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Organ-specific variability and inheritance of maize proteins revealed by two-dimensional electrophoresis

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Summary

Using two-dimensional polyacrylamide gel electrophoresis (2-D PAGE), the genetic variation of proteins was investigated in three organs (mesocotyl, sheath and blade of second leaf) from two maize lines and their hybrids. Nine hundred and eighty-six spots were numbered over the three organs. One hundred and fifty-five polypeptides were found to be genetically variable, due to allelic polymorphism of structural genes and/or to polymorphism of any genetic elements controlling protein amounts. Of these 155 variants 12% clearly showed complete dominance effects in the hybrid patterns, which could reveal dominance effects in the regulation of the protein biosynthesis. Comparison of the three organs showed that (i) the level of variability between lines depended upon the organ, since it varied from 7·5% for the blade to 12·6% for the mesocotyl and 13·2% for the sheath; (ii) 68 polypeptides displayed different kinds of variation in different organs and (iii) in all cases but one the dominant inheritance was organ-specific.

1. Introduction

Genetic differences in the level of expression, the tissue distribution or the developmental stage have often been demonstrated for particular enzymes, in plants as in animals (Paigen, 1979; Das & Messing, 1987; King & McDonald, 1987; Kuhlemeier, Green & Chua, 1987). The molecular basis of these differences may be multiple, from the regulation of transcription to post-translational modifications. As a prerequisite for molecular studies, and in order to get general information about the way the protein amounts are inherited, we have undertaken the comparison of two genetically distant maize lines with their F_1 hybrids using two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) of denatured proteins (O'Farrell, 1975; Garrels, 1979). If the two lines differ for many loci involved in regulation of gene product synthesis and degradation, their hybrids are expected to display various schemes of inheritance for protein amounts, due to dominance and/or epistatic interactions.

In a previous paper (Leonardi, Damerval & de Vienne, 1987) we described the results for one organ, the sheath of the second leaf. The same intergenotypic comparison is here extended to three different organs, the mesocotyl, and the sheath and blade of the second leaf.

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2. Materials and methods

Two maize (*Zea mays* L.) lines, W_{117} (dent type) and F_{1254} (flint type) and their two reciprocal hybrids ($♀W_{117} \times ♂F_{1254}$ and $♀F_{1254} \times ♂W_{117}$) were grown as described in Leonardi, Damerval & de Vienne (1987). The mesocotyl, the basal etiolated part of the sheath and the green blade of the second leaf were harvested at the same developmental stage (3-week-old plantlets). Three individuals were taken per line and per F_1 hybrid, and constituted repetitions of the genotypes.

(i) *Protein extraction and electrophoresis*

The extraction procedure was according to Zivy (in Damerval *et al.* (1986)) except that 40 μl of the resolubilization solution was used to resuspend 1 mg of pellet. The isoelectrofocusing (IEF) was as described by Leonardi, Damerval & de Vienne (1987). The second dimension followed the procedure of Damerval *et al.* (1986) and the 2-D gels were silver stained according to Granier & de Vienne (1985) and Damerval *et al.* (1987). These procedures were developed to ensure a very good reproducibility of the 2-D gels.

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(ii) *Scoring procedure*

The inheritance of amounts of individual proteins are described in terms of spot intensity: (i) 'co-dominance' corresponds to a hybrid spot intensity half way between the two parental spot intensities; (ii) 'dominance' means that the hybrid spot is similar to one of the parental spots; and (iii) 'over-dominance' means that the hybrid spot is more intense than either of the parental spots. Although various cases of partial dominance were suspected, they were all considered as co-dominance ones. These definitions hold as well if the spot is present in only one line.

For each organ of each genotype, three gels (one per individual plant), representing three repetitions, were visually scored by a first observer and then independently revised by a second one. Moreover the dominance cases in the sheath were checked by a third one. Differences in presence/absence or intensity of spots between the two parental lines were only retained for further study if they were clearly visible in the three repetitions.

The inheritance of spot intensities was determined by comparing the hybrid pattern with a 1:1 co-migration of the parental lines, which represents the 'theoretical' hybrid pattern under the hypothesis of co-dominance of protein amounts.

