amylase), *1B* (labelled by HMW glutelins), and *2R* (labelled by γ-secalins); they are observed in all the progenies. However, the expression of some *1B*-coded glutenin genes in the T 4 x parent is weak, and some heterogeneity occurs in the progeny for the R5 and R7 γ-secalin components.

Two chromosomes (*1R* and *5R*) are present in both parents and show heterogeneous labelling. For *1R*, the R3 and R4 HMW secalin subunits segreate close to 1:1 (17:24). In some plants, however, both markers are observed even in sister seeds. This suggests that either R3 and R4 subunits are not completely allelic or the two parental chromosomes are present, indicating a strong residual heterozygosity at this level of generation for this character.

For chromosome *5R*, the β-amylase allele contributed by the octoploid parent is not detectable. Therefore, the only visible marker is that apparently coming from the tetraploid parent, which is observed in about one-half of the progenies (23/52).

Three chromosomes (*6A*, *1D*, and *4D*) are contributed only by the octoploid parent. Relevant markers were detected to some extent in 18, 31, and 12 progenies, respectively.

Concerning chromosome *1D*, markers were available on both arms (*Glu-1DL*) and (*Gli-1DS*). The *Glu* markers were clearly present in 31 progenies and were weakly expressed in 8 others. This proportion is different from that expected according to a probability of 0.5. On the other hand, among the 31 progenies with the *Glu* marker, only 12 also showed the *Gli* marker. In contrast, the *Gli* marker was never observed without the *Glu* one.

The results suggest a complex expression of these genes involving a regulatory system. Whether the regulatory system is close to the locus on the same chromosome or another chromosome is not established. Moreover, since the initial hybrid situation involved only one chromosome *1D*, the variations in the expression could be explained by: (i) a segregation in the remaining part of the genome, involving the regulatory systems; (ii) some internal rearrangements in chromosome *1D*; (iii) a homoeologous recombination process involving mainly chromosome *1A*.

On this last assumption, the observed results suggest that the only existing recombinants would be *1DL/1AS*, excluding the reciprocal *1DS/1AL*.

Several markers for the rye genome have been identified in all the triticale lines. Segregating rye genome markers originate either from the T 4 x parent (in 29 lines) or from the T 8 x parent (in 16 lines), or from both (in 6 lines). The 75-K γ-secalin group, however, is not expressed as a whole in all lines, but only in the T 4 x parent and in 37 progenies.

By crossing octoploid and tetraploid triticales, it has been possible to produce hexaploid triticale lines that proved to have introgressed some chromosomes of the D genome, certain ones exhibiting very good agronomic performance. Although it is difficult to state whether the chromosomes of the *D* genomes are selected against or not, chromosomes *1D* and *4D* are present with a frequency quite close to the theoretical one. This approach, therefore, can be suggested as readily practicable for transfering *D* chromosomes into hexaploid triticale.

As expected, substituting some chromosomes of the D genome to chromosome of the A or B – instead of the R – genome appears easier to put into practice. This enables lines with multiple substitutions to be obtained.

However, phenotypes of these triticale are not very different from phenotypes of classical 6 x lines. Consequently, at this ploidy level, the substitution of D chromosome to other wheat chromosomes does not have phenotypic effects as marked as that of D to R chromosomes.

This material must now be tested with respect to baking quality, in order to detect substitutions leading to increased values for this trait. It remains perhaps more important to delete some parts of the R genome involved in the genetic control of protein synthesis and quality expression.

A comprehensive study of the structuring of (AB) of (ABD) mixogenomes should obviously include at least two marker loci per chromosome. This is difficult with biochemical markers, because not enough are available to identify each chromosome arm. The availability of molecular probes, specific to either a given sequence or a chromosome, could permit a better understanding of these newly created structures.

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