Theor Appl Genet (1986) 73: 246-251

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Regulatory effects of homoeologous chromosome arms on wheat proteins at two developmental stages

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Received June 20, 1986; Accepted July 28, 1986
Communicated by F. Salamini

Summary. Two-dimensional gel electrophoresis was conducted on denatured proteins of the 10-day-old first leaf (1F stage) of 18 homoeologous ditelosomic (DT) lines of wheat cultivar 'Chinese Spring'. The observations, compared to the euploid control and relative to previous data found on 7-day-old etiolated seedlings (G7 stage) of the same lines lead to the following statements: 1) the structural genes of 24 spots can be assigned to 12 chromosome arms; 2) regulatory effects are completely different between the 1F and the G7 stages which may indicate that the regulation of protein amounts is often stage-specific; 3) no case of complete gene dosage compensation is observed among 4 groups of hypothesized homoeoallelic products; 4) homoeologous DT lines do not manifest similar effects which suggest the absence of homoeology for the detected regulatory effects.

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

Introduction

The allohexaploid nature of the common wheat *Triticum aestivum* L. ($2n=6x=42$) has permitted the development of aneuploids (Sears 1954) and the use of ditelosomic (DT) lines, where one pair of chromosone arms is lacking, has enabled the chromosomal assignment of many structural genes (see Hart 1983, for a review).

We have recently studied a series of DT lines by comparing the two dimensional (2D) denatured protein patterns at a "G7" stage, i.e. the aerial part of 7-day-old dark germinated seedling (Colas des Francs and Thiellement 1985) in order to localize structural genes and regulators. We defined a regulator as any regulatory sequence, gene, or element of a system that affects the amount of a protein, whatever the level where this regulation takes place. The following simple hypothesis was used: the lack of one chromosome pair results in: 1) the absence of the polypeptides whose structural genes are located on the missing arm and 2) the modification in quantity of the polypeptides whose regulators are located on the missing chromosome arm.

From this analysis we found that very frequently a spot is not quantitatively affected in the same way by the absence of different homoeologous chromosome arms. In order to further define this finding and to determine if the regulatory effects are similar during development, we have undertaken the comparison of the same 2D protein patterns on the "1F" stage: the first leaf at ten days. Experiments were conducted on a restricted set of 18 homoeologous DT lines.

Material and methods

Plant material

The following ditelosomic lines of *Triticum aestivum* ($2n=6x=42$) c.v. 'Chinese Spring' (CS) were used: 1AL, 1BL, 1DL, 3AS, 3BS, 3DS, 3AL, 3BL, 3DL, 5AL, 5BL, 5DL, 6AS, 6BS, 6DS, 7AS, 7BS, 7DS. These lines, originally produced by Sears, were furnished by Dr. Y. Cauderon (I.N.R.A. Versailles, France), Dr. J.W. Snape (P.B.I. Cambridge, England) and Prof. E.R. Sears (University of Missouri, Columbia, USA). They were chosen because the 3 homoeologues were available and because they do not manifest noticeable phenotypic change as compared to the euploid, at the two stages studied.

After 4 days of germination in the dark in Petri dishes, the plantlets were picked out in the greenhouse (16 h photo-

period). On the tenth day, the first leaf (1F) was cut out and immediately frozen in liquid nitrogen until extraction. The length of this first leaf was between 9 and 17 cm and its weight between 50 and 160 mg. This variation between individual plants and between lines also exists between individuals from the euploid CS control.

Protein extraction and electrophoresis

The proteins were extracted and denatured according to the method described by Bahrman et al. (1985) and Colas des Francs et al. (1985) except for the final protein solubilisation solution which was as in Damerval et al. (1986). The electrophoresis were performed as previously described (Colas des Francs and Thiellement 1985). The $16.5 \times 16 \times 0.08$ cm 2D gels, bound to Gelbond PAG (Marine Colloids) were silver-stained according to Oakley et al. (1980) with twice diluted developer, ten gels being simultaneously treated as described by Granier and de Vienne (1986).

