Theoretical and Applied Genetics 45, 290–293 (1975)

Genetic Control of Esterases in Common Wheat*

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Summary. A refined technique of gel electrofocusing revealed the esterases in caryopses of *Triticum aestivum*, *T. durum* and *Triticum timopheevi*. In *T. aestivum*, 17 isoenzymatic bands were ascertained in the pH 5–8 range: 11 were of higher intensity, 4 were weak and two very weak. Using Chinese Spring nulli-tetrasomic lines it was possible to locate the genetic control of several isoenzymes in the chromosomes of the homoeologous group 3. In chromosome 3A three bands are coded; in 3B four bands are coded; and in 3D two bands out of the eleven of higher intensity.

T. durum, as expected, lacks bands coded in *T. aestivum* by chromosome 3D. *T. timopheevi* presents a quite distinct isoenzyme pattern, thus confirming its different speciation. Two major bands do not disappear in any of the nulli-tetra lines analyzed: it is supposed that these isoenzymes could be coded by at least two of the chromosomes of the group three involved in esterase control. The presence of several esterase isoenzymes in wheat is both evidence of their additivity with increasing ploidy level and biochemical support for the hypothesis that there is a higher possibility of adaptation of polyploids compared with diploid species.

Introduction

In recent years several investigations have been undertaken to study the genetic control of synthesis of reserve and enzymatic proteins in higher plants, for instance, in maize (Schwartz *et al.*, 1965; McDonald and Brewbaker, 1972; Scandalios, 1969), in barley (Solari and Favret, 1969), *Solanum* (Desborough and Peloquin, 1967), *Gossypium* (Cherry *et al.* 1970, 1971), *Avena* (Marshall and Allard, 1970; Murray *et al.*, 1970), sugar beet (Lerch and Wolf, 1972), etc.

Studies on these proteins have also been an interesting tool to investigate the phylogenetical relationships between related species (Johnson, 1972; Bozzini *et al.*, 1970; Nakai *et al.*, 1971).

Wheat is an interesting model because of its polyploid constitution, the presence of some ancestors at diploid and tetraploid levels, the availability at hexaploid level of aneuploid series (nullisomics, nullitetrasomics, ditelocentrics, etc.) which allow detailed chromosomal analyses.

Using nulli-tetrasomic lines Shepherd (1968) found that a consistent fraction of gliadins, fractionated by starch gel electrophoresis, are controlled by factors carried in chromosomes of the homoeologous groups 1 and 6 of bread wheat.

Bozzini *et al.* (1970) found, by immunochemical methods, genes in 3D and 4D chromosomes controlling the synthesis of two albumines present in *T. aestivum* and absent in *T. durum*. Cubadda (1973) found that in homoeologous chromosomes of group 3 are located genes involved in the synthesis of seven albumin fractions. Brewer *et al.* (1969) reported that isoenzymes of alcalin-phosphatase are coded by 4B and 4D chromosomes. Hart (1970) demonstrated that triplicate genes of alcohol dehydrogenase are located in chromosomes 4A, 4B and 4D. Esterases have also been the object of some studies. Barber *et al.* (1968), using roots, leaves and caryopses of bread wheat aneuploids, of *Secale* and *Triticale*, located genes coding esterases in the left arm of the 3A chromosome. Bergman and Williams (1972) located esterases in chromosomes of homoeologous group 3. In the present study further information is reported on the genetic control of wheat esterases. These enzymes have previously been separated by Cubadda *et al.* (1973) using gel-electrofocusing and classified, by substrate specificity and selective inhibitors, as isoenzymes belonging to carbossil-esterhydrolases or aliaesterases (3.1.1.1.1 following the international code).

Material and Methods

The method used to ascertain whether a chromosome controls the synthesis of a particular esterase fraction relies on the comparison of zymograms of aneuploid lines (nullitetra) with those of the control disomic Chinese Spring. The differences found in zymograms are attributed to the absent chromosome, which, presumably, should carry the gene(s) coding disappearing isoenzymatic fractions.

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* Contribution n. 406 from the Laboratorio Applicazioni in Agricoltura, C.N.E.N., Centro Studi Nucleari Casaccia, S. Maria di Galeria, Roma, Italy.

This work was partly supported by C.N.R. special program „Problemi inerenti l'utilizzazione delle proteine e ricerche di nuove fonti proteiche" contract n° 70-01545/32-17.43.3.

The material examined consisted of nullitetrasomic lines of Chinese Spring in all possible combinations except nulli 2A tetra 2B and 2D, nulli 4A tetra 4B and 4D; nulli 4D tetra 4B and nulli 6B tetra 6D. Further information was obtained by analysis of the ditelos 3AL, 3BL, 3DRt and several varieties of *T. durum*, *T. timopheevi* and *T. aestivum*.

Enzymatic extracts were obtained by treating milled caryopses with a solution of $NaHCO_3$ 0.24 M, pH 7.4, in the ratio W/v = 1/4. The solution was then centrifuged at 16.000 × g at 4 °C. Gel electrofocusing was conducted at 4 °C using acrylamide gels, recrystallized before use, with ampholines (LKB, Sweden) at a range of pH 5–8. Migration was obtained using a constant current of 2 mA per tube, with a voltage of 350 V, for about 5 hrs. Esterasic activity was then revealed with a mixture of α-naphthylacetate in acetone and Fast Blue RR in phosphate buffer pH 7.2. Further details of the technique have been reported in previous papers (Cubadda *et al.*, 1973).

Results

Fig. 1 shows zymograms of Chinese Spring of nulli 3A tetra 3B and tetra 3D; of nulli 3B tetra 3A and 3D; of tetra 3D and nulli 3A and 3B.

Chinese Spring disomics present 17 enzymatic bands in the range pH 5.3–7.4, of which 11 have higher intensity, 4 are weak and two very weak. Only the 11 stronger ones are considered here.

All nulli-tetra lines, except for the homoeologous group 3, show zymograms identical to those typical of Chinese Spring. In nulli 3A tetra 3B and 3D bands, 1, 2 and 6 are absent; nulli 3B tetra 3A and 3D lack bands 3, 8, 11 and 13; in nulli 3D tetra 3A and 3B, bands 5 and 12 are absent. Bands 4 and 10 were always present in all analyzed aneuploids. The results show that the homoeologous chromosomes of group 3 have the genes responsible for the synthesis of 9 out of the 11 major fractions revealed with this technique.

Fig. 2 presents zymograms of *T. aestivum* cv. Chinese Spring and cv. Marzotto, of nulli 3D tetra 3D, of *T. durum* cv. Cappelli and of *T. timopheevi*.

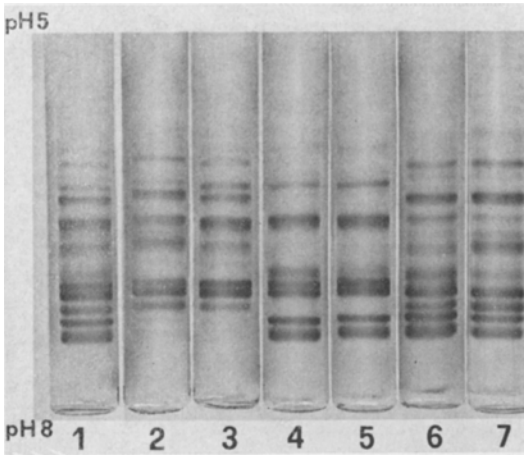

Fig. 1. Zymograms of esterases from Chinese Spring (1); nulli-tetra 3A–3B (2); 3A–3D (3); 3B–3A (4); 3B–3D (5); 3D to 3A (6); 3D–3B (7)

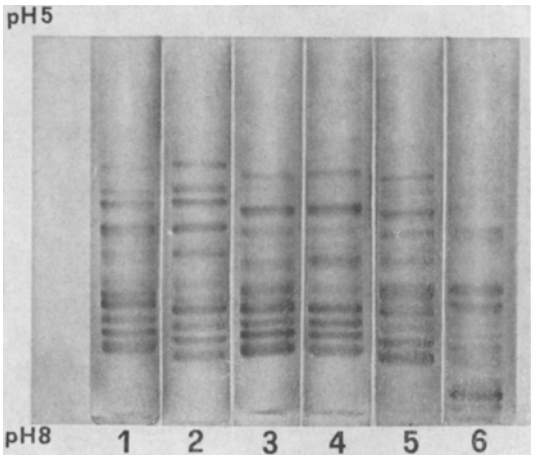

Fig. 2. Zymograms of esterases from Chinese Spring (1); *T. aestivum* cv. Marzotto (2); nulli-tetra 3D–3A (3); nulli-tetra 3D–3B (4); *T. durum* cv. Cappelli (5); *T. timopheevi* (6)

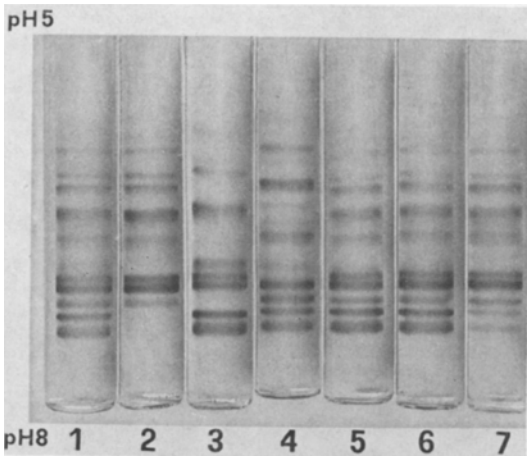

Fig. 3. Zymograms of esterases from Chinese Spring (1); nulli-tetra 3A–3B (2); 3B–3A (3); 3D–3B (4); ditelos 3AL (5); 3 BL (6); 3D RT (7)

Zymograms of the two hexaploid wheats appear to be identical, while bands 5 and 12, coded by the D genome, are absent in *T. durum* (genome AABB). *T. timopheevi* (genome AAGG) shows a zymogram quite different from the one typical of *T. durum*.

Fig. 3 shows zymograms of Chinese Spring of nulli 3A tetra 3B, of nulli 3B tetra 3A, of nulli 3D and tetra 3B and of ditelos 3AL, 3BL and 3DRt. Ditelocentrics show a zymogram identical to that of the Chinese Spring disomic. This could be considered as indirect evidence of the location of genes controlling the above esterasic fractions in the opposite arms of the homoeologous chromosomes of group 3.

Discussion

In the present work 17 isoenzymatic bands have been revealed, many more than those obtained by several other authors. This could be a result of differences in

both technique and material used (caryopses instead of roots, leaves of immature seeds). Moreover, the results show a difference in the number of bands between tetraploid and hexaploid wheats, not previously found by other authors (Bathia, 1968). In tetraploids belonging to the *T. turgidum* group, the two bands lacking correspond to those coded in *T. aestivum* by the genome D. This corrobrates the hypothesis that chromosome 3D carries gene(s) coding isoenzymes different from those present in genomes A and B. *T. timopheevi* and *T. turgidum*, although distinct, have some bands in common: this could be due to the presence of the A genome, shared by both species.

Results obtained with the nulli-tetra and ditelos available for homologous group 3 seem to limit the coding of most of the bands revealed to only one arm. It was, however, clearly demonstrated that all 3 chromosomes carry genes coding different isoenzymes. Only two bands (no'.s 4 and 10) were present in all nulli-tetra analyzed. Two hypotheses may be advanced: 1) these isoenzymes are coded by chromosomes 4A and 2A (not available for analysis) 2) these bands are coded by at least two genes (or group of genes) common to the genomes A, B or D: further analyses will demonstrate which one of these two possibilities is correct.

Since we found isoenzymatic variations coded by each of these chromosomes, it might be concluded that differentiation took place in the evolution of these genomes. At least two mutational events may safely be assumed. This is valid also if hybrid isoenzymes (dimers, trimers or tetramers, etc.) are expected, since only if variants are present can different polymers be formed. However, the fact that isoenzymes present are coded by homoeologous chromosomes (and probably arms) is in line with the hypothesis that differenitation of genomes A, B and D was fairly recent.

The presence of several isoenzymes in wheats, clearly increasing from tetraploid to hexaploid level, besides being a demonstration of their additivity, leads to the more general consideration of how several possibilities of utilizing the esterase system are available: this could contribute to increasing the plasticity of polyploids and their adaptation to different environmental conditions. This is in line — on a biochemical basis — with the observation, often made in plants, that polyploids are able to colonize areas much larger or more extreme than those colonized by related diploid species.

The complexity of isoenzymatic patterns found with this technique in this material is comparable to the condition found in several higher animals. Therefore, interpretation of these bands as enzymatic hybrids (dimers, etc.) becomes extremely complicated. Probably better interpretations will be possible when the isoenzymatic patterns of the diploid donor species are known.

Acknowledgement

We are grateful to Mr. P. Fantauzzi for excellent technical assistance.

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Received April 3, 1974

Communicated by H. Stubbe

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