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Study on nuclear and cytoplasmic genome expression in wheat by two-dimensional gel electrophoresis

2. Genetic differences between two lines and two groups of cytoplasms at five developmental stages or organs

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Summary. Two-dimensional gel electrophoresis of denaturated proteins were performed at five developmental stages or organs (hereafter referred to as stage-organs) on two wheat lines with four different cytoplasms. Five hundred and fifty to 712 reproducible spots were scored depending on the stage-organ. Each stage-organ is unambiguously characterized and several types of control of protein quantity are recorded. Post-translational modifications are hypothetized and may sometimes be stagespecific. Two cytoplasmic patterns are found: one for the euplasmic lines with *Triticum aestivum* cytoplasm and one for the alloplasmic lines with *Aegilops juvenalis*, *Ae. ventricosa* and *Ae. kotschyi* cytoplasms. Cytoplasmic variation is observed for 28 spots showing position difference, all of which are probably products of the *LS* gene, and for four spots showing differences for regulation of protein quantity. Nuclear variation between 'Chinese Spring' and 'Selkirk' is found for 20 allelic differences and for 20 regulatory systems, the latter number being probably underestimated.

Key words: *Triticum aestivum* – 2D electrophoresis – Developmental genetics – Cytoplasmic inheritance – Genetic regulation

Introduction

The population geneticists as well as the plant or animal breeders are facing the problem of frequent inadequacy between genetic variability assessed by isozymes or some other protein coding genes on the one hand and the variability observed at the phenotypic level on the other hand (Prager et al. 1976; Wilson et al. 1977; Jensen et al. 1979; McNeill and Jain 1983). The reasons may be that the gene sample is too small and that most isozyme genes are selectively neutral, which is not the case for biometrical character genes. We believe that one way of approaching this problem is to have access both to the variability of a great number of genes and to the variability of the regulatory systems involved in the developmental process leading to phenotypic traits.

The use of two-dimensional (2D) gel electrophoresis of proteins (O'Farrell 1975) can provide this information. We have previously shown that 2D gel patterns exhibiting several hundreds of reproducible spots can be obtained from wheat tissues (8 day old first leaves) (Zivy et al. 1983). We reported the genetic variation observed between 18 euplasmic and alloplasmic lines: it was possible to discriminate between them on the protein coding gene variation (allelism) but also on the regulatory systems by taking into account the intensity differences observed on the spots.

The effect of a regulatory system may evolve according to the organ and/or to the developmental stage considered in the plant. In order to observe the products of a larger number of genes and to examine their regulation during development, we have studied at five stages and/or organs two wheat lines and two groups of cytoplasms, using euplasmic and alloplasmic lines of 'Chinese Spring' (CS) and 'Selkirk' (Sk).

Materials and methods

Plant material

Eight lines were used in this study: the CS lines with the cytoplasms of *Triticum aestivum* (euplasmic), *Aegilops juvenalis*, *Ae. ventricosa*, *Ae. kotschyi* (alloplasmic) that were fur-

nished by Dr. K. Tsunewaki, Kyoto University, Japan, and the four equivalent euplasmic and alloplasmic lines of Sk that were furnished by Dr. S. S. Maan, North Dakota State University, Fargo, USA.

After 4 days of dark germination in water-imbibed Petri dishes, the plantlets were transferred into small pots and kept at 4 °C from day 11 to day 19. When 4 weeks old the plantlets were brought into the greenhouse and the genotypes were distributed in a randomized experimental design.

The following five developmental stages and/or organs (hereafter referred to as stage-organs) were studied:

G: the etiolated aerial part of 4 day old dark grown seedlings,
1F: the 8 day old first leaf,
2F: the same first leaf when the 4 week old plantlet has four leaves,
3F: the flag leaf when the ear is emerging,
3CE: the culm beneath the ear, at the same emerging stage.

No delay in development was noticed between the lines until heading. Heading was synchronous for genotypes having the same nuclear genome as reported by Tsunewaki (1980), but CS lines were late compared to Sk lines.

Extraction and electrophoresis

At each stage-organ, each of the eight genotypes was represented by two different samples and each sample was obtained from the mixture or three plants.

The proteins were extracted and the 2D electrophoresis performed as previously described (Zivy et al. 1983), except for the G and 3CE stage-organ where the pellet of proteins was resuspended in 100 µl of "lysis buffer" instead of 200 µl for 1F, 2F and 3F. This modification was made because of the abundance of Ribulose bisphosphate carboxylase/oxygenase (Rubisco) in the leaf stage-organs. The molecular weight standards were from Pharmacia (electrophoresis calibration kit).

The 2D gels were silver stained according to Oakley et al. (1980), except for the revelator which was twice diluted.

Comparison method

The 80 2D gels (5 stage-organs × 8 genotypes × 2 replicates) were visually scored by superposition of transparent positive films. Only the reproducible spots were retained. If a residual heterozygosity still existed in the lines here studied the spots corresponding to such a phenomenon should not be reproducible and therefore not retained.

To take into account a very high number of spots, the stage-organs were compared as if all the genotypes at one stage-organ were replicates, except for the spots showing genetic differences.

For easier comparisons, one 2D gel of G and one of 1F were chosen which best represented these two stage-organs. Then, every 2D gel form 2F and 3F was compared to the 1F standard and every 3CE gel to the G standard. The two standards G and 1F were also compared. In the case of ambiguity, the other 2D gels of the G and 1F stage-organs were used for the comparison.

Since some spots could be hidden by other ones depending on their intensity in different stage-organs, not all the spots could be taken into account in each comparison.

Quantitative variation for one spot was assessed relative to other spots in the same area of the gel. When qualitative variation (presence/absence of a spot) was observed between stage-organs, we recorded it as a quantitative variation since disappearance of a spot may also correspond to a decrease in protein quantity behind the level of detection.

As visual examination allowed only rough analysis, three classes of variation were defined: decrease, stability and increase of the protein quantity. When G and 1F were compared only two classes of variation were recorded because the proteins in G were twice as concentrated as those in 1F: the spots showing no evolution from G to 1F were recorded as increasing in intensity whereas the spots decreasing in intensity were recorded as stable or decreasing in intensity.

The maps where the stage-organs are compared show the spots of Sk on *Aegilops*. We recall that no differences were observed between the *Aegilops* cytoplasms studied (Zivy et al. 1983).

Interpretation of allelism

In a first approach a slight position difference of a spot in the isoelectrofocusing (IEF) direction between gels from two different lines is attributed to allelism. In wheat, however, this is complicated by allohexaploidy (AABBDD, 2n = 6x = 42). Figure 1 illustrates the 10 different situations that can occur when the homologous polypeptides of two lines differ by one charge change and when the three homoeologous genes are expressed. These 10 situations can be classified in type 1: position difference of a spot (three situations), type 2: presence/absence of a spot (six situations) and type 3: intensity difference (one situation).

Spot nomenclature

The spots are designated by four numerals, the first two defining a region of the gel and the last two defining a spot in its region.

When we refer to "allelic" spots showing a position difference between two lines or two groups of cytoplasms, the first of the two numbers corresponds to the spot position in the CS line or to the spot position in *T. aestivum* cytoplasm.

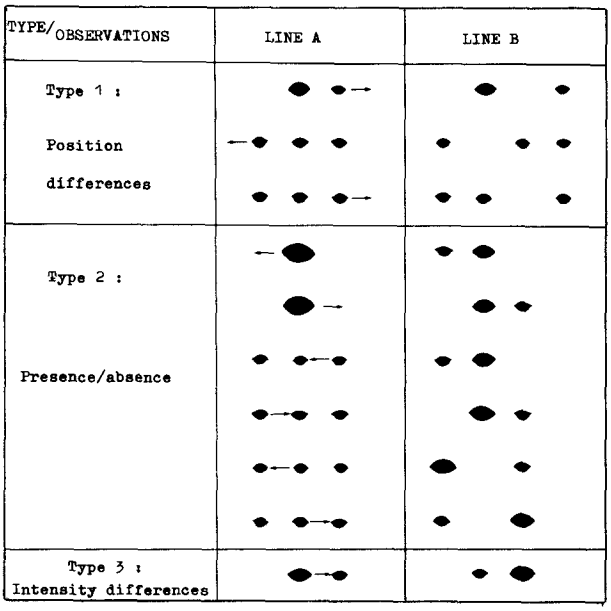

Fig. 1. Allelic situations between two wheat lines when the three genomes are expressed and when one of the three homologous protein differs by one charge change (in the IEF dimension). The three spot sizes are proportional to the protein quantity

Results

Differences between stages or organs

On the basis of their reproducibility 712 spots have been retained in G and 550 spots in 1F. In the previous analysis only 440 spots were retained in 1F (Zivy et al. 1983).

The most stringent differences are observed between the two standards G and 1F (Fig. 2): 111 spots are found in 1F that are not present in G, and all the major spots in 1F are either not seen or are weak in G. One can observe, for instance, spots 1201 and 2207, corresponding respectively to the large (LS) and small (SS) subunit of the Rubisco. In the other comparisons between 1F and 2F (Fig. 3), 1F and 3F (Fig. 4) and between G and 3CE (Fig. 5), many differences are noted even between leaf stages. They are summarized in Table 1.

Many spots that increase in intensity from G to 3CE (Fig. 5) also increase from G to 1F (Fig. 2). This is the case of spots 1201, 2207, 1603 and 1703 for instance. Conversely, some other spots that increase from G to 3CE (Fig. 5), as spots 0822, 1316, 2208, 2209 and 2210, are not detectable in the leaf stage-organs. Moreover, some spots, as spots 0901 or 1217, decrease from G to 3CE (Fig. 5) and increase from G to 1F (Fig. 2). Another example of variation is shown by spots 0601, 0602 and 0603, which are major spots in G, less intense in 1F, and absent in 3CE. Most of the spots that increase from G to 1F (Fig. 2) are stable or increase from 1F to 2F (Fig. 3) and to 3F (Fig. 4) but spots 0604 to 0607 are absent in G, 2F and 3F, and present in 1F. Another kind of variation is displayed by spots 0805 and 0806: they decrease from G to 1F, are absent in 2F, but present in 3F.

For the LS spot (1201) we noticed a slight decrease from 1F to 2F (Fig. 3) but an increase from 1F to 3F (Fig. 4). At this stage-organ (3F), Rubisco appears to be in excess and some additional spots can be found on both sides of spot 1201 but they are not reproducible.

Cytoplasmic variation

Whatever the stage-organ studied we observe two different patterns: one for the euplasmic lines (*T. aestivum* cytoplasm) and one for the alloplasmic lines (*Aegilops* cytoplasms). All the cytoplasmically variable spots are more intense or present only in the leaf stages (1F, 2F and 3F).

Figure 6 shows the differences observed between *T. aestivum* and *Aegilops* cytoplasms at the 3F stage-organ. Twenty-eight spots show position differences. Some of them, for instance 1203/1204 were not in 1F but can be detected in 3F and spots 1712/1713, although present in 1F, were not previously scored (Zivy et al. 1983). In addition to position differences, spots 0705, 1306, 1307, 1308 are present only or more intensely in the *Aegilops* cytoplasms and spots 1304, 1305, 1119, 1120, 1121, 1122 are present only or more intensely in the *T. aestivum* cytoplasm.

Table 1. Evolution of the intensity of the spots between stage-organs

Comparison	No. of spots showing intensity		
	Increase	Stability	Decrease
G versus 1F	334	487	
2F versus 1F	100	317	133
3F versus 1F	196	169	135
3CE versus G	58	229	425

At the G and 3CE stage-organs (not shown) only three position differences are seen (1102/1201, 1602/1603, 1604/1703) together with the presence/absence of spots 1304 and 1305. Spots 1306 and 1307 are detected at these stage-organs but they do not differ between the cytoplasms.

Nuclear variation

At each stage-organ, the two lines CS and Sk can be clearly recognized. The number of differences between them is maximum at the G stage-organ (Fig. 7a) where 48 spots display genetic variation. In 3CE (Fig. 7b), the stage-organ which is the most similar to G for 2D gel examinations, several spots are absent or are too small so that no intensity difference can be noticed between the two lines. Three other differences are observed: a new spot (1211) is present in Sk and absent in CS and two other spots exhibit intensity differences in 3CE but not in G (0817, 1214). Some other variations are clearer in 3CE because the spots are more intense (e.g. spots 0815, 0816).

At the 1F stage-organ (Fig. 7c) numerous spots that were different between the two lines in G are absent or are very faint, but some new differences are observed which do not occur in G or 3CE (spots 0705, 1310, 1311, 1209 and 1210). Spot 1202, present only in Sk, and spot 1802, more intense in Sk at the G stage-organ, are present at the same level in the two lines in 1F.

In 2F and 3F no difference between the two lines was further recorded but these stage-organs were of interest for the interpretation, as exemplified in Fig. 8. The two spots, 1310 and 1311, were previously interpretated in 1F as allelic (called I1 and I2 in Zivy et al. 1983). However, in 2F and 3F, spot 1311 is observed in the two lines whereas 1310 is absent in the two lines. Thus, the study of 2F and 3F stage-organs allows a better interpretation.

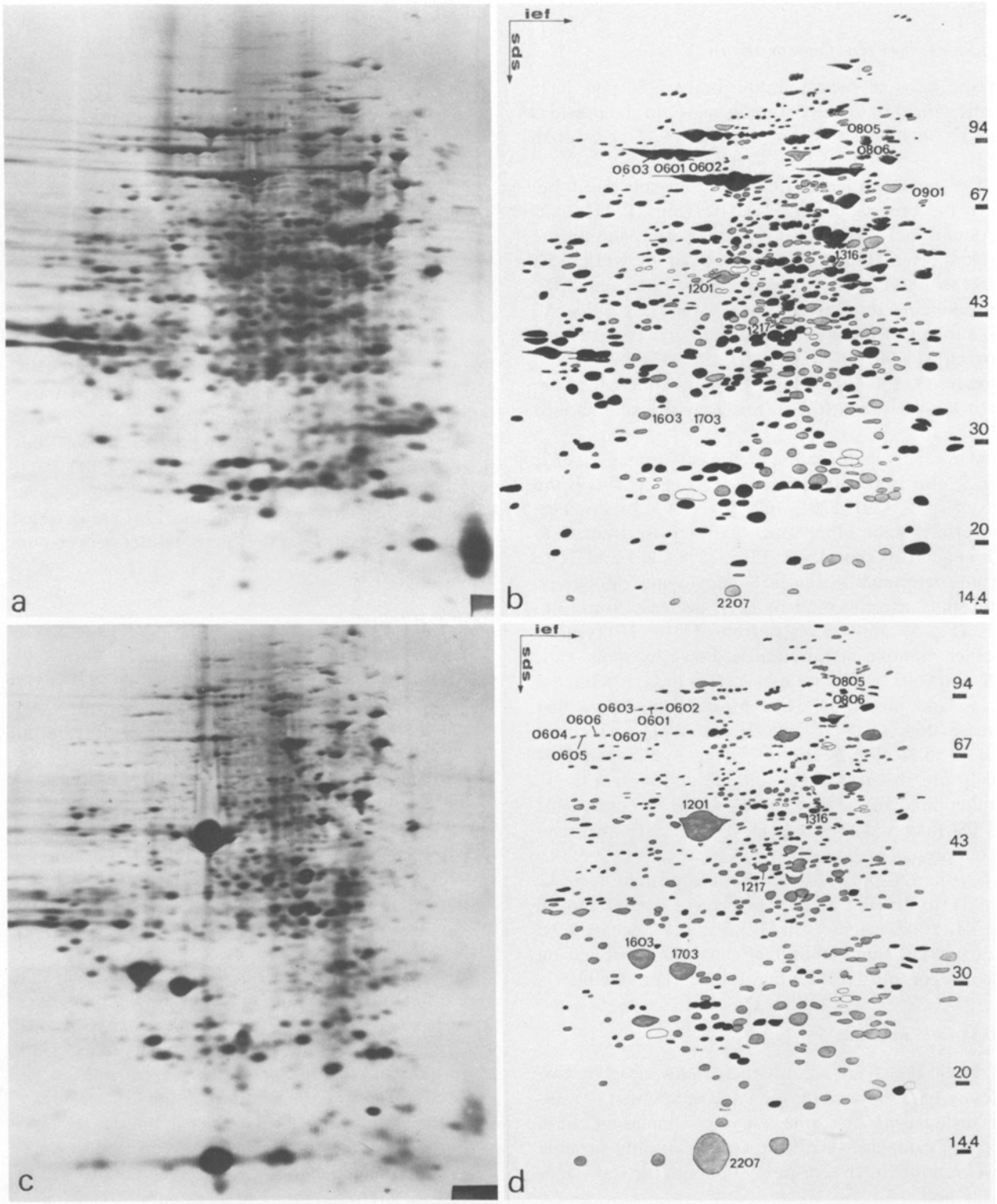

Fig. 2 a–d. G and 1F stage-organs. **a** 2D gel from G. **b** G map where the black spots are those showing intensity decrease or stability from G to 1F and the grey spots are those showing intensity increase from G to 1F. White spots are those where no observation was made because of superposition. Labelled spots are those we refer to in the text. On the right are indicated the molecular weights of standard proteins in kilodaltons. **c** 2D gel from 1F. **d** 1F map where spot symbols are as in **b** (from G to 1F)

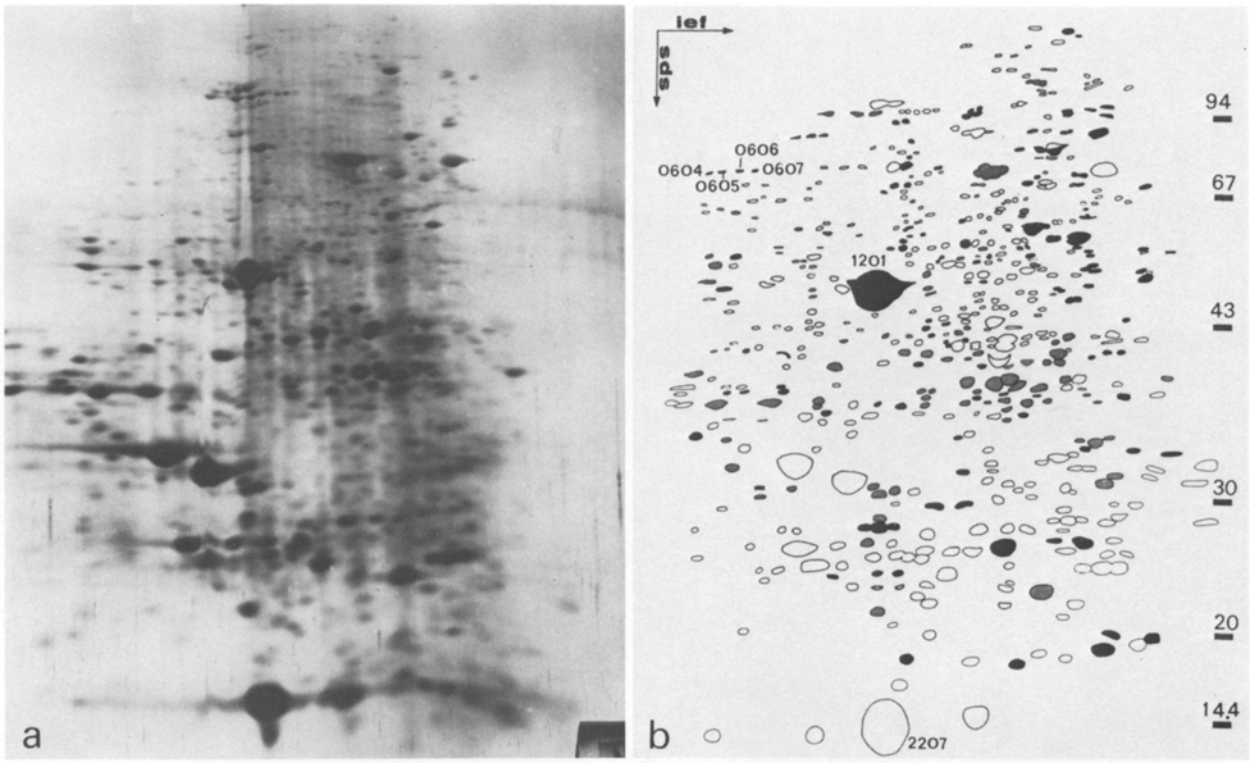

Fig. 3 a, b. 2F stage-organ. **a** 2D gel from 2F. **b** 1F map where black spots are those disappearing or decreasing in intensity, white spots are those showing intensity stability and grey spots are those appearing or increasing in intensity from 1F to 2F

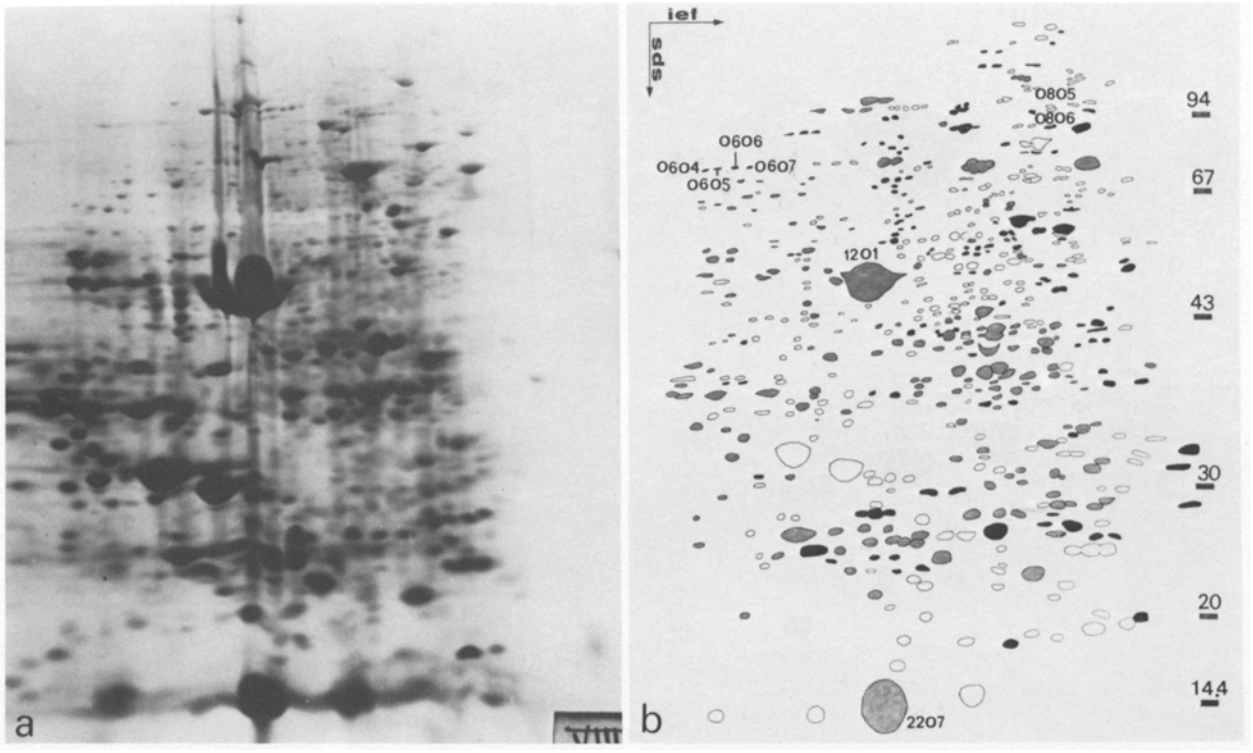

Fig. 4 a, b. 3F stage-organ. **a** 2D gel from 3F. **b** 1F map where spot symbols are as in Fig. 3, from 1F to 3F

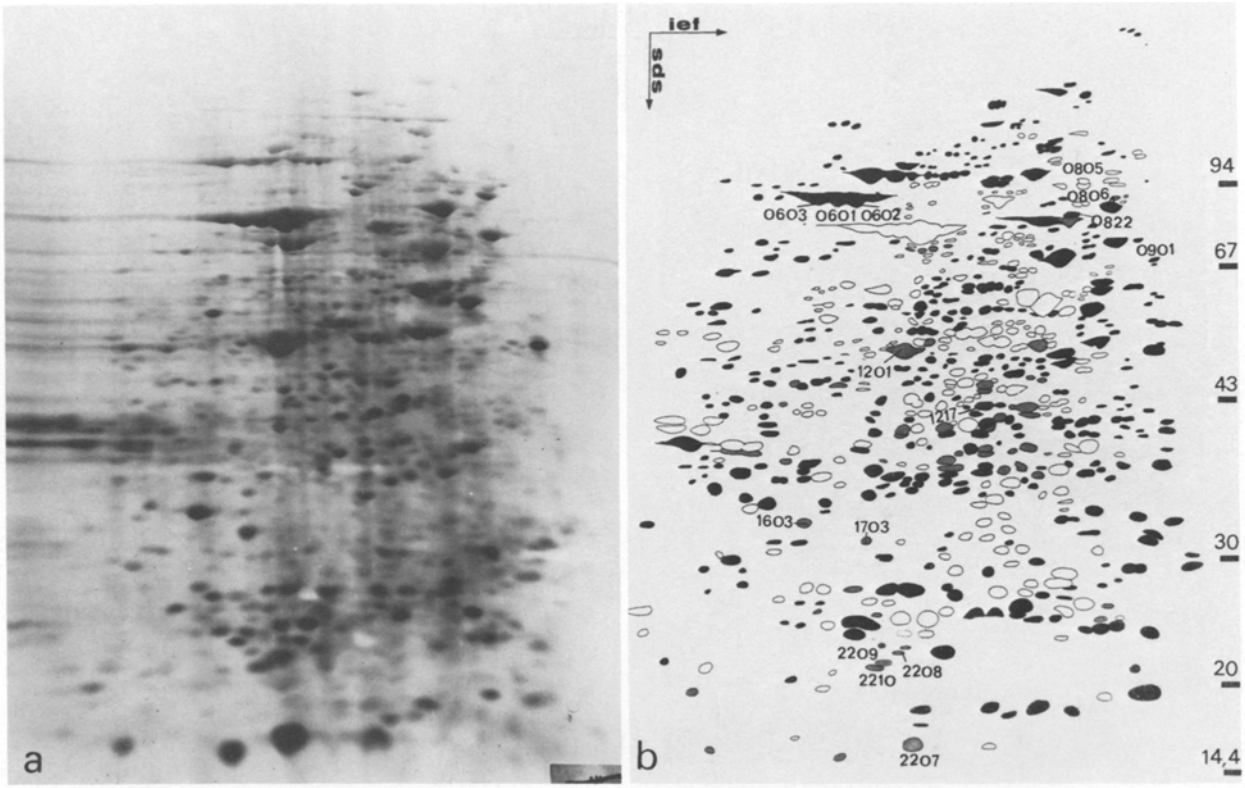

Fig. 5 a, b. 3CE stage-organ. **a** 2D gel from 3CE. **b** G map where spot symbols are as in Fig. 3, from G to 3CE

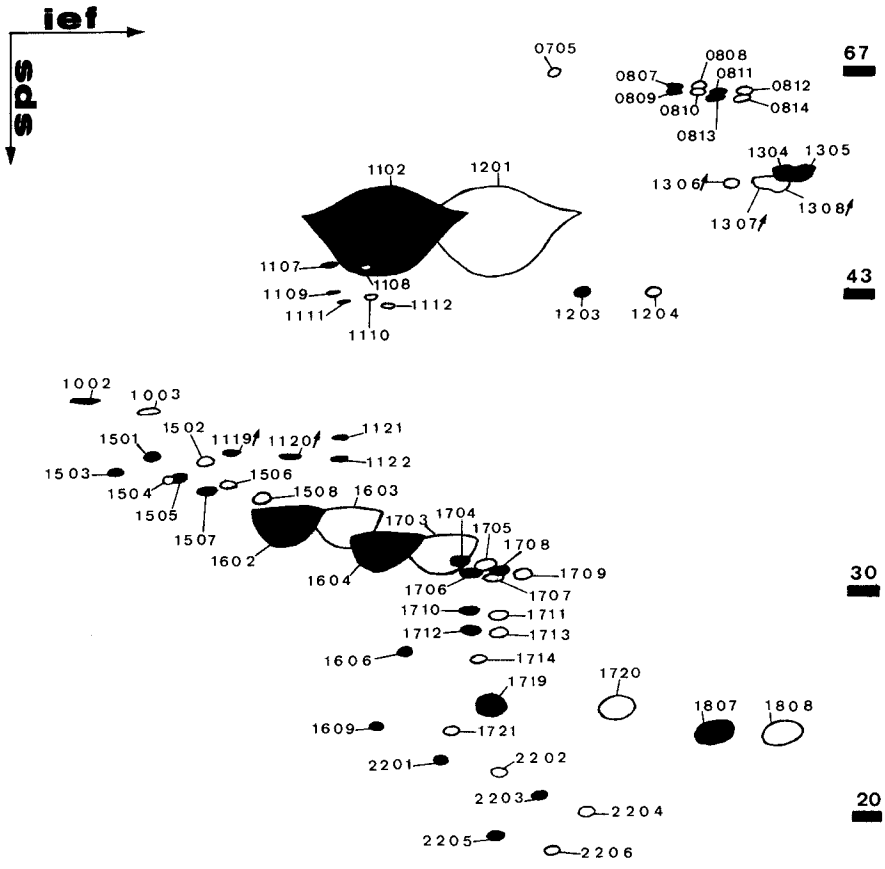

Fig. 6. Cytoplasmic variation at the 3F stage-organ. On this map indicated in black are the spots present or more intense in the *Triticum aestivum* cytoplasm and in white the spots present or more intense in the *Aegilops* cytoplasms. The numbers of the spots more intense in one of the two cytoplasmic types are followed by an *arrow*. On the *right* are indicated the molecular weights of standard proteins in kilodaltons

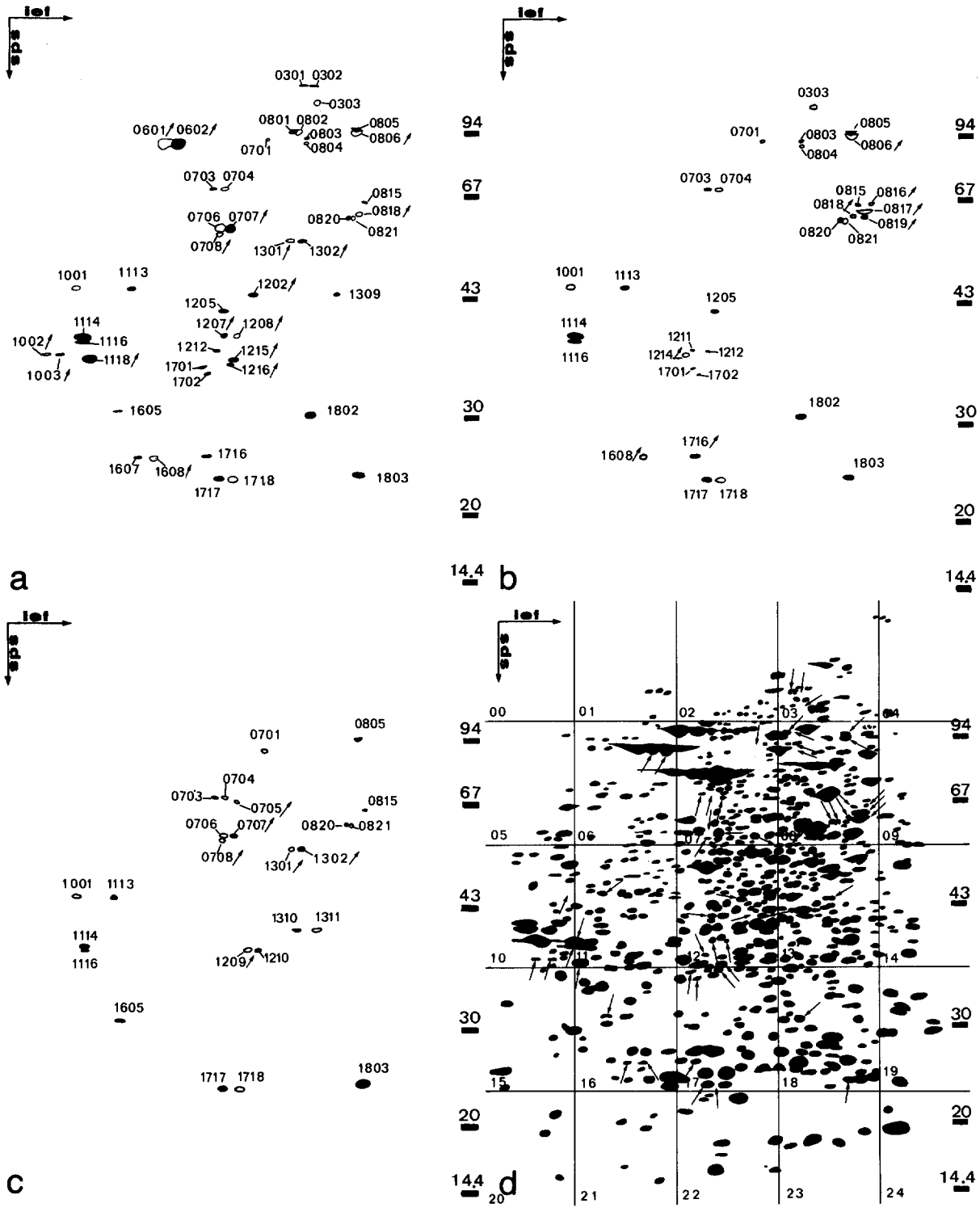

Fig. 7 a–d. Nuclear variation between 'Chinese Spring' (CS) and 'Selkirk' (Sk). **a** Spot differences at the G stage-organ. The spots present or more intense in Sk are in black and the spots present or more intense in CS are in white. The numbers of the spots more intense in one of the two lines are followed by an arrow. On the *right* are indicated the molecular weights of standard proteins in kilodaltons. **b** Spot differences at the 3CE stage-organ. Symbols are as in **a. c** Spot differences at the 1F stage-organ. Symbols are as in **a. d** Map of Sk with *Aegilops* cytoplasm at the G stage-organ where the different regions are indicated (which give the first two numerals of the spot numbers). *Arrows* indicate the locations of the spot involved in nuclear variation. Some of them cannot be indicated since they are not seen at the G stage-organ

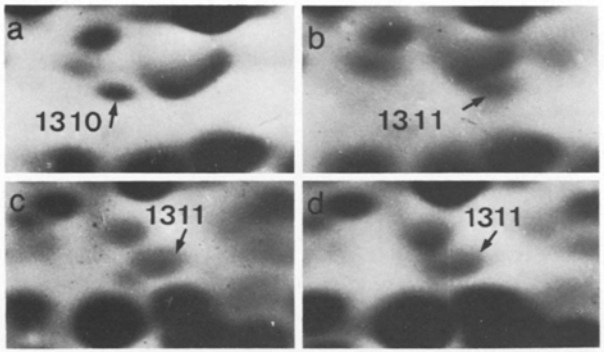

Fig. 8. Portions of 2D gels from 'Selkirk' (**a** and **c**) and from 'Chinese Spring' (**b** and **d**) at the 1F stage-organ (**a** and **b**) and at the 3F stage-organ (**c** and **d**). See text

Nucleo-cytoplasmic interaction

Spot 0705 (Figs. 6 and 7c) presents an interesting behaviour: present in the *Aegilops* cytoplasms and absent in the *T. aestivum* cytoplasm, it is less intense in Sk than in CS at the 1F stage-organ. Thus, this polypeptide is under the control of both the nuclear and cytoplasmic genomes. The effect of the nuclear genome on the synthesis of proteins encoded by the mitochondria has already been reported, for instance in maize (Forde and Leaver 1980). Nucleo-cytoplasmic interactions may be more frequent but the probability of finding such a phenomenon was very low because of the method used to compare the 2D gels.

Allelism in the SDS dimension

As mentioned above, the allelic variation of a protein coding gene is observed in the IEF horizontal dimension. Nevertheless, in two cases (spots 0804/0803 and 0806/0805) a position difference is observed in the SDS dimension between the two lines (Figs. 7 and 9). Such an observation is currently interpretated as a molecular weight change. In that case the difference between the two proteins may result from the deletion or addition of a neutrally charged oligopeptide. However, some amino acid substitutions without any charge change can greatly affect the polypeptide migration in the SDS dimension although the polypeptide keeps the same molecular weight (de Jong et al. 1978; Noel et al. 1979; Wilson et al. 1983). Thus, we record the two cases as a protein coding gene variation (allelic situation).

Associated spots

In several instances, a similar variation affects simultaneously two or more adjacent spots. This is noticed in one or the other dimension for cytoplasmic dependent differences, as for the 3 spots 1306, 1307 and 1308 or for the four spots 1119, 1120, 1121 and 1122 (Fig. 6). This is also noticed for nuclear dependent differences as for spots 1002 and 1003 or for spots 1701 and 1702 (Fig. 7a).

An interesting point is that the behaviour or the associated spots sometimes appears stage specific. This is exemplified in Fig. 10 for four spots observed in G. In 1F they are less intense and additional spots are present in their vicinity at more acidic isoelectric points. In 2F the four "original" spots are very faint whereas additional spots are more intense. At the 3F stage-organ all the spots are found but less intense than in 1F. None of them is detectable in 3CE.

In these cases it seems likely that the multiple spots correspond to various products of a single gene. Such associated spots have already been reported and can be due to post-translational 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).

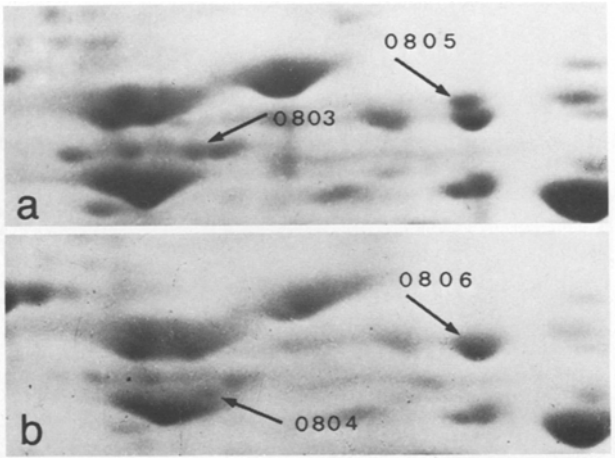

Fig. 9. Allelism in the SDS dimension as observed for spots 0804/0803 and hypothetized for spots 0806/0805 (**a** 'Selkirk', **b** 'Chinese Spring')

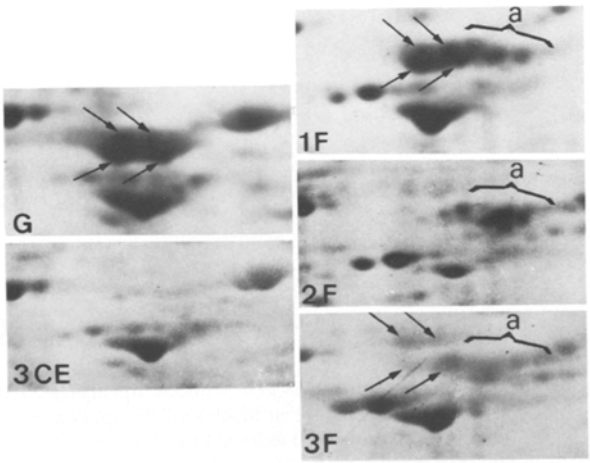

Fig. 10. Parts of 2D gels from the five stage-organs showing hypothetical posttranslational modifications. *Arrows* indicate "original" spots and additional spots are under the "a" double bracket

We consider a variation observed on two or more adjacent spots as a single genetic difference. Such a group of associated spots will be referred to as in the following example: the four spots 1119, 1120, 1121 and 1122 will be noted 1119+.

Discussion

Variation between stages or organs

In order to visualize different states of genome expression 2D gels were obtained from different development stages and different organs in wheat. Each stage-organ can be unambiguously characterized, i.e. clear-cut differences are noticed between them, even between leaf stages. Some spots are stage-specific but most differences are seen as intensity changes.

The way we define three classes of variation at each comparison, due to our rough visual analysis, induces a great heterogeneity inside each class. For instance, a slight decrease or increase in intensity is not considered and major as well as minor spots are identically classified. Therefore, no conclusion can be drawn about a common regulatory control for the spots belonging to the same class of intensity evolution.

Nevertheless, as indicated in the 'Results' section, the use of 2D electrophoresis at different stage-organs allows several different types of control of the protein quantity to be defined. Moreover, the behaviour of the group of spots shown in Fig. 10 suggests that post-translational modifications may be stage-specific, as indicated by Van Blerkom (1981).

In higher plants, 2D electrophoresis has been used to study in vitro embryogenesis in cotton (Dure et al. 1980), the incidence of heat shocks or more generally environmental changes on the genome expression (Sachs et al. 1980; Monroy and Schwartzbach 1983) and also to characterize differential expression of two different tissues of the same organ (Harrison and Black 1982).

Our goal was different since we were looking for the highest number of genetic markers, including regulatory systems. Theoretical as well as experimental studies (Wilson et al. 1977; Hedrick and McDonald 1980; Klose 1982) have shown or suggested the importance of the genetic variability of regulatory systems.

That is the reason why we take those regulatory systems into account to describe the genetic differences between the wheat lines and compare them at the five stage-organs described here.

Cytoplasmic variation

Taking into account the five stage-organs only two cytoplasmic patterns are found: one for the *T. aestivum* and one for the *Aegilops* cytoplasms studied here. Two spots are more intense or present only in *T. aestivum* (spots 1304+ and 1119+) and two spots are more intense or present only in *Aegilops* (spots 1306+ and 0705). In addition 28 polypeptides show position differences.

We suggested in a previous paper (Zivy et al. 1983) that the polypeptides showing an identical shift in both dimensions are structurally related to LS of Rubisco i.e. spot 1102/1201. It has recently been shown in our laboratory (Colas des Francs et al. 1984) that most spots are in fact degradation products of the LS, occurring during the extraction procedure.

The IEF shift for LS spot is larger than one charge change, as exhibited by standard charge proteins (data not shown). The molecular weight change observed in the SDS dimension is further evidenced for the LS spot in coelectrophoresis of two cytoplasmic types at the G stage-organ (Fig. 11). These two observations support our suggestion (Zivy et al. 1983) that the LS polypeptide is shorter in the three *Aegilops* cytoplasms here studied than in *T. aestivum*. According to Bowman et al. (1983) the LS coding region of the chloroplastic DNA can indeed show size variation between *T. aestivum* and *Aegilops*.

Twenty-eight polypeptides reflect the variation of only one protein coding gene. For the other four genes whose regulatory systems are under cytoplasmic control, spots 0705, 1304+, 1306+ and 1119+ are present only or more intense in the leaf stage-organs; this might suggest that they are also related to the chloroplastic apparatus.

We discriminate two types in the four cytoplasms, although they induce similar phenotypical effects (Tsunewaki 1980). However, they can be subdivided in three chloroplastic DNA types (Ogihara and Tsunewaki 1982; Tsunewaki and Ogihara 1983). Our method is thus less sensitive than the cpDNA restriction analysis but 1) we study the gene products and thus reveal the expressed part of the genome and 2) four new proteins have been recorded that can be used as markers for a more extensive cytoplasmic diversity survey.

Nuclear variation

By considering the five stage-organs, the associated spots and allelism in the SDS dimension, we scored 20 allelic differences between CS and Sk. Every situation

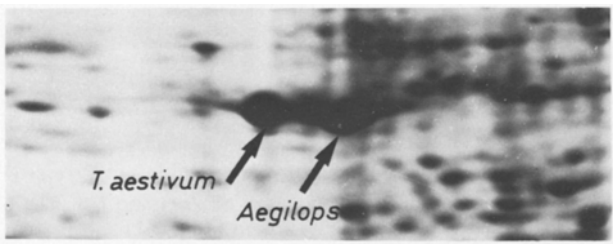

Fig. 11. Coelectrophoresis of a 1/1 mixture of the two cytoplasmic types at the G stage-organ. LS spots are indicated by *arrows*

expected in Fig. 1 has been observed and is illustrated in Fig. 12. Among the 20 allelic differences we found seven cases of type 1, two of them being in the SDS dimension, ten cases of type 2 (presence/absence) and three cases of type 3 (intensity differences).

We must remember that some of these allelic cases may result from regulatory changes. Galili and Feldmann (1983) reported that a gliadin coding gene can be expressed or silent according to the genotype and this can be another interpretation of type 2 allelism. It can also be suggested that such a situation results from partial phosphorylation or glycosylation of a protein in only one genotype. The latter kind of genetic variation has been reported by Steinberg et al. (1977).

Looking at the genetic variability in hexaploid wheat by IEF, Fejer et al. (1979) did not observe the position differences of the bands corresponding to the three homoeologous genes but they noticed intensity differences. The preferential expression of one or the other homoeologous gene according to the genotype may also be an interpretation of a type 3 situation.

Thus, when interpreting all these observations as allelism we underestimate the number of variations for regulatory systems. In any case, to support our allelic interpretations, the hybrid plant resulting from crossing CS to Sk must be studied.

Twenty other genetic differences are scored between the two genotypes that are interpreted as variations of regulatory systems. Paigen (1979) and Scandalios and Baum (1982) have defined "systemic" regulation for control of protein quantity independently of the developmental stage and "spatial" or "temporal" regulation for control of protein quantity depending upon the organ or the developmental stage. Among the 20 regulatory systems recorded here, 12 can be termed systemic and eight spatial or temporal.

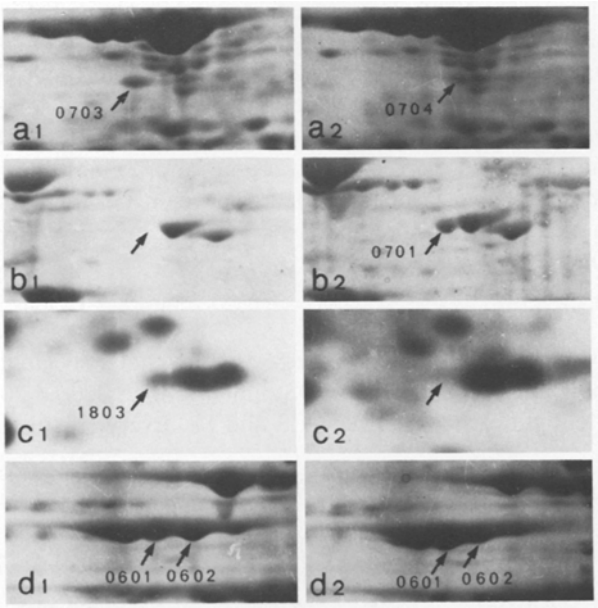

Fig. 12a–d. Examples of different allelic situation. **a** Type 1: position difference of a spot between Sk (**a1**) and CS (**a2**). **b** Type 2: presence/absence. Spot absent in Sk (**b1**) and present in CS (**b2**). **c** Type 2: presence/absence. Spot present in Sk (**c1**) and absent in CS (**c2**). **d** Type 3: intensity change between Sk (**d1**) and CS (**d2**)

One spot corresponds to the product of one gene but one protein coding gene can give rise to several spots (associated spots) due either to in vivo post-translational and catabolic events or to in vitro degradations. So we may wonder how many genes are revealed in this analysis. One way of answering is to consider the genetically variable spots as a representative sample of all the spots. Before doing this we must exclude the LS from this sample for its extreme abundance in the leaf stage-organs. Twenty-three spots are degradation products that shared the same different segment. Thus, it is likely that at least as many spots are degradation products present in the gel but we cannot notice them since they share the other common part of the molecule. So in a rough approximation, about 50 spots are products of the LS gene in the leaf stages.

Taking into account all the studied stage-organs, 835 spots have been retained and 785 remain after deduction of the LS products. Considering the genetically variable spots, 43 variations are recorded on 55 spots because of the previously discussed phenomenon of associated spots. Thus a protein coding gene leads on the average to 1.28 spots and we revealed about 610 protein coding genes. This rough estimate, 22% additional spots, is the same as the one (23%) given by Klose (1982).

Considering the allelic situations recorded between CS and Sk the maximum percentage of variations is 3.3% for the protein coding genes. It is difficult to know if this value is lower than the genetic variation assessed from classical native ID electrophoresis. In spite of a high genetic divergence between these two lines it is likely that, according to Rosenblum et al. (1983), the number of variants in "crowded" regions of silver stained 2D gels is underestimated. In any case the allohexaploid structure of wheat makes a comparison of our result to those from other species difficult.

We record an equal number of variations for protein coding genes and for regulatory systems although we always favoured the allelism interpretation. One must also keep in mind the poor estimation of intensity variations resulting from visual analysis. Taking into account these underestimations the genetic variability of regulatory systems is probably much greater than that of protein coding genes. Klose (1982) working in a comparable way on lines of mice has reported a very important genetic variability for regulatory systems.

Up to now, most of the geneticists have revealed genetic variation mainly on enzyme genes. The reason for the frequent inadequacy between phenotypic divergence and molecular diversity may be that most enzyme variants are roughly neutral in their effect on the phenotype (Kacser and Burns 1981). The analysis of genetic variability of a very large number of proteins and of their regulatory systems could help to find better markers of phenotypic divergence. This study is currently being conducted in our laboratory on wheat and on other species.

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