Method of comparing gels

The euploid control 2D pattern was defined by taking into account every CS gel obtained from plants of the same age but with different leaf lengths and weights. A synthetic map was made where only the reproducible spots of the analysis were noted.

We observed at least three well-defined gels from different individual plant extractions and different runs of the same DT line. Comparisons were made relative to control gels by superimposing gels dried on Gelbond. Each spot was observed on each gel of the analysis relative to the control, and intensity changes, appearance, or disappearance were noted. A difference between a DT line and the euploid was retained only when it was observed on every gel of this DT line.

Each variation retained was then searched on the gels from the previous G7 analysis in order to compare for each spot the variations observed (or not) at the two "developmental stages": G7 and 1F. To define which spots are commonly found at the two stages, the comparison was done on the CS control with the help of coelectrophoresis, i.e. where the initial sample was a 1/1 or a 1/2 mixture of the two protein extracts.

The map was divided into 12 quadrants. The varying spots were designated by four numerals, the first two defining a quadrant of the gel and the last two defining a spot in this quadrant.

Results

In order to easily compare the observations between the present 1F investigation and the previous G7 one we chose to keep exactly the same electrophoresis procedure although it could have been optimized for these leaf samples.

The 1F pattern (Fig. 1) is characterized by the abundance of the large subunit (LS) of ribulose bisphosphate carboxylase/oxygenase (Rubisco). Many spots are hidden under the LS spot and many others, when more basic than the LS, are no longer accurately resolved since such a major protein disturbs the isoelectrofocusing.

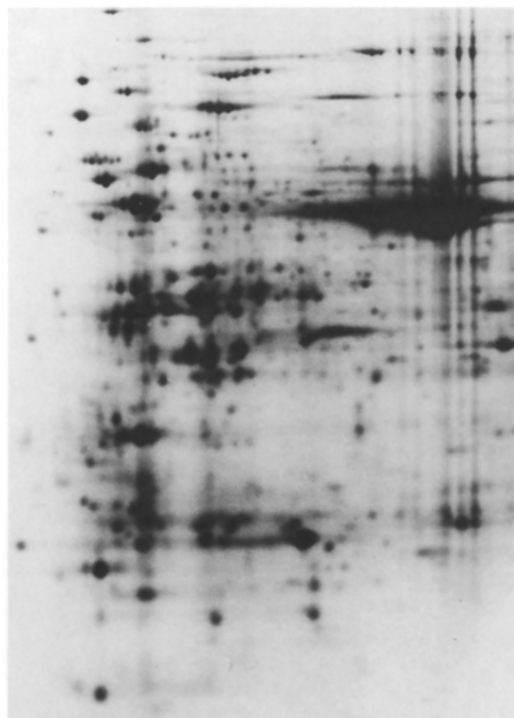

Fig. 1. 2 D protein pattern obtained from a 10-day-old first leaf (1F) of the euploid 'Chinese Spring'

Another difficulty is raised by the great number of abundant proteins at this stage (see Fig. 1). A good stain of faint spots leads to the saturation of the big ones precluding an easy estimation of the quantitative variations on such saturated spots.

In the CS 1F map (Fig. 2) 477 spots, reproducibly and clearly observed, are retained to be compared to the 766 spots of the G7 analysis. The majority of the 1F spots (402) are present on the G7 gels (Fig. 2 a) but most of them in different relative amounts. This finding is in accordance with data obtained in a previous study (Zivy et al. 1984).

The spots found variable in the G7 analysis and present on the 1F pattern are indicated in Fig. 2 a by their number. The spots varying in 1F, regardless of their presence or their behaviour in G7, are indicated in Fig. 2 b.