The spot positions were identified from one organ to another with the help of a 1:1:1 co-electrophoresis of the three organs, sheath, blade and mesocotyl. Intensity differences for a given spot between different organs were considered only relative to the other spots.

3. Results

(i) *Differences in protein patterns of different organs*

The 2-D gels of every organ are shown in Fig. 1. The number of reproducible spots was equivalent in the

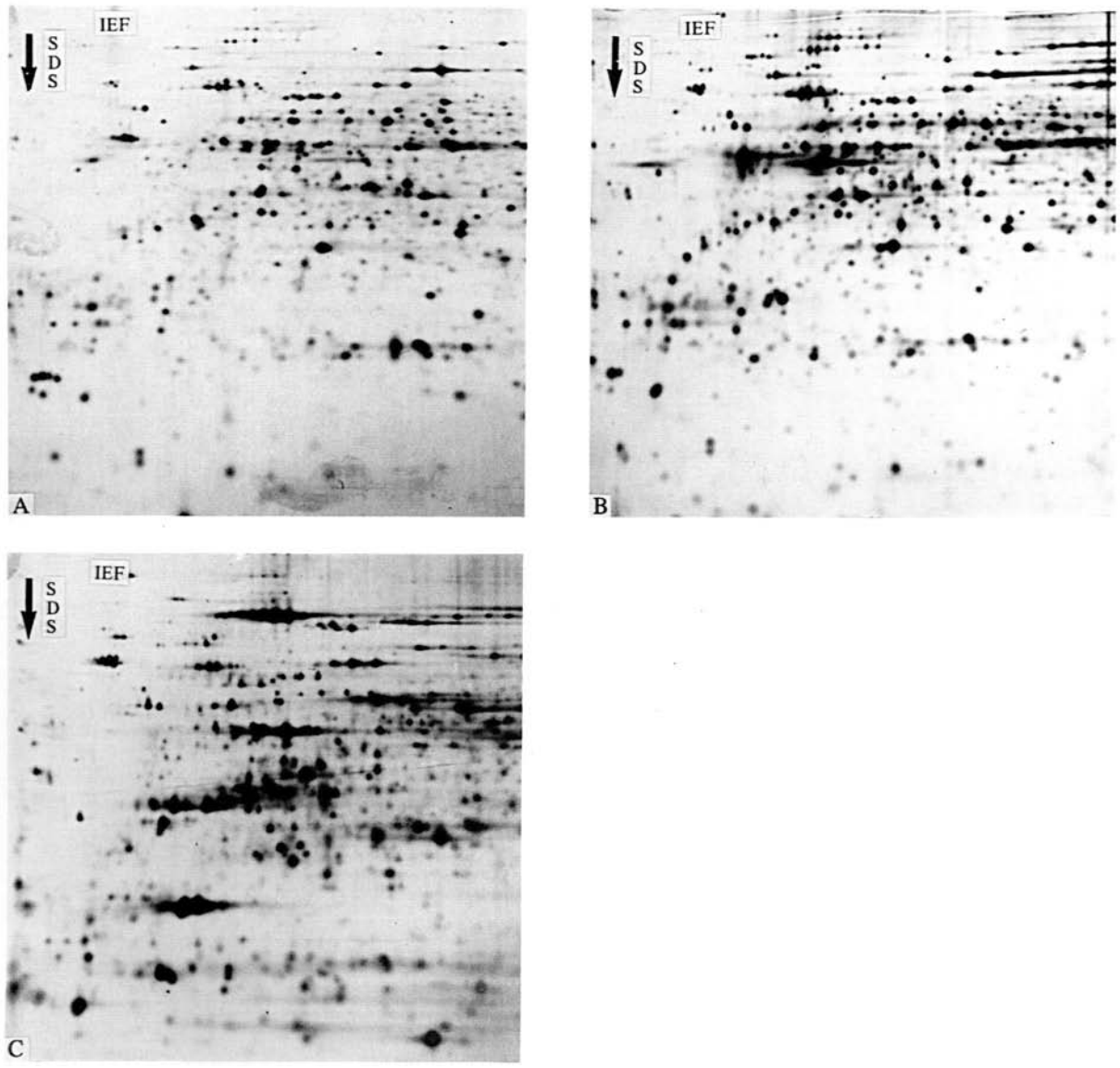

Fig. 1. 2-D gels of the $W_{117} \times F_{1254}$ hybrid proteins from the three organs studied. A, mesocotyl; B, sheath; C, blade.

