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Chromosomal localization of structural genes and regulators in wheat by 2D electrophoresis of ditelosomic lines

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Summary. Among the 782 spots observed in two-dimensional gel electrophoresis of denatured proteins from etiolated wheat shoots, 185 were found to be variable between the euploid and 26 ditelosomic lines of 'Chinese Spring'. Thirty-five structural genes were located on 17 chromosome arms. Numerous intensity changes showing alterations in protein levels were observed and led to the following statements: 1) regulators are frequently found and can be assigned for a same polypeptide to various chromosome arms; 2) for most polypeptides homoeologous arms do not manifest similar effects; 3) nevertheless, when affecting the same polypeptide, homoeologous arms display in most cases identical regulatory effects; 4) gene dosage compensation is observed in only one out of four homoeoallelic situations.

Key words: *Triticum aestivum* – 2D electrophoresis – Gene localization – Homoeologous genes – Genetic regulation

Introduction

The regulation of gene expression is one of the most important questions facing biologists today in areas varying from developmental to evolutionary studies. It is also of primary importance that the plant or animal breeder takes into account the genetic variability of regulatory genes or systems (Korochkin 1978; Paigen 1979; Hedrick and MacDonald 1980; Scandalios and Baum 1982; MacIntyre 1982). The commonly used approach to undertake the genetics of regulation is to study variation in activity or in quantity of a protein between genotypes or developmental stages and organs.

The use of two-dimensional (2D) gel electrophoresis of denatured proteins (O'Farrell 1975) provides a powerful tool to describe the genetic variability of the regulation of numerous gene products (Klose 1982, Zivy et al. 1983, 1984). Comparing various genotypes allows the observation of 1) the shift of some spots, which is assumed to reflect allelic variation of structural genes and 2) the variation in intensity of the spots, which reveals the effects of regulators (regulatory sequences, genes or systems) on the quantity of the polypeptides.

Such defined regulators can exert their effect at numerous levels: pretranscriptional, transcriptional, posttranscriptional or posttranslational, the latter including the turnover of the gene product. Therefore, they can also be structural genes, i.e. encoding the primary structure of proteins interacting with the one under study.

We used here ditelosomic lines of 'Chinese Spring' to localize on chromosome arms the structural genes of the polypeptides revealed on 2D gels, and the regulators affecting their quantity.

Material and methods

Plant material

Ditelosomic (DT) lines of *Triticum aestivum* (2n=6x=42) cv. 'Chinese Spring' (CS) were used: such lines lack an arm of both chromosomes of a chromosome pair. Only 26 of the 42 possible DT lines were available. These lines, originally produced by Sears (1954), were kindly provided to us by Dr. Y. Cauderon (I.N.R.A. Versailles, France). The euploid CS line was taken as control.

Three seeds of each DT line were sown in Petri dishes and allowed to germinate in the dark. Each etiolated seedling of about 100 mg fresh weight constituted one sample. The following DT lines were harvested 7 or 8 days after sowing: 1AS, 1BL, 1DL, 2BL, 2BS, 2DL, 3AS, 3BS, 3DS, 4AS, 4DS, 4DL, 5AL, 5BL, 5DL, 6AS, 6BS, 6DS, 6DL, 7AL, 7BL, 7DS (DT 3AS means the line is ditelosomic for the short arm of chromosome 3 of the A genome). The DT lines which had not grown enough were harvested at 13–15 days: they are DT lines 1BS, 3BL, 4BL and 7BS. In every case, each DT sample was compared to CS euploid of the same age.

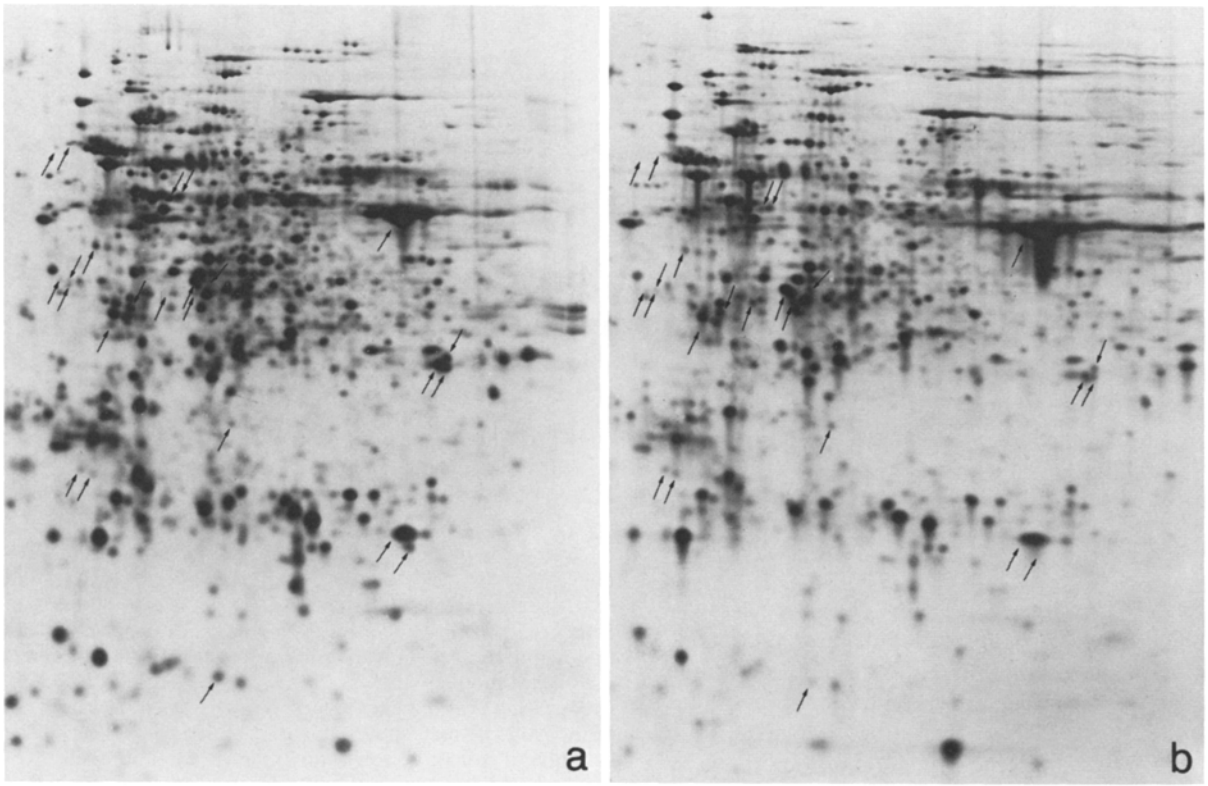

Fig. 1. **a** 2D gel from euploid 'Chinese Spring'. **b** 2D gel from DT 3AS. Each genotype was checked on three gels and arrows indicate the reproducible variable spots

Protein extraction and electrophoresis

Proteins were extracted according to the method described by Bahrman et al. (1985) and Colas des Francs et al. (1985) which prevents protease action during extraction.

The first dimension isoelectric focusing (IEF) was performed in 160 mm long glass tubes with 1 mm inner diameter. Gel composition was 4% acrylamide, 9.2 M urea, 4% carrier ampholytes (1/10 LKB pH 3.5–9.5, 1/10 Serva pH 3–10, 2/5 Pharmacia pH 5–8 and 2/5 LKB pH 5–8) and 2% Triton X-100. Samples of 10 µl were applied on the gel tops and IEF was performed for 20 h at 750 V. The electrode solutions were 20 mM NaOH and 10 mM H_3PO_4. The 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 (SDS dimension) was performed on slab gels (160 × 165 × 1 mm) bound to Gelbond PAG (Marine Colloids) in a Dalt tank (Anderson and Anderson 1978). Uniform gel composition was 11% acrylamide, 0.5 M Tris, 0.15% SDS, 1% sucrose. Ten gels were simultaneously run: each batch usually contained one euploid CS sample and 9 DT line samples.

The silver stain was adapted from Oakley et al. (1980). Ten gels were simultaneously stained under standardized conditions at 20 °C. The apparatus and the method will be described in detail elsewhere (Granier and de Vienne, in preparation).

Method of comparing gels

The reported results were obtained after observing at least three well defined gels from different extractions and different runs of the same DT line. Comparisons were made relative to control gels by superposing 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. This type of comparison is illustrated in Fig. 1.

A synthetic map was made where only the reproducible spots of the analysis were noted. Due to the large number of spots, 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 (Fig. 2).

Results

A 2D gel from euploid CS is shown in Fig. 1 a. The 782 spots retained in the analysis are schematized in Fig. 2: with the exception of 9 spots that are present only in some DT lines, all are observed on every control gel. Variation in 185 spots is dependent upon the chromosome arm pair lacking (qualitative or quantitative changes).

The number of spots affected in each DT line are listed in Table 1. Several chromosome arms do not appear to have a major effect on the polypeptides revealed at this stage as only one or two spots are

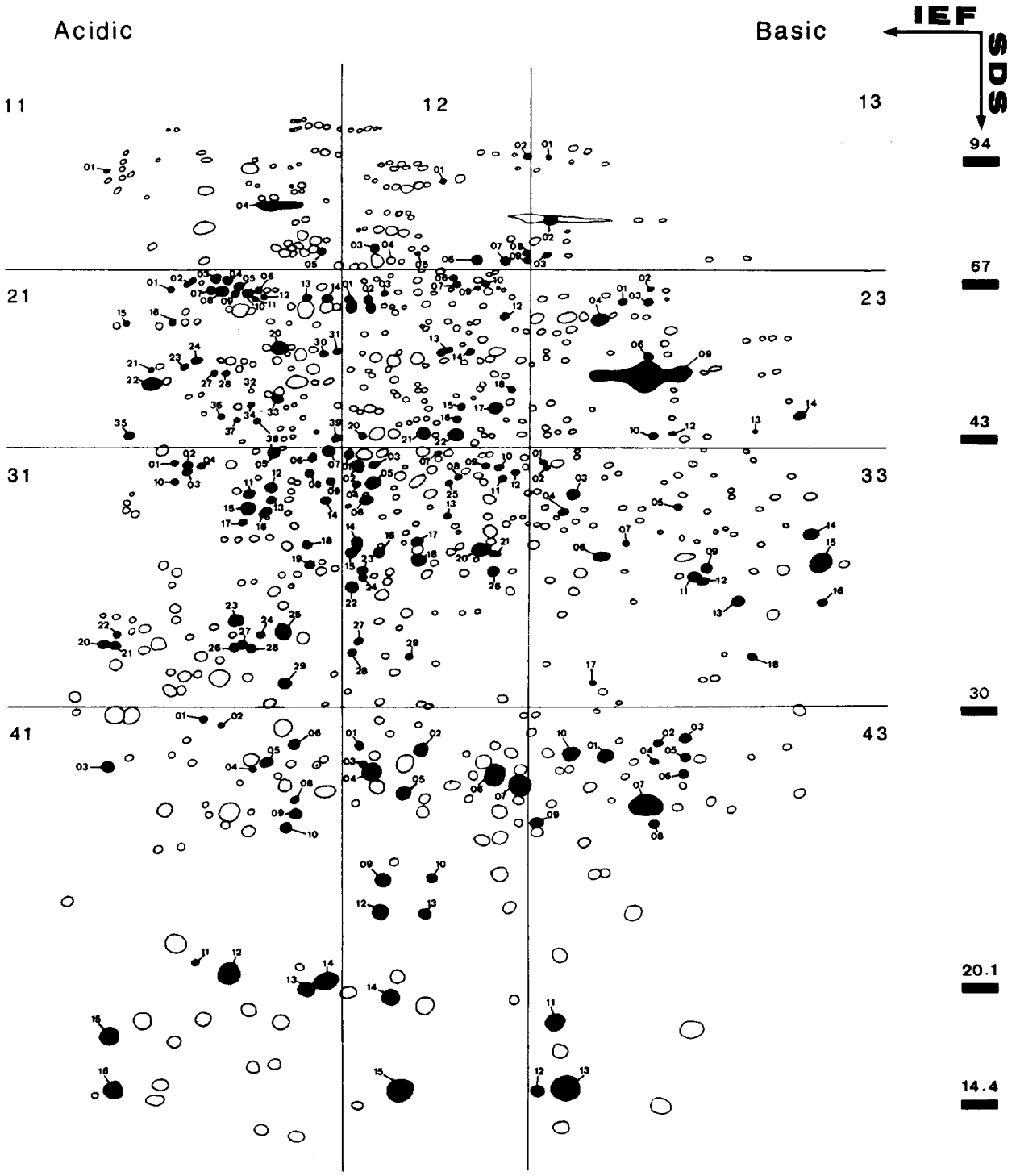

Fig. 2. Map of the spots retained in the analysis. Black spots are the varying ones and are labelled by two numerals which define them in their quadrant. Molecular weights in kilodaltons are indicated on the right

Table 1. Number of spot variations observed relative to each DT line studied

DT line	No. of variations a-i-d-di-T[a]	DT line	No. of variations a-i-d-di-T	DT line	No. of variations a-i-d-di-T
1 AS	0-0-2-0-2	1 BL	1-6-7-0-14	1 DL	1-4-8-2-15
3 AS	2-7-13-2-24	1 BS	0-7-9-5-21	2 DL	0-1-0-0-1
4 AS	0-1-6-1-8	2 BL	0-9-24-4-37	2 DS	1-0-0-0-1
5 AL	1-0-4-0-5	3 BS	1-2-11-1-15	3 DS	1-4-2-0-7
6 AS	0-5-6-4-15	3 BL	1-5-1-1-8	4 DL	0-3-0-0-3
7 AL	0-0-1-1-2	4 BL	4-12-3-1-20	4 DS	1-12-4-3-20
		5 BL	0-5-7-3-15	5 DL	0-4-0-0-4
		6 BS	0-2-1-4-7	6 DS	2-17-2-4-25
		7 BL	1-1-6-2-10	6 DL	0-0-0-1-1
		7 BS	0-4-10-0-14	7 DS	0-4-3-1-8
Total A	3-13-32-8-58	Total B	8-53-79-21-161	Total D	6-49-19-11-85
Total	17-115-130-40-302				

[a] a = spot appearance; i = spot increase; d = spot decrease; di = spot disappearance; T = total

affected in DT lines 1AS, 2DS, 2DL, 6DL and 7AL. Despite the more active appearance of the B genome, the great variation in the number of spots affected in each DT line (Table 1) prevents us from drawing any conclusion as to the differential action of the three genomes.

Among the 185 varying spots, we distinguish between "single" and "coaffected" spots. The latter are close to each other on the 2D gels and affected either by the same DT line(s) or by DT lines lacking homoeologous arm pairs. Only spots having the same colour in our silver stain method are classified as coaffected. Such spots were grouped together because they can be:

1) the products of homoeoalleles: two or three homoeologous genes can be expressed, giving rise on the 2D gels to polypeptides close to each other and of identical colour, having the same biochemical properties (Nielsen and Brown 1984).

2) various products of the same gene, as frequently described in 2D analysis, resulting from posttranslational events such as phosphorylation, glycosylation, maturation of a precursor or degradation of the protein (Anderson and Anderson 1979; Zannis and Breslow 1981; Klose 1982; Rosenblum et al. 1983; Zivy et al. 1984).

Only two pairs of spots were not retained as coaffected because they had different colours.

1 Localization of structural genes

The disappearance of 40 spots can be attributed to 17 absent chromosome arm pairs (Table 2). For a disappearing spot, two main hypotheses can be put forward: the missing chromosome arm pairs carries either 1) the structural gene coding for the polypeptide or 2) a regulator necessary for this gene to be expressed at our detection level. Since each of these spots disappears in one DT line only, the first hypothesis has been considered as the more likely. It is commonly used to localize enzyme loci (see Hart 1983, for a review) and the structural genes of storage proteins (Brown and Flavell 1981; Payne et al. 1984). Thus, if a spot is absent in DT 5BL for instance, its structural gene is located on the short arm of chromosome 5B.

As indicated in Table 2, we observed 5 cases of two coaffected spots disappearing in the same DT line: they

Table 2. Assignment of structural genes to chromosome arms. Spots in brackets are considered to be products of the same gene and spots followed by the same letter to be homoeoallelic products. Arms 4AS, 4BL and 4DS are homoeologous

DT line examined	Disappearing spots	Localization of the corresponding structural genes
1BS	3201, (3306, 3307), 4111, 4312	1BL
1DL	2218, 4303	1DS
2BL	2107, 2304, 3213, 4106	2BS
3AS	4308[a], 2101	3AL
3BS	4309[a]	3BL
3BL	3304	3BS
4AS	3312[b]	4AL
4BL	3311[b]	4BS
4DS	3309[b], 1207, 4301	4DL
5BL	1101, (4302, 4304)	5BS
6AS	1208[c], 3301[d], (2215, 2216)	6AL
6BS	1303[c], 3302[d], (1202, 1301)	6BL
6DS	1209[c], (3127, 3128), 3210	6DL
6DL	2313	6DS
7AL	3129	7AS
7BL	3208, 3212	7BS
7DS	3315	7DL

are considered as products of the same gene. Some other spots disappear in response to homoeologous DT lines: they are likely products of homoeoalleles. Spots 4308 and 4309 have their structural genes located on chromosome arms 3AL and 3BL; spots 3301 and 3302 on 6AL and 6BL; spots 1208, 1303 and 1209 on 6AL, 6BL and 6DL; and spots 3312, 3311 and 3309 on 4AL, 4BS and 4DL. The three latter arms have been previously shown to be homoeologous (Sears and Sears 1978) and to carry homoeoalleles of enzyme genes (Hart and Langston 1977; Benito et al. 1984).

2 Intensity changes and regulators

When a spot increases or decreases in intensity in one DT line, we consider that this gene product is under the regulatory control of the chromosome arm missing in this DT line. This interpretation, which is discussed below, is also used for the appearing spots: in the observed DT line the corresponding structural genes are either expressed (silent in the euploid) or increased in expression (expressed in the euploid, but below our detection level).

Single spot variations (Table 3-1). Fifty-five spots decrease or increase in intensity (including the appearing ones) in only one DT line. Thirty-four other spots are affected in two or more (up to 7) different DT lines. The chromosome arms involved are either unrelated (26 spots), or homoeologous (2 spots), or both (6 spots). All but one (4206) of the spots affected in homoeologous DT lines are modified in the same direction. For 5 spots (already listed in Table 2), structural genes and regulators are located on different chromosomes. This is also the case with spot 2309 which is the large subunit (LS) of Ribulose bisphosphate carboxylase/oxygenase (Rubisco), as demonstrated by alloplasmic line comparisons (Zivy et al. 1983) and immunochemical observations (unpublished results). Its structural gene is located on the chloroplastic DNA (Kawashima and Wildmann 1972; Coen et al. 1977). This major protein is affected by 7 chromosome arms whereas the nuclearly encoded small subunit of Rubisco (spot 4313) is affected by only two chromosome arms.

Coaffected spot variations (Table 3-2). Excluding the 5 groups of co-disappearing spots, 16 groups of coaffected spots are modified in only one DT line. In most cases they are coaffected in the same direction: intensity increase for 7 groups, intensity decrease for 7 groups. The two spots 3105 and 3107 are coaffected in opposite directions and for 3126, 3127 and 3128, two spots disappear as the third one increases.

Seven other groups of spots are coaffected by unrelated arms (at least one of the spots in a group varies in intensity in more than one DT line). In 3 instances, one of the two coaffected spots has its structural gene localized, i.e. listed in Table 2.

Eleven groups of spots are coaffected by homoeologous arms, including the four sets of homoeollelic products. If the spots listed in Table 3-2 are considered independently, 30 of them are affected in two or more DT lines. Among them, 15 spots have their intensities modified in the same direction by homoeologous arms. The three homoeoallelic products 1208, 1209, 1303 are included in this list: when one of them is missing, the two remaining spots increase in intensity. This is the expected manifestation of gene dosage compensation. It is not observed for two other sets of homoeoalleles whose products are spots 4308, 4309 and 3309, 3311, 3312. An opposite effect is noticed for the last set: when 3301 is lacking, 3302 decreases.

Discussion

The assumption that variation in protein quantity reflects the effect of a regulator is complicated by the allohexaploid structure of the wheat genome. Zivy et al. (1984) previously discussed how allelic variation may appear as intensity changes on wheat 2D gels. In the present analysis interpretation difficulties are also encountered.

The absence of a structural gene may be interpreted as a regulatory effect: if 2 or 3 homoeoallelic products are electrophoretically identical, the lack of one of them in a DT line can give rise to the intensity decrease of the spot. We consider 262 effects (Table 1) as regulatory (17 appearances, 115 increases, 130 decreases). Only a fraction of the spot decreases may be due to the absence of one out of two or three identical products. This is not the case when 1) the spot decrease is observed in only one of the three homoeologous DT lines (in groups 3S, 4AS, 4BL, 4DS, 5L and 6S); 2) the structural gene of this spot has been located; 3) the spot decreases in intensity in another non-homoeologous DT line (only one of the decrease effects may not be regulatory). The remaining 65 spot intensity decreases may be due to the absence of a structural gene. Therefore, we have observed at least 197 regulatory effects among the 302 spot variations of Table 1.

On the other hand, if the phenomenon of gene dosage compensation occurs between homoeoalleles, we may observe a spot intensity increase in the absence of one homoeoallele, but we may also observe no intensity change if two or three homoeoallelic products are identical and one of them absent. In this last case, we underestimate the number of varying spots.

In addition, our visual analysis leads to a rough estimation of intensity variations since only clear cut

Table 3. Spot variations

3-1: Single spots

1104	↗DT1DL	2133	(+)DT2DS	3122	↘DT4DS	3221	(+)DT1BL	3318	↘DT1AS
1105	↗DT1BS	2135	↗DT6DS	3123	↘DT4AS	3223	↘DT7BS	4103	↘DT6AS
1201	↘DT5AL	2203	↘DT3BS	3124	↗DT6DS	3224	↗DT7BL	4104	↗DT7DS
1203	(+)DT5AL	2212	↗DT2BL	3125	↘DT7BS	3225	↘DT4BL	4109	↘DT7BL
1204	↘DT7BL	2217	↗DT5DL	3203	(+)DT3AS	3226	↗DT7DS	4112	↘DT7BS
1206	↘DT4AS	2220	↗DT1DL	3207	↘DT7BS	3229	↗DT3AS	4202	↘DT7DS
1302	↘DT5BL	2302	↗DT1DL	3209	↘DT6DS	3303	↘DT2BL	4203	↘DT1DL
2102	↘DT3AS	3104	↘DT7BS	3215	(+)DT4BL	3305	↘DT5BL	4204	↗DT5BL
2120	↘DT5BL	3114	↗DT4BL	3217	↗DT2BL	3313	↗DT1BS	4205	↘DT4AS
2122	↘DT2BL	3118	↗DT6DS	3218	↘DT2BL	3316	↗DT4DS	4310	↘DT4AS
2132	↘DT3BS	3119	↗DT4DS	3220	↘DT1BL	3317	↘DT3BL	4311	↘DT3BS

2304 (−)DT2BL, ↗DT1BS, ↘DT4AS, ↘DT7DS
3129 (−)DT7AL, ↘DT7DS
3210 (−)DT6DS, ↘DT4DS, ↘DT7BL, ↗DT7DS
3212 (−)DT7BL, ↗DT4DS
4312 (−)DT1BS, ↗DT4BL

2105 ↘DT2BL, ↘DT3BS
2134 ↘DT2BL, ↘DT3BS
2139 ↘DT5BL, ↗DT6DS, ↘DT7BS
2209 ↗DT1BS, ↗DT4DL
2306 ↗DT1BS, ↗DT2DL, ↗DT7BS
3106 ↘DT3DS, ↗DT4BL
3109 ↘DT1BS, ↗DT2BL, ↘DT5BL
3110 ↘DT1BS, ↘DT7BS
3115 ↘DT3BS, ↘DT6DS
3116 ↘DT2BL, ↘DT3BS, ↗DT4BL
3117 ↘DT3AS, ↗DT4BL
3205 ↗DT2BL, ↗DT6DS
3206 ↘DT2BL, ↗DT6DS
3314 ↗DT3DS, ↗DT4DS
4105 ↘DT2BL, ↘DT6BS
4201 (+)DT1DL, (+)DT7BL
4209 ↘DT1BS, ↘DT5BL, ↘DT6AS
4214 ↘DT1DL, ↘DT3AS, ↘DT4AS
4306 (+)DT4DL, (+)DT6DS
4307 ↘DT2BL, ↘DT3AS, ↘DT6AS
4313 ↗DT4BL, ↗DT6DS

affected in homoeologous DT lines:

1205 ↗DT7BS, ↗DT7DS
2309 ↘DT1BS, ↗DT2BL, ↗DT3AS, ↗DT3BS, ↗DT3DS, ↗DT4BL, ↘DT7BS
3108 (+)DT3AS, (+)DT3BS, (+)DT3DS, (+)DT3BL, (+)DT4BL, (+)DT4DS
3222 ↘DT7AL, ↘DT7BL, ↗DT7BS
4206 ↗DT1BL, ↘DT1DL, ↗DT2BL
4207 ↘DT1BL, ↘DT1DL, ↘DT2BL
4210 ↗DT4BL, ↗DT5BL, ↗DT6AS, ↗DT6DS
4215 ↗DT4AS, ↗DT4BL

3-2: Coaffected spots

2103, 2104, 2106, 2108, 2109, 2110	↘DT2BL	2213, 2214	↘DT6AS	3214, 3216	↗DT2BL	2111, 2112	↗DT1BL, ↘DT2BL
		2221, 2222	↘DT2BL	3227, 3228	↗DT3BL	2136	↘DT2BL, ↘DT3AS, ↘DT4DS, ↗DT7BS
		2301, 2303	↗DT5BL	4101, 4102	↗DT3AS	2137	↘DT2BL
2113, 2114	(+)DT4BL	3101, 3102, 3103	↘DT3AS	4108, 4110	↘DT7BL	2138	↘DT2BL, ↘DT4DS
2115, 2116	↗DT4DS	3105, 3107	↘DT1BS, ↗DT1BS	4113, 4114	↘DT1DL	2206, 2207	↗DT1BL, ↗DT5DL
2121, 2123, 2124	↗DT3BL	3126	↗DT6DS	4115, 4116	↘DT1BL	2313	(−)DT6DL, ↗DT4DS
		3127	(−)DT6DS			2314	↗DT4DS
		3128	(−)DT6DS			3111	↗DT6DS
						3112	↗DT6DS
						3113	↗DT3AS, ↗DT6DS
						3211	↗DT4DS
						3212	(−)DT7BL, ↗DT4DS
						4303	(−)DT1DL, ↗DT4DL, ↘DT4BL, ↘DT7BS
						4305	↘DT1DL, ↗DT4DL, ↘DT7BS

affected in homoeologous DT lines:

1208 (−)DT6AS, ↗DT6BS, ↗DT6DS
1209 (−)DT6DS, ↗DT6AS, ↗DT6BS
1303 (−)DT6BS, ↗DT6AS, ↗DT6DS
2127, 2128 ↘DT3BS, ↘DT3DS, ↘DT5AL
2130 ↘DT1BS, ↘DT3AS, ↘DT3BS
2131 ↗DT1BS, ↘DT3AS, ↘DT3BS
2201 ↗DT1BL, ↗DT1DL
2202 ↘DT1BL, ↘DT1DL
2310 ↘DT1AS
2312 ↘DT1BS
3120, 3121 ↗DT4BL, ↗DT4DS, ↗DT6AS
3202 ↗DT2BL, ↗DT3AS, ↗DT3DS, ↗DT6DS
3204 ↘DT2BL, ↗DT3AS, ↗DT3BS, ↗DT6DS
3301 (−)DT6AS, ↘DT1BS, ↘DT1BL
3302 (−)DT6BS, ↘DT1BS, ↘DT1BL, ↘DT6AS
3309 (−)DT4DS, ↘DT3AS
3311 (−)DT4BL, ↘DT3AS
3312 (−)DT4AS, ↘DT3AS
4212 ↗DT4BL, ↘DT5AL, ↘DT5BL
4213 ↗DT4BL, ↗DT5BL, ↗DT5DL, ↗I
4308 (−)DT3AS
4309 (−)DT3BS

(+) = appearance; ↗ = increase; ↘ = decrease; (−) = disappearance

reproducible differences between the DT lines and the euploid CS are retained. All these phenomena can explain why only 185 among 782 spots were found variable in this analysis, although a ratio close to 26/42 (26 of the 42 possible DT lines) might have been expected.

Since each of the disappearing spots is lacking in only one DT line, we feel confident that the simplest hypothesis is the more likely: the structural gene of this polypeptide is located on the missing chromosome arm. This leads to the localization of 35 such genes, corresponding to 40 spots, on 17 chromosome arms. Four different genes are assigned to chromosome 2B (Table 2) although its genetic map is still very poor (Hart 1982, 1983). We observe two sets of homoeoallelic products where the D homoeoallele does not appear to be expressed (Table 2). The relatively small number of localized structural genes may be due to a great proportion of two- or three-dose spots. It may also result from the predominant expression of one of the three homoeoalleles in conjunction with the biased sample of DT lines examined, i.e. most of the polypeptides revealed have their structural genes located on chromosome arms which have not been studied, the corresponding DT lines being lethal or sterile.

For 17 spots, regulatory effects have been noticed, and considered to be due to chromosome arms other than the one carrying the structural gene. Thus, the existence of regulators located far from the structural gene is unambiguously demonstrated. The same statement can be made for the numerous other spots affected by at least two non-homoeologous chromosome arms. Although genetically distant regulators have been previously suggested in wheat for phosphodiesterases (Wolf et al. 1977) and for storage proteins (Brown and Flavell 1981), the present analysis extends this notion to a great number of different gene products, including the LS of Rubisco (spot 2309). This statement will remain valid even if some intensity decreases are due to the absence of structural genes.

As shown by Aragoncillo et al. (1978) for storage proteins, the gene dosage compensation, observed in one out of four cases (see "Results"), can not be considered as a general rule. Moreover, regulatory effects between homoeologous chromosome arms can lead to a decrease of the remaining product.

Homoeoallelic products may be coaffected (Table 3-2) but by only one of the two or three homoeologous DT lines available. Moreover, homoeologous arms do not generally manifest similar effects (Table 1) and only a few spots are jointly affected by homoeologous arms. Thus, we cannot deduce from one DT line what will be observed in the other homoeologous ones. This may indicate that a regulator, being specific to one or several gene products, cannot always be replaced by its homoeologous on the other genomes. As structural genes, regulators may have diverged in the different genomes and either each of the three homoeologous regulators has acquired its own specific role, or one (or two) of the three is (are) no longer expressed, at least at this stage.

Nevertheless, among 22 spots affected by homoeologous DT lines (Table 3), 21 have their intensity modified in the same direction by homoeologous chromosome arms. This suggests the persistance of duplicate or triplicate sets of regulators.

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