The numbers of variations observed according to the DT line examined are indicated in Table 1 where what has been observed at the G7 stage is also noted. Only 67 variations were retained on the 18 DT lines at the 1F stage whereas 230 were found for the same lines on G7 proteins. This results from a smaller percentage of variable spots (11% versus 21%) and from a smaller number of lines where a spot is affected (1.26 in average versus 1.39).

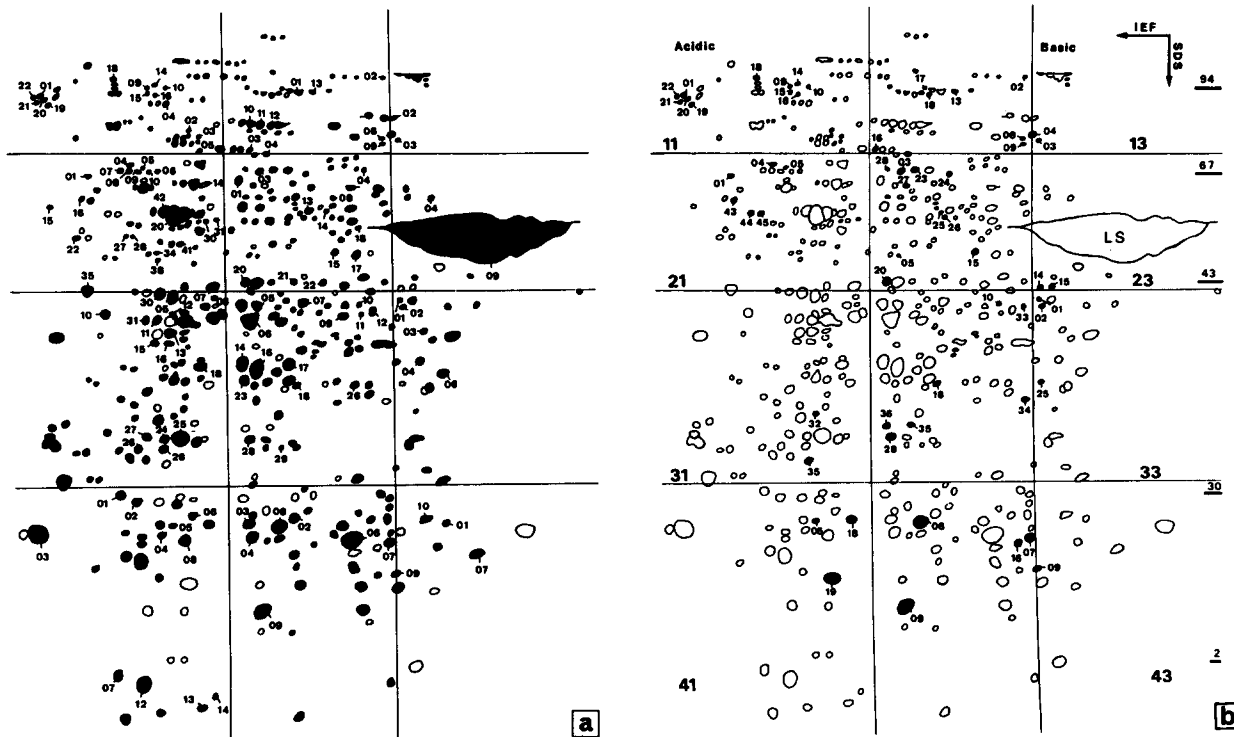

Fig. 2a, b. Map of the 1F spots. **a** The spots present in G7 are in black and those varying in G7 are numbered. **b** The spots found variable in this 1F analysis are in black and numbered. *On the right* the molecular weights in Kilodaltons are indicated. LS = large subunit of ribulose bisphosphate carboxylase/oxygenase

Table 1. Number of spot variations observed at the 1F and G7 stages relative to each DT line studied

DT line	No. of variations a-i-d-di-T[a]	
	1F[b] stage	G7[b] stage
1AL	0- 0- 0- 0- 0	0- 3- 19- 1- 23
1BL	0- 0- 0- 0- 0	1- 6- 7- 0- 14
1DL	0- 0- 0- 0- 0	1- 4- 8- 2- 15
3AS	0- 0- 2- 1- 3	2- 7- 13- 2- 24
3BS	0- 1- 7- 3-11	1- 2- 11- 1- 15
3DS	0- 1- 4- 2- 7	1- 4- 2- 0- 7
3AL	0- 3- 0- 2- 5	2- 1- 4- 4- 11
3BL	0- 0- 0- 0- 0	1- 3- 1- 1- 6
3DL	0- 1- 2- 1- 4	1- 0- 4- 4- 9
5AL	0- 4- 0- 3- 7	1- 0- 4- 0- 5
5BL	0- 3- 0- 3- 6	0- 5- 7- 3- 15
5DL	1- 0- 1- 1- 3	0- 2- 0- 0- 2
6AS	0- 2- 0- 3- 5	0- 5- 6- 4- 15
6BS	0- 0- 2- 4- 6	0- 2- 1- 4- 7
6DS	0- 0- 0- 2- 2	1-17- 2- 4- 24
7AS	0- 0- 1- 0- 1	0-11- 5- 1- 17
7BS	0- 1- 3- 0- 4	0- 2- 11- 0- 13
7DS	0- 2- 0- 1- 3	0- 4- 3- 1- 8
Total	1-18-22-26-67	12-78-108-32-230

[a] a = spot appearance; i = spot increase; d = spot decrease; di = spot disappearance; T = Total
[b] see text

No spot was found reproducibly different between the euploid and the 1AL, 1BL, 1DL, and 3BL DT lines. Most lines are characterized by a very small number of varying spots (Table 1).

Localization of structural genes

As stated in the introduction and discussed previously (Colas des Francs and Thiellement 1985) we consider that if a spot reproducibly found in the euploid pattern is absent in every gel of a given DT line, it is likely that the structural gene coding for this polypeptide is located on the missing chromosome arm. We found 26 such spots (Table 2). Most of them (22) are absent on the two patterns (G7 and 1F) of the same DT line, as expected. Among them, six spots (1118, 1119, 1120, 1121, 2143 and 3155) were not considered in the G7 analysis because of bad resolution in the euploid, but were indeed absent in the corresponding DT lines (Table 2).

Two spots (1116 and 1217) do not exist on the G7 patterns. Only the variation of two spots is not consistant in the two stages: spots 2203 and 2228 absent in DT 3BS at the 1F stage are present in the same line at the G7 stage where they were noticed as decreasing in intensity. This may by due to the overlap of two spots

Table 2. Spot variations

	Spot no.	1F analysis	G7 analysis
(a)	1101, 1122 (b)	(–) 5BL	(–) 5BL
(a)	1120, 1121 (b)	(–) 5AL	(–) 5AL (c)
(a)	1119	(–) 5DL	(–) 5DL (c)
	1114	↑ 5BL	absent
	1115	↑ 5BL	absent
	1116	(–) 5BL, ↑ 5AL	(–) 5BL (c)
(a)	1109	(–) 3DL, ↑ 5BL	(–) 3DL
(a)	1110	(–) 3AL	(–) 3AL
	1118	(–) 3DS	(–) 3DS (c)
	1202	(–) 6BS	(–) 6BS
(a)	1208	(–) 6AS	(–) 6AS, ↑ 6BS, ↑ 6DS
(a)	1209	(–) 6DS, ↑ 6AS	(–) 6DS, ↑ 6BS, ↑ 6AS
(a)	1303	(–) 6BS	(–) 6BS, ↑ 6AS, ↑ 6DS
	1213	↑ 5AL, ↓ 5DL	↓ 1AL
	1217	(–) 5AL	absent
	1218	↑ 5AL	–
	1304	↑ 3DS	–
	2101	(–) 3AS	(–) 3AS
	2104	↓ 3DL, ↓ 3DS	–
	2105	↓ 3DL, ↓ 3DS	↓ 3BS
	2143	(–) 3DS	(–) 3DS (c)
	2144	↓ 3BS	absent
	2145	↓ 3BS	–
	2203	(–) 3BS	↓ 3BS
	2205	↑ 7BS	↑ 1AL
	2215	(–) 6AS	(–) 6AS
	2220	↓ 3AS	↑ 1DL
	2223	↓ 3AS	–
	2224	↑ 3AL	–
	2225	↑ 7DS	–
	2226	↑ 7DS	–
	2227	↑ 3BS	–
	2228	(–) 3BS	↓ 3BS
	2314	(–) 6BS, ↑ 3AL	absent
	2315	↑ 3AL	absent
	3132	(–) 3AL, ↑ 3DL	(–) 3AL
	3135	(–) 7DS, ↓ 7AS	(–) 7DS, ↓ 7AS (c)
	3210	(–) 6DS	(–) 6DS, ↑ 7DS
	3218	↑ 6AS	–
	3233	(+) 5DL	absent
	3234	↑ 5AL	–
(a)	3301	(–) 6AS	(–) 6AS, ↓ 1BL
(a)	3302	(–) 6BS	(–) 6BS, ↓ 1BL, ↑ 6AS
	3325	↓ 3BS	–
	4105	↓ 6BS	↓ 6BS
	4118	↓ 3BS	–
	4119	↓ 3DS	absent
	4207	↓ 7BS	↑ 7AS, ↓ 1BL, ↓ 1DL
	4208	↓ 6BS	↓ 6AS, ↓ 5BL
	4216	↓ 7BS	–
	4309	(–) 3BS	(–) 3BS

(+) = appearance; (–) = disappearance; ↑ = increase; ↓ = decrease; – = no variation
(a) Groups of hypothesized homoeoallelic products
(b) Hypothesized products of the same gene
(c) Disappearance in G7 was not previously noticed

in G7: the disappearance of one spot would then be observed as an intensity decrease. This may also result from a decrease in intensity in 1F below our detection level.

Four groups of spots are hypothesized as homoeoallelic products on the basis of their disappearance in homoeologous DT lines and their proximity on the gels (Table 2).

Intensity changes and regulators

The modification in intensity of a spot in every gel from a DT line, compared to the euploid control, is due, in the previously suggested hypothesis (Colas des Francs and Thiellement 1985), to the absence of a regulator located on the missing chromosome arm. The number of regulatory effects exerted by the 18 DT lines are indicated in Table 1. Forty-one are retained in 1F whereas 198 were found in G7 for the same DT lines.

The variations observed for the 1F spots are indicated in Table 2 where their behaviour in G7 is also listed.

Among the spots which are detectable in the euploid CS at both stages, only 3 spots (1209, 3135 and 4105) show a similar quantitative variation according to the same DT line in the two analyses (Table 2). Six spots vary in intensity at both stages but are not affected by the same DT lines, and 112 spots vary at one stage but not at the other (18 at 1F, 94 at G7).

In addition, in 1F, when a spot is affected in one DT line, it is not affected in the two other homoeologous DT lines.

Among the 4 groups of hypothesized homoeoallelic products (Table 2) only spot 1209 increases in the absence of spot 1208. The other homoeoallelic products of the same group (1208, 1303) and of the other groups (1109, 1110; 1101–22, 1120–21, 1119; 3301, 3302) do not manifest any noticeable dosage compensation in this 1F analysis.

Discussion

As far as structural genes are concerned, this 1F analysis is in very good accordance with the G7 one and confirms most of the chromosomal localizations hypothesized in G7. As in the G7 analysis, each spot disappears in only one of the DT lines examined.

The number of localized structural genes is relatively small. As 18 out of the 42 possible DT lines were examined, one may have expected to localize about 18/42 of the 477 spots (when not considering the cytoplasmically encoded proteins). The explanation is probably that most polypeptides exist in two or three doses, i.e. electrophoretically identical products are syn-

thesized by homoeoallelic genes on two or three genomes. It has been shown, using the same 2D procedure in comparing the G7 protein patterns from diploid and tetraploid *Triticum* to the CS wheat pattern, that 70 to 80% of the wheat spots may exist in two or three doses (Colas des Francs 1985; Thiellement and Colas des Francs 1986).

Opposite to what is observed for structural genes localization, regulatory effects are completely different between the two stages studied. The G7 observations do not permit one to deduce the 1F ones (Table 1). There is no correlation between the regulatory effects exerted by the different chromosome arms at the two stages. This is true for the number of effects by the DT line examined (r = –0.31 NS, from Table 1) as well as for each individual spot (Table 2).

Except for the disappearing ones, 3 spots only are similarly affected in intensity in the two stages. In most cases no variation has been observed at one of the 2 stages and in 6 cases the protein quantity is regulated by different arms in 1F and in G7 (Table 2).

A polypeptide amount can be under the control of several regulators at one stage, as it is more clearly shown in G7 (Colas des Francs and Thiellement 1985) and additional regulators appear preponderant when another stage is observed.

The observations on regulators a posteriori strengthen the hypothesis used to localize structural genes. The absence of a polypeptide in a DT line relative to the euploid can be due to the absence of the structural gene coding for this polypeptide or to the absence of a regulator necessary for this gene to be expressed at a detectable level. Since the two analysis are in accordance for most disappearances and since it is very infrequent that the same regulation is observed in both stages, the first assumption is the more likely.

The modification in a protein amount is hypothesized to result from the absence of a regulator. But the reverse proposition is not true: the lack of a regulator will change the protein amount only in the following situations:

1) two or three doses of regulators are necessary to control the amount of a protein. The spot should vary in intensity with 2 or 3 homoeologous DT lines. This was observed for only 25 spots in G7 where 30 DT lines were studied (Colas des Francs 1985; Colas des Francs and Thiellement 1985). This situation, corresponding to homoeologous regulators, whose absence lead to identical effect, was never observed in this analysis (Table 2).

2) only one among the three possible regulators is preponderant and its absence cannot be compensated for either by the 2 remaining ones or by other regulators located on non homoeologous chromosomes. This situation of non-homoeology for the regulators is observed for every spot that varies in intensity in the present 1F analysis.

Numerous wheat polypeptides revealed by this 2D procedure are probably constituted by 2 or 3 homoeoallelic gene products. In the absence of gene dosage compensation we should have observed many intensity decreases, corresponding to one dose disappearances, in at least two homoeologous DT lines for one spot. No such case, which is identical to the effect of homoeologous regulators, was observed in the present analysis although gene dosage compensation seems to be a rare phenomenon, noticed only once in G7 (Table 2). The explanation may lie in the inability to detect a one-third or even a one-half intensity decrease on the numerous saturated spots.

The data discussed above were obtained using a high-resolutive and reproducible 2D procedure that can be and will be improved (Anderson et al. 1985; Damerval et al. 1986). The visual analysis need to be replaced by precise scanning quantification and automatic analysis. In addition, the phenotypic variation within and between lines may also contribute to a decrease in the number of the reproducible variations retained. Therefore, the number of quantitative variations that really occur between the studied DT lines is probably underevaluated. Moreover, many spots are saturated at this foliar 1F stage: a more precise estimation of quantitative variations may have been obtained using a multiple step staining procedure for the same gel.

Further experiments are needed to estimate the extent of the homoeology for the regulation of protein amounts betweeen the three genomes of wheat. In any case, it clearly results from the data that the regulatory effects detected on protein amounts are very different between the two stages studied.

Acknowledgements. We thank Joël Blaisonneau, Marie-Pierre Digard and Martine Le Guilloux for their technical assistance and Catherine Damerval, Dominique de Vienne and Michel Zivy for their comments and suggestions.

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