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Table 1. *Spot intensity differences between the 3 organs in the hybrids*

Present in only one organ	Mesocotyl		47	112
	Sheath		15	
	Blade		50	
Present in only two organs	Mesocotyl = 0			218
	Sheath = blade	20	42	
	Sheath ≠ blade	22		
	Blade = 0			
	sheath = mesocotyl	150	174	
	sheath ≠ mesocotyl	24		
	Sheath = 0			
	mesocotyl = blade		2	
Present in three organs with different intensities	Mesocotyl > sheath > blade		17	299
	Blade > sheath > mesocotyl		7	
	Mesocotyl = sheath ≠ blade		221	
	Blade = sheath ≠ mesocotyl		35	
	Blade = mesocotyl ≠ sheath		19	
Present in the three organs with the same intensity				357
Total				986

mesocotyl and the sheath (879 *vs.* 887) but lower in the blade (750). A different spot intensity distribution was observed in the blade: more spots of high or very high intensity, together with fewer spots of average intensity. This is further demonstrated by the high number of spots present in both sheath and mesocotyl and absent in the blade (Table 1).

Among the 986 reproducible spots found in the co-migration of the three organs, 656 spots were common to the three organs, and numerous intensity differences were found. Forty-seven, 15 and 50 spots were specific to the mesocotyl, the sheath and the blade, respectively. The sheath pattern was closer to that of the mesocotyl than to that of the blade. Its intermediacy is demonstrated by a low number of specific spots and the fact that a spot present in both the blade and the mesocotyl was almost always present in the sheath.

(ii) *Genetic variability of protein amounts from different organs*

The numbers of variable spots and the different kinds of inheritance of protein amounts for each organ are given in Table 2. Spot variations between lines are described in this table as either qualitative (presence or absence of a spot) or quantitative (spot more or less intense), without regard to their genetic meaning and possible ambiguities in the partition (see discussion).

The level of variation between the two parental lines was different in the three organs, and varied from 7·5% for the blade to 12·6% for the mesocotyl and 13·2% for the sheath, the value for the blade being significantly less than those for mesocotyl and sheath.

All in all, 155 spots displayed interline variations in at least one organ. Twenty-two pairs of likely allelic polypeptides could be defined: for a given pair, each parent had one spot and both spots close together were found in the F_1's. Allelic polypeptides can differ in pI, or apparent molecular weight, or both (an example of allelic molecular weight difference is shown in Fig. 2, first line). Thirty-three spots were present in one line and absent in the other line without possible pairing. The variation of these 77 spots was visible in all the organs which displayed them.

Thirty-two other spots were qualitatively variable in one (or two) organ(s) but quantitatively variable or constant in other organ(s). The remaining 46 spots were either quantitatively variable in all organs where they can be found (for 10 of them) or quantitatively variable in one (or two) organ(s) and invariant in other(s) (for the last 36 spots) (Fig. 3).

Table 2. *Protein variations between the lines and their inheritance in the F_1's*

Organ	Mesocotyl	Sheath	Blade	3 organs together*
Number of spots	879	887	750	986
Number of variants	111	117	56	155
Qualitative				
Co-dominant	62	54	40	69
Dominant	2	7	0	8
Quantitative				
Co-dominant	42	50	16	67
Dominant	5	6	0	11

* Since many variants are overlapping from one organ to another, the numbers in this column are not the sum of values for each organ.

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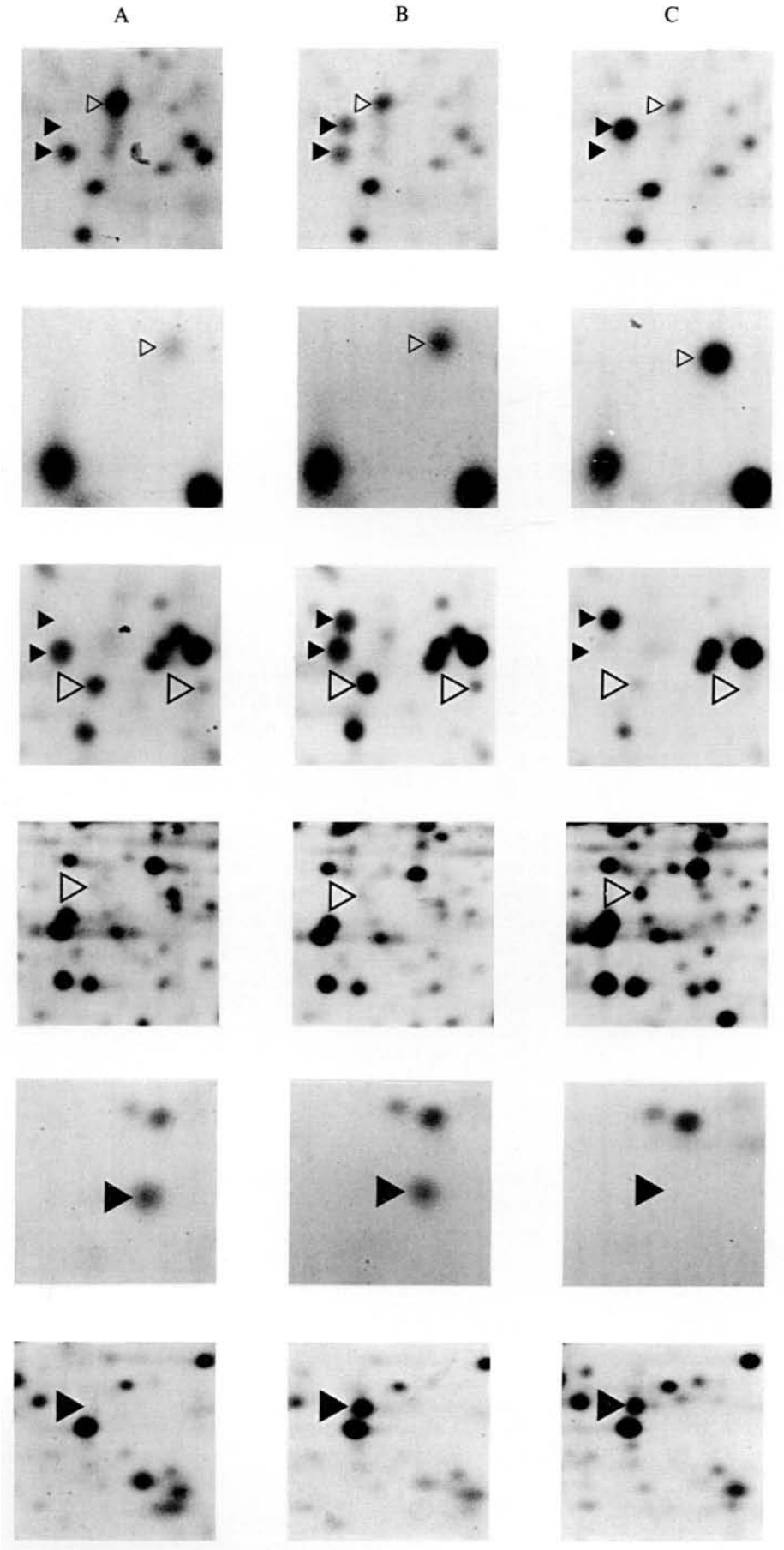

Fig. 2. Examples of variation between the lines and their inheritance in the hybrid. A, B and C are the patterns of F_{1254}, $W_{117} \times F_{1254}$ and W_{117}, respectively. ▶, qualitative variants; ▷, quantitative variants. The small symbols are for the co-dominance cases and the large ones for dominance cases.

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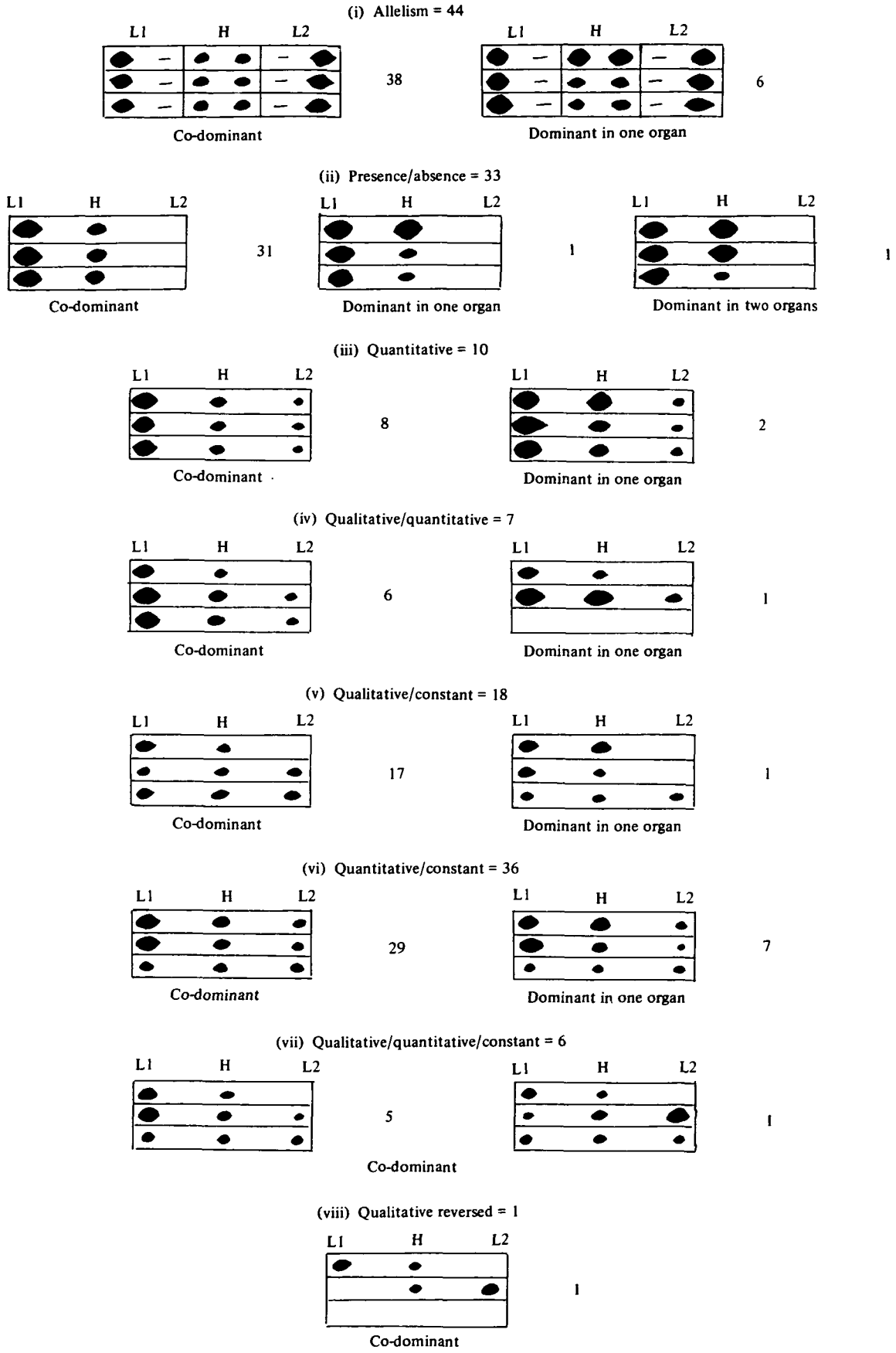

Fig. 3. Kinds of variation and inheritance of the 155 genetic variants according to the three organs. L1 and L2 represent the parents, and H the hybrid. The three lines in each case represent the three organs indiscriminately. For the allelism cases, the dash in L1 (L2) indicates the polypeptide lacking in L2 (L1).

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(iii) *Inheritance of protein amounts from different organs*

Examples of the different kinds of inheritance found in the different organs are shown in Fig. 2. Neither of the hybrids had any protein spots that were not found in one or both of the parents; conversely, when a spot was observed in one or both parents, it was always present in the hybrids. One hundred and thirty-six of the variable spots showed co-dominant inheritance. In the blade, no case of dominance was found. Nevertheless, seven spots in the mesocotyl and 12 spots in the sheath showed very clear complete dominance effects (Fig. 2). All the cases but one of dominant/recessive inheritance of protein amounts concerned only one organ. In most cases the spot was present in other organs but either with a co-dominant inheritance, or even invariant between the lines. In the mesocotyl, high intensity (or presence) was dominant over low intensity (or absence) in four cases, and the situation was reversed in the three other cases. In the sheath, high intensity (or presence) was always dominant over low intensity (or absence), as previously reported in Leonardi, Damerval & de Vienne (1987).

One spot appeared over-dominant in the sheath pattern, since the hybrid $\varphi W_{117} \times \sigma F_{1254}$ spot was more intense than both the W_{117} and F_{1254} spots, these two being visually identical. However F_{1254} displayed a spot (not visible in the hybrids) very close to the over-dominant spot, and the intensities added of these two spots in F_{1254} seemed equivalent to that of the over-dominant spot. Moreover one replicate of F_{1254} displayed only one spot instead of two, whose localization and intensity correspond to the hybrid $\varphi W_{117} \times \sigma F_{1254}$ spot. Thus it might be that the 'over-dominant' case is actually due to doubling of one spot in most F_{1254} 2-D gels (this spot is noted with dominant ones in Table 2 and in Fig. 3). In the reciprocal hybrid $\varphi F_{1254} \times \sigma W_{117}$ this spot displayed an intensity intermediate between these of $\varphi W_{117} \times \sigma F_{1254}$ and W_{117}. This was the only reciprocal effect observed in the sheath; two reciprocal effects were observed between the two hybrids in the mesocotyl, i.e. for two spots, the reciprocal hybrids differed, each displaying the maternal intensity (or position); none was found in the blade.

4. Discussion

When comparing two-dimensional gels from different genotypes, the variability appears either as presence/absence of protein spots or differences in spot intensities. The genetic meaning of these variations is not straightforward in terms of structural versus regulatory mutations, as discussed by Damerval, Hébert & de Vienne (1987). In this regard, the study of the three organs was very informative.

Twenty-two pairs of qualitative variants have a behavior – identical over the three organs – which may be expected for allelic polypeptides differing for one or a small number of amino acids (Anderson *et al.* 1985; McLellan & Inouye, 1986). Thus these 44 spots very likely correspond to allelic gene products of 22 structural genes. The genetic meaning of the 33 other qualitative variants remains to be determined.

On the other hand, some polypeptides could not be classified with the presence/absence variants, since a line lacking the spot in one organ can display it in at least one other. These variants and all the quantitative ones (78 spots) could reveal a polymorphism of genetic factors controlling protein quantities, including mutations inside the structural gene of the protein itself.

The two lines studied in this experiment differ for a large number of genes, as attested by the high percentages of variable spots. Moreover, a similar protein quantity in two lines does not prove that they share the same alleles for the loci influencing this gene product. For these reasons it was *a priori* possible that the hybrids exhibit often various unexpected situations, due to interactions between two or several genes, e.g. appearance or disappearance of polypeptides in the hybrids, frequent over- or under-dominance, reciprocal effects. Actually this was not the case, since spot intensities in the F_1's were always in the range of the parental spot intensities, and were similar to one of the parental values in only 12% of the cases.

Among the 357 protein spots invariant between the organs, only 13 (3·6%) displayed variation between lines, whereas among the remaining 629 spots with organ-specific intensity, 142 (22·6%) are variable between lines. This follows the trends of an observation made by Klose (1982), according to whom 'organ-specific proteins' are more variable between different mouse strains than 'organ-unspecific proteins'. Moreover, the comparison between organs revealed the complexity of patterns of variation since as many as 95% of the variants in spot intensity displayed organ-specific variation and/or organ-specific inheritance.

Lastly, the level of variation differed significantly according to the organ, and the sets of polypeptides involved were not identical. Thus, the estimation of genetic divergence can be influenced by the choice of the organ or stage.

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