Proc. 4th Int. Wheat Genet. Symp. - Missouri (1973)

GENOME ANALYSIS IN THE TRITICINAE USING ISOENZYMES OF PHOSPHODIESTERASE

G. WOLF AND B. LERCH

Institut für Pflanzenpathologie der
Universität Gottingen
and Institut für Biochemie der
Biologischen Bundesanstalt,
Braunschweig

INTRODUCTION

The hitherto accepted concept of the origin of the three different genomes of hexaploid wheat has been questioned in recent times because the origin of the B genome is not clear (see the review by SEARS, 1969). This concept was based mainly on cytological and morphological investigations. In recent years electrophoretical investigations have been used increasingly to elucidate the relationships in the *Triticinae*. In particular the spectra of the seed storage proteins (JOHNSON, 1972) and of several enzymes of different species (JAASKA, 1971; MITRA and BHATIA, 1971) have been compared by electrophoresis. However, efforts so far to elucidate the relationships in the *Triticinae* by comparing the isoenzymes of twelve different enzymes have been unsuccessful (BREWER *et al.*, 1969). While working on nucleic-acid-degrading enzymes in wheat, we found three isoenzymes of phosphodiesterase (PDE) in *Triticum aestivum* ssp. *vulgare* using disc electrophoresis in polyacrylamide gels (WOLF, 1968). We presumed that the three enzymes could be traced back to the three genomes of the hexaploid wheat. In the work presented here we compared the patterns of the isoenzymes of phosphodiesterase of 18 species or subspecies of *Triticum* and *Aegilops*, hoping that by this means we might obtain information about the donors of the three genomes of the hexaploid wheat.

MATERIALS AND METHODS

Extraction

Leaves (about 1-8 g fresh weight) were ground at 4° C with sand in a mortar with 3x the amount (v/w) of 0.05 M Tris-HCl buffer (pH 7.5), containing 0.5 M KCl. The extracts were centrifuged at 20,000 g for 10 minutes and concentrated to about 1/3 of the original volume by polyethylene glycol (Aquacide, Calbiochem).

For some experiments (see legend to Figure 5) the enzyme was partially purified by slowly heating the crude extract to 60° C in a water bath. Under these conditions about 50% of the proteins were precipitated without loss of PDE activity or changes of electrophoretic mobility (LERCH and WOLF, 1972).

Electrophoresis

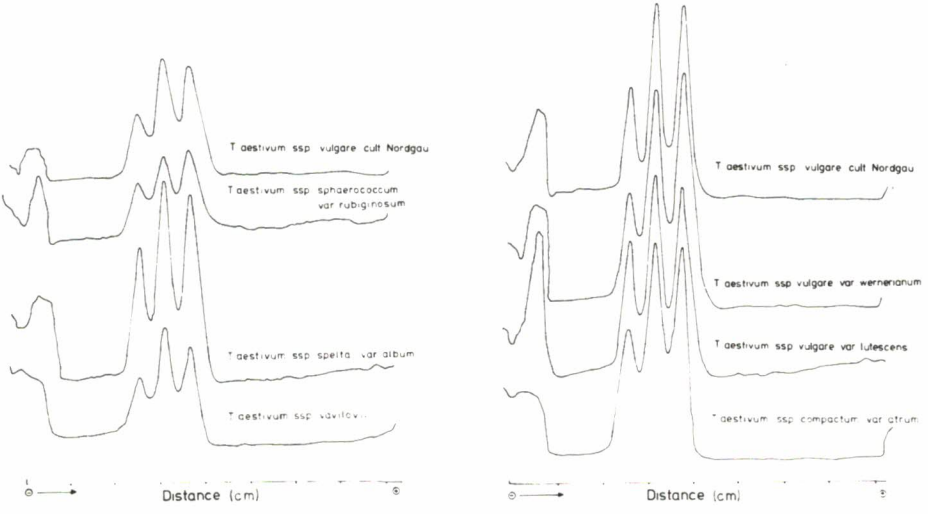
Disc electrophoresis was performed in 7% polyacrylamide gels (0.6 x 9 cm) at 4^0 C according to DAVIS (1964). Two hundred V were applied until the proteins had entered the running gel (after about 30 min) and 300 V until the bromphenolblue band had reached the bottom of the gel. Under these standard conditions the electrophoresis was completed after about 3 1/2 hours.

Detection of Enzyme

Each gel was stained for PDE (orthophosphoric diester phosphohydrolase, EC 3.1.4.1) according to LERCH (1968) by incubation in a solution of 2.5 mg 2'-deoxythymidine-5'-naphthylphosphate (Merck, Darmstadt) and 15 mg fast red RC (Serva, Heidelberg) in 25 ml 0.05 M Tris-HCl (pH 7.5) for 15 minutes to one hour at room temperature. After staining, the gels were washed in a solution of 7% acetic acid and scanned in a Joyce-Loeble densitometer at 460 nm.

RESULTS

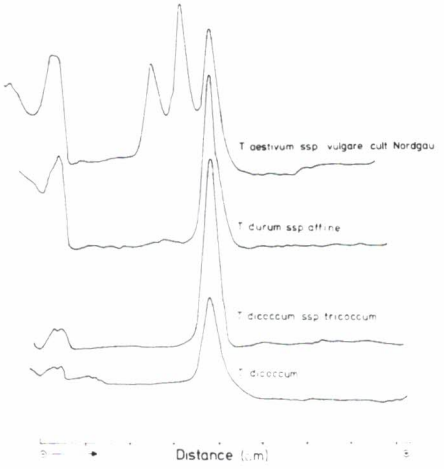
All tested hexaploid species and subspecies have 3 PDE isoenzymes with the same electrophoretic mobility (Figures 1 and 2). Each of the two faster moving bands is always about twice as active as the slowest one.

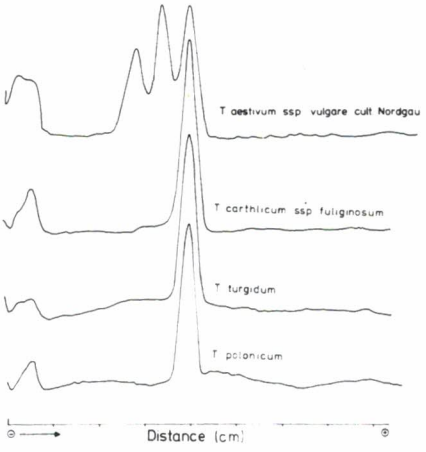


Figures 1 (left) and 2. Densitometer tracings of phosphodiesterase of different hexaploid wheats after electrophoresis in polyacrylamid gels. (The peak in the upper part of the gel is caused by nonspecific adsorption of fast red to fraction-I protein).

The tetraploids contain only one band (Figures 3 and 4), which is identical with the fastest band of the hexaploids.

The band of three diploid species and one subspecies was fast moving, and under standard conditions it was difficult to decide whether this band and that of the tetraploids moved electrophoretically as fast or at a different rate as the fastest of the hexaploids.





Figures 3 (left) and 4. Densitometer tracings of phosphodiesterase of different tetraploid wheats after electrophoresis in polyacrylamide gels. (The peak in the upper part of the gels is caused by unspecific adsorption of fast red to fraction-I protein).

Unambiguous results were obtained only by coelectrophoresis at a prolonged separation time (5 1/2 hours). Thus, by coelectrophoresis of partially purified extracts of *T. aestivum* ssp. *vulgare* var. *wernerianum* and extracts of the four diploid species or subspecies, it was shown that the diploid band was not identical with the fastest band of the hexaploids (Figure 5). Coelectrophoresis also demonstrated that the slow-moving band of *Ae. squarrosa* is identical with the slowest band of the hexapoids (Figure 5).

These results imply that the PDE isoenzymes of the four diploid species or subspecies are also not identical with the PDE of the tetraploids. Coelectrophoresis of extracts of *T. aestivum* ssp. *vulgare* var. *wernerianum* with extracts of the other hexaploid species and with those of the tetraploids

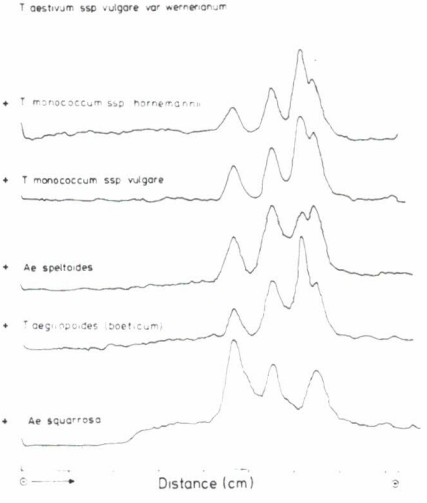


Figure 5. Densitometer tracings of phosphodiesterase after electrophoresis of protein mixtures of *T. aestivum* ssp. *vulgare* var. *wernerianum* and different diploid *Triticum* and *Aegilops* species.

also confirmed the results obtained under standard conditions; that is, all the bands of the hexaploids are identical and the fastest of these is identical with the band of the tetraploids.

DISCUSSION

The use of seed proteins and of many of the enzymes employed in genetic investigations of wheat presents several problems. The seed proteins are very numerous and only characterized by their electrophoretic mobility. Some enzymes, e.g., the phosphatases and esterases, also give many bands, and their substrate specifity is not well defined. With other enzymes, such as esterase, alcohol dehydrogenase, catalase or aminopeptidase, the picture may be complicated by polymorphism (MacDONALD and BREWBAKER, 1972).

Phosphodiesterase, however, has a well-defined substrate specifity and occurs in the *Triticinae* only in the form of at most three isozymes, which are not composed of dissociable subunits (WOLF, unpublished). Therefore, in our opinion, it is particularly suited for genome analysis.

It is striking that the pattern of the isoenzymes of phosphodiesterase is the same within the di-, tetra-, and hexaploid group. Assuming the validity of the one-gene, one-protein hypothesis and considering the PDE as a genome marker, we draw the following conclusions from a comparison of the isoenzyme pattern:

1. The identity of the slowest moving band of the hexaploids and the band of *Aegilops squarrosa* confirms *Ae. squarrosa* as the donor of the D genome.

2. The identity of the band of the tetraploids and the fastest migrating band of the hexaploids confirms that the hexaploids derive from a tetraploid species.

3. No band corresponding to the middle band of the hexaploids could be found in the di- and tetraploids. However, the synthetic hexaploid *T. durum* x *Ae. squarrosa* displayed three isoenzymes of phosphodiesterase. As the parents possess only one band each, we conclude that the middle band in the hexaploids is a hybrid enzyme (WOLF, unpublished).

4. The occurrence of only one band in the tetraploids can be explained best by the hypothesis of autotetraploidy (CAMARA, 1935). The hypothesis is supported further by the observation that the fastest band of the hexaploids (originating from the tetraploids) contains about twice the activity of the slowest one (originating from *Ae. squarrosa*).

5. *T. monococcum*, *T. aegilopoides*, and *Ae. speltoides*, which are said to possess the genomes A or B, have an identical band which is different, however, from that of *Ae. squarrosa* and does not correspond to any band of the tetra- or hexaploids. We therefore conclude that the tetraploids have originated from a diploid species as yet unknown. Studies on occurrence and properties of hybrid enzymes of PDE in Triticum are nearly completed and will be published elsewhere.

ACKNOWLEDGEMENTS

We are grateful to Mrs. U. Oulevey for technical assistance and Dr. Ch. Lehmann, Gatersleben, Dr. E. R. Sears, Columbia, Dr. E. Fuchs, Braunschweig, and Dr. G. Röbbelen, Göttingen, for supplying seeds. We thank Dr. E. R. Sears, Columbia, for helpful discussions.

LITERATURE CITED

BREWER, G., C. F. SING and E. R. SEARS 1969. Studies of isozyme patterns in nullisomic-tetrasomic combinations of hexaploid wheat. Proc. Nat. Acad. Sci. USA, 64:1224-1229.

CAMARA, A. 1935. Efeitos dos Raios-X nos chromosomas do *Triticum monococcum*. Sua análise na apreciação da filogenia do Trigo. Ann. Inst. Sup. Agron. 7:5-38.

DAVIS, J. 1964. Disc-electrophoresis II. Method and application to human serum proteins. Ann. N. Y. Acad. Sci. 121:404.

JAASKA, V. 1971. Phylogenetic differentiation of tetraploid wheats. Eesti NSV Ta Toimet., Biol. 20:202-205.

JOHNSON, B. L. 1972. Seed protein profiles and the origin of the hexaploid wheats. Am. J. Bot. 59:952-960.

LERCH, B. 1968. Phosphodiesterