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Parental genome expression in synthetic wheats (*Triticum turgidum* sp. × *T. tauschii* sp.) revealed by two-dimensional electrophoresis of seedling proteins

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Summary. Two-dimensional gel electrophoresis was conducted on etiolated seedling proteins from two distinct amphiploids (ABD1, ABD2) and their parental lines (AB1, D1 and AB2, D2), AB1 and AB2 being used as female. On the amphiploid patterns were found all the parental spots except 8 D spots of which 3 are cytoplasmically encoded. One exceptional polypeptide observed in ABD1 was present neither in AB1 nor D1. The patterns fromt the amphiploids very closely resemble the co-electrophoresis done with ⅓ D protein extract and ⅔ AB protein extract. Thus it is very likely that for most gene products revealed the genomes act independently of each other.

Key words: *Triticum* – 2D electrophoresis – Amphiploid – Homoeology – Gene regulation

Introduction

Homoeology and evolutionary relationships between the three A, B and D genomes of the common wheat *Triticum aestivum* (L.) Thell. have been examined at various levels, from the pioneer cytological studies (Kihara 1954, 1963; Sears 1954, 1958; Riley and Chapman 1960; Okamoto 1962) to more recent biochemical and molecular investigations (Flavell et al. 1979; Jaaska 1980; Appels and Dvorak 1982; Bowman et al. 1983; Tsunewaki and Ogihara 1983).

It is now well established that the hexaploid wheat *Triticum aestivum* originates from a cross between *T. turgidum* (L.) Thell. AABB as the female parent and *T. tauschii* (Coss.) Schmal. DD (formerly *Aegilops squarrosa* L.), followed by a doubling of the chromosome number. Since these spontaneous phenomena occurred circa 10,000 years ago, artificial selection led to today's wheat varieties.

The three genomes are homoeologous (Sears 1952, 1975; Kihara 1954) and each chromosome in the A, B and D genomes has two homoeologous in the other two genomes, with the exception of chromosome 4. Chromosome 4 "A" also originates from the B genome (Lane Rayburn and Gill 1985; Wazuddin and Driscoll 1986).

The experiments reported below were undertaken (1) to assess to which extent the homoeology described mainly at the chromosome level is also true for numerous individual genes and (2) to contribute to a better understanding of the regulation between homoeologous genes, for instance intergenomic suppression (Galili and Feldman 1984)

We examined using two-dimensional (2D) gel electrophoresis, the expression of several hundreds of genes from *Triticum turgidum, T. tauschii* and their amphiploid (synthetic wheat). Two different amphiploids and their parental lines were studied and the 2D patterns of etiolated seedling proteins from these six genotypes were compared with each other and with the variety Chinese Spring.

Materials and methods

Plant Material

The plant material was supplied by Dr. Y. Cauderon (INRA Versailles) who made the amphiploids:

(1) amphiploid no. 364, hereafter called ABD1 (2n = 42), resulting from the cross ♀ *Triticum turgidum* ssp. *dicoccoides spontaneo-nigrum* (accesion no. 1262), AB1 (2n = 28) × ♂ *T. tauschii* (*Aegilops squarrosa typica*, accession no. 1267), D1 (2n = 14).
(2) amphiploid no. 367, ABD2 (2n = 42), resulting from the cross ♀ *T. turgidum* ssp. *persicum stramineun* (accession no.

1265), AB2 (2n=28)×♂*T. tauschii* (*Aegilops squarrosa meyeri*, accession no. 1269), D2 (2n=14).

The amphiploid seeds used in the experiments were harvested on the selfed plants resulting from self-pollinated colchicine treated hybrids. In addition to these 6 genotypes, we also used the following lines provided by G. Doussinault (INRA Rennes): the wheat variety Chinese Spring (CS), the alloplasmic CS on the cytoplasm of *T. turgidum* and the alloplasmic CS on the cytoplasm of *T. tauschii*.

The seeds were placed on water-soaked paper in Petri dishes and allowed to germinate in the dark for 6 days at 20 °C in a regulated growth chamber. The aerial part of the seedlings was cut off and immediately frozen in liquid nitrogen until extraction.

At least three samples were used for each genotype, each sample consisting of one or two hexaploid or tetraploid individuals and two or more diploid individuals.

Protein extraction and electrophoresis

The proteins were extracted according to Zivy (Damerval et al. 1986). The isoelectrofocusing (IEF) rod gels were 24 cm long and had a 1 mm inner diameter. The gel mixture was 4% acrylamide, 9.2 M urea, 2% Triton X-100 and 4% carrier ampholytes (¾ Pharmalyte pH 5–8, ¼ Pharmalyte pH 5–6). The IEF was performed for 40,000 Vh. The rod gels were then equilibrated for 15 min in a Tris-Cl^- buffer, pH 6.8 (62.5 mM Tris, 2.3% SDS, 10% sucrose). The second dimension was performed on slab gels (215×240×1 mm) bound to Gelbond PAG (Marine Colloids) in a Dalt tank (Electronucleonics). Uniform gel composition was 11% acrylamide, 0.5 M Tris-Cl^-, 0.15% SDS, 1% sucrose. Then 19 gels were simultaneously run and silver stained using the method and the apparatus described by Granier and de Vienne (1986).

Gel comparison

The gels were visually scored using co-electrophoresis between different genotypes and by superimposing the gels dried on Gelbond. Each genotype was compared with the others. We noted the spots present or absent in each of the genotypes and those common to all. Quantitative variations were also retained when clear and reproducible. A difference was retained between two genotypes when scored on every gel of the comparison (at least three gels for each genotype). In addition, since the two parental lines of the amphiploids possess different cytoplasms we compared the euplasmic CS line with its two alloplasmic lines having *T. turgidum* and *T. tauschii* cytoplasms to define the cytoplasmically encoded proteins. Finally we compared the expression of seven nuclear genotypes (D1, D2, AB1, AB2, ABD1, ABD2, CS) and three cytoplasmic constitutions (cytoplasms of *T. tauschii*, *T. turgidum* and *T. aestivum*). We also calculated a similarity index between two genotypes (Is) as the number of common spots divided by the total number of spots found in the two genotypes.

Results

One of the 2D gels obtained is presented in Fig. 1. On the synthetic "master" map (Fig. 2), 988 spots are retained, all those reproducibly found in one or the other genotype examined. Among them, 401 (in black) are present in every genotype. We scored approximately the same number of spots in the D diploids and the AB tetraploids (around 700) and more in the hexaploids (867 to 878). For the two amphiploids, the number of spots of each parental line and their behaviour in the amphiploid are indicated in Table 1.

Fig. 1. The 2D gel produced by a mixture of the two genotypes AB1 and D1 (co-electrophoresis)

T. turgidum polypeptides in the amphiploids

All the spots reproducibly found in AB1 are also present in the ABD1 pattern and all the AB2 spots are also found in ABD2 (Table 1). It was found that 46 AB1 specific spots and 16 AB2 specific spots are less intense in the corresponding amphiploid. Three spots common to AB1 and D1 are less intense in ABD1 and in D1 than in AB1. One spot in AB1 and one in AB2 are more intense in the corresponding amphiploid and the D parent.

T. tauschii polypeptides in the amphiploids

Eight D1 specific spots are not present on the ABD1 pattern and 4 D2 specific polypeptides are not found in ABD2. Quantitative variation was observed for most of the D specific spots, decreasing in intensity in the amphiploid. Three D1 spots are more intense in ABD1 and in AB1 and 3 D2 spots are more intense in ABD2 and in AB2. Seven D1 spots are less intense in ABD1 and in AB1 and 3 D2 spots are less intense in ABD2 and in AB2.

Cytoplasmically encoded polypeptides

Since both amphiploids have been produced using *T. turgidum* as the female parent, some spots found on the D pattern and not in ABD can be cytoplasmically

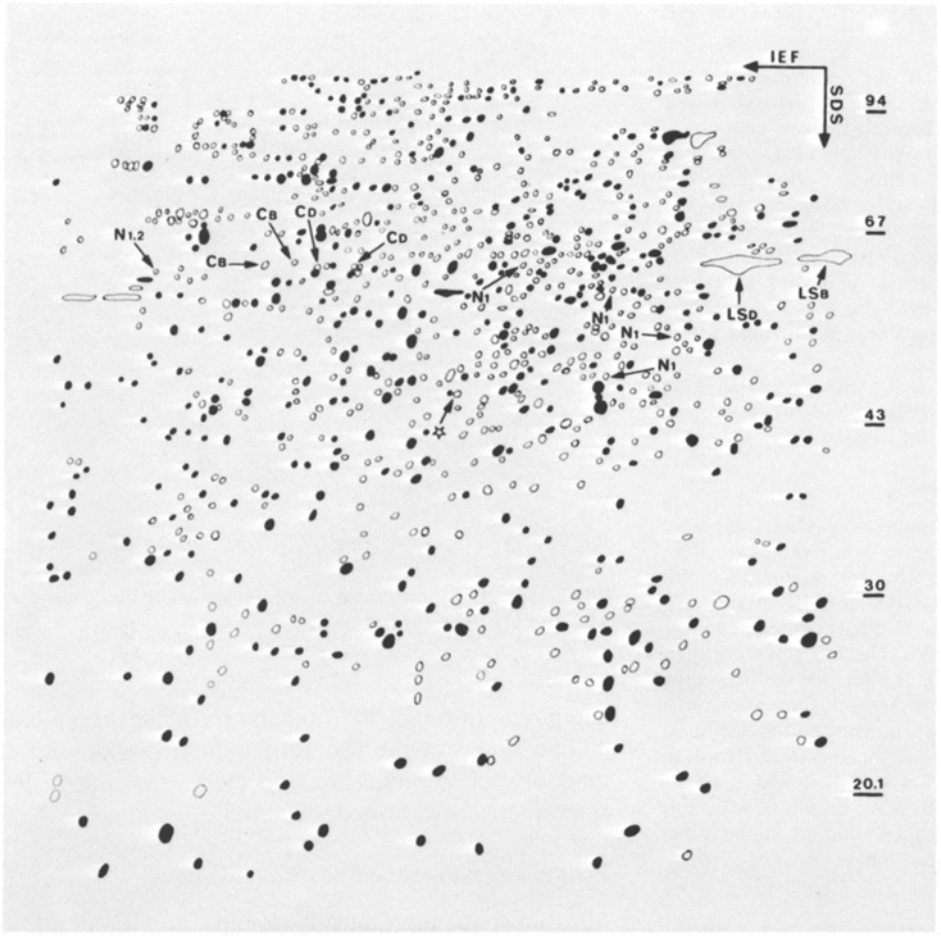

Fig. 2. "Master" map of all the spots of the analysis. In black: spots found in every genotype. *LS:* large subunit of ribulose bisphosphate carboxylase/oxygenase; *C:* cytoplasmically encoded polypeptide from *T. tauschii* (*D*) or from *T. turgidum* (*B*); N:D genome encoded polypeptides supressed in ABD1 (N1) or in ABD2 (N2). The star indicates the ABD1 specific spot. The molecular weights are indicated in kilodaltons on the right

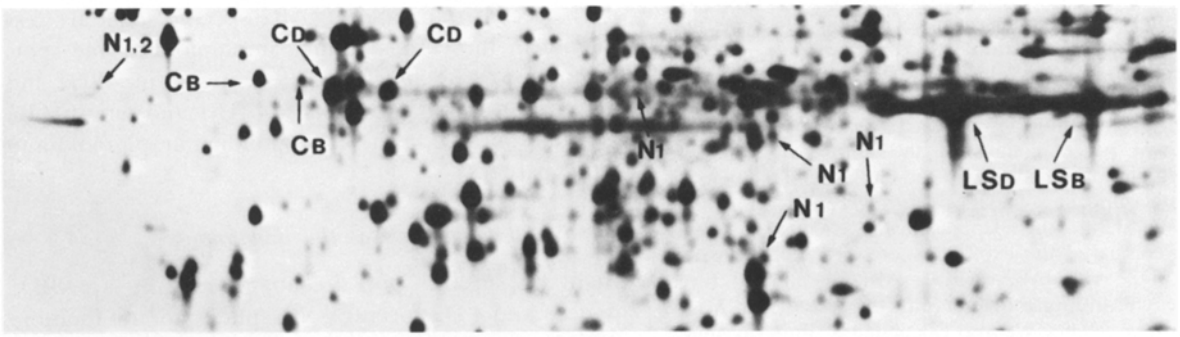

Fig. 3. A cutting from Fig. 1: symbols as in Fig. 2

encoded. To distinguish those spots we compared the two cytoplasmic genomes using the alloplasmic lines of CS on *T. turgidum* and *T. tauschii* cytoplasms.

The two CS lines on *T. aestivum* and on *T. turgidum* cytoplasms gave strictly identical patterns, as reported previously (Zivy et al. 1983). However, we found that the large subunit (LS) of Ribulose bisphosphate carboxylase/oxygenase and two other spots (indicated in Figs. 2 and 3) behave as cytoplasmically encoded "allelic" products. Three spots are present in *T. aestivum* and *T. turgidum* cytoplasms and absent in *T. tauschii* cytoplasm, whereas three others with different isoelectric points and molecular weights are present in *T. tauschii* but absent in the two other cytoplasms.

ABD1 spot

Except for a few *T. tauschii* spots, the amphiploid pattern is the sum of the spots found on the patterns of its

Table 1. Number of spots in the parental lines and their behaviour in the amphiploid

Genotype	No. of spots	Present in ABD	Decreasing in intensity	Increasing in intensity
D1	695 (192[a])	184	144	
			(7[d])	(3[f])
AB1	682 (503[b])	503	(3[e])	(1[g])
ABD1	(179[c])	179	46	
		867		
D2	688 (181[a])	177	130	
			(3[d])	(3[f])
AB2	697 (507[b])	507	(0)	(1[g])
ABD2	(190[c])	190	16	
		874		

[a] Spots only found in the corresponding *T. tauschii*
[b] Spots found common to both parental lines
[c] Spots only found in the corresponding *T. turgidum*
[d] Spots common to both parents, less intense in ABD and AB than in D
[e] Spots common to both parents, less intense in ABD and D than in AB
[f] Spots common to both parents, more intense in ABD and AB than in D
[g] Spots common to both parents, more intense in ABD and D than in AB

Table 2. Comparisons between the three hexaploids (ABD1, ABD2 and CS)

Comparison X/Y	No. of spots				
	in X	in Y	common to X and Y	more intense in X	more intense in Y
CS/ABD1	878	867	799	6	14
CS/ABD2	878	874	825	5	6
ABD1/ABD2	867	874	796	16	15

two parental lines. Surprisingly, we found one spot in ABD1 which was not present in the parental patterns. This unique spot (Fig. 2) is present only in this amphiploid and is reproducible.

Comparison of amphiploids and wheat variety Chinese Spring

The differences observed between the ABD1 and the ABD2 amphiploids and between each of them and CS are indicated in Table 2. Most spots are electrophoretically identical and the number of quantitative variations retained, i.e. clear cut and reproducible, among the common spots, is small.

Table 3. Similarity indices between genotypes: number of spots common to the two genotypes divided by the total number of spots found in both

	D2	AB1	AB2	ABD1	ABD2	CS
D1	0.79	0.58	0.56	0.79	0.71	0.71
D2		0.55	0.58	0.70	0.78	0.73
AB1			0.79	0.78	0.71	0.70
AB2				0.73	0.80	0.74
ABD1					0.84	0.84
ABD2						0.89

Similarity of the patterns at the three ploidy levels

In Table 3 the similarity indices calculated for each couple of genotypes are indicated. As reported previously (Colas des Francs 1985; Thiellement and Colas des Francs 1986) and as can be deduced from Tables 1 and 2, more than half of the polypeptides are electrophoretically identical between two *Triticum* genotypes, whatever the ploidy levels.

Discussion

Quantitative variations between ploidy levels

The same amount of protein extract is electrophoresed whatever the ploidy level of the genotype. If each genome acts independently of the other ones, the D genome, for instance, will account for all the proteins found on the D pattern and for one third of those on the ABD pattern. Thus the spots common to both parents will appear unchanged whatever the ploidy level, whereas the D or AB specific spots will be less intense on the amphiploid patterns. This simple explanation very likely holds, since this phenomenon is observed for almost all the common polypeptides in ABD1 and in ABD2. It is also observed for 75% and 72% of the D1 and D2 specific spots that decrease in intensity in their respective amphiploids. However, it is not found for the AB specific spots (26% and 8% decreased in intensity). This results, in our opinion, from the difficulty in visually detecting a one third intensity decrease. An indirect confirmation is given by the comparison between the amphiploid pattern and a co-electrophoresis where the initial load was ⅓ of D extract and ⅔ of AB extract: the two patterns appear undistinguishable for most proteins, common or specific. The discrepancy between the two amphiploids for the AB specific spots probably results from the very high quality of the 2D gels from AB1 and ABD1.

This inability to see a one third intensity decrease was suggested in previous experiments. Only a few spots are less intense than in the euploid in two or three

homoeologous ditelosomic lines, although this phenomenon should have been observed on the numerous polypeptides that correspond to 2 or 3 electrophoretically identical homoeoallelic products (Thiellement et al. 1986).

Similarity of the 2D patterns and homoeology

Most of the spots found in one genotype are electrophoretically identical to those of other genotypes, whatever the ploidy level. The indices given in Table 3 are an estimation of this similarity. Between the two *T. tauschii* genotypes and between the two *T. turgidum* subspecies, similarity indices are around 0.8, which may indicate the intraspecific variability. Each subspecies is represented by only one line but as most gene products must derive from the more frequent allelic state, the great number of genes revealed permits this comparison. The variability observed in the hexaploid group is slightly less important than in the other two groups and, surprisingly, the newly created amphiploid ABD2 is very similar to the CS wheat variety (Is = 0.89).

The smaller indices are between the D and AB groups, as expected (Is from 0.55 to 0.58). The validity of this comparison between ploidy levels can be questioned, but nevertheless more than half the polypeptides are common to the D and AB groups. Thus many genes on the D genome and on the A and/or B genomes synthesize electrophoretically identical products.

There are 401 polypeptides common to the 5 genotypes examined (D1, D2, AB1, AB2, CS). Thus allelic variation seems very rare at these loci, encoding identical products. The same deduction was made in a previous study of a polyploid series of *Triticum* (Thiellement and Colas des Francs 1986). Homoeology is very likely the rule for most individual genes on the chromosomes since in most cases the structural genes are very similar on at least two (D and A or B) homoeologous chromosomes of wheat. Recent experiments in our laboratory on representatives of the A genome and on *Sitopsis* species provided additional evidence to support this finding, as does the analysis of the ditelosomic series (Colas des Francs and Thiellement 1985; Thiellement et al. 1986). By contrast, homoeology does not seem to be the rule for regulators (defined as any sequence, gene or element of a system that affects the amount of a protein), as reported recently (Thiellement et al. 1986).

Interactive regulations in the amphiploids

Galili and Feldman (1984) have shown that some new gene products detected in AB were not present in ABD in their study of the endosperm proteins of the tetraploid extracted from an ABD wheat variety. Our experiments were partly designed to assess the generality of this "intergenomic suppression". We found that all the AB gene products are present in the amphiploid. For the D gene products, eight were no longer present in the ABD1 genotype and four in the ABD2. The disappearance of three polypeptides in each case is due to their cytoplasmic determinism. The remaining D2 spot, absent in ABD2, is the same as one of the D1 spots which is absent in ABD1. Among the other four D1 "suppressed" polypeptides, two are found only in D1 and the other two are common to D1 and D2 but are present in ABD2. The spot disappearances were verified by their absence on gels obtained by increasing the initial amphiploid protein load although they were present in ⅓ D, ⅔ AB co-electrophoresis.

If gene suppression takes place during the course of wheat evolution, i.e. in fact a loss of redundant gene expression (Garcia-Olmedo et al. 1977), we should have observed fewer gene products in today's variety of CS than in the newly created amphiploids. As this is not the case (see Table 2, 878 spots are scored in CS, 874 in ABD2 and 867 in ABD1) and as suppression is only observed for 5 D gene products, this phenomenon is very unfrequent, at least for the proteins studied. However, this may have occurred for homoeologous genes that give rise to indistinguishable products.

There is one ABD1 specific spot (Fig. 2) which appears in this amphiploid although it was absent in the two parental lines AB1 and D1. This polypeptide is found in every *Sitopsis* species so far examined in our laboratory but is absent in *T. monococcum monococcum, T. monococcum boeoticum* and *T. urartu* (unpublished results). A possible explanation is that this hypothetical B product is suppressed in the AB constitution and can be reexpressed in the presence of the D genome.

Some polypeptides are not synthesized at the level expected if each genome is acting independently of the others. This is true for the above mentioned disappearing D products and the ABD1 specific spot. It can also be the case for common, D or AB specific spots but visual examination does not reveal the extent of this phenomenon. Precise quantitative variations must be obtained using automatic gel scanning and comparisons between the amphiploid patterns and the ⅓ D, ⅔ AB co-electrophoresis.

The data reported above, however, demonstrate that new regulatory effects resulting from the presence of three genomes in the same cell do not drastically affect the expression of most of the revealed genes. This may be due to the genetic proximity of the A, B and D genomes. The same kind of investigations on primary *Triticale*, for instance, will be of interest since less related genomes will interact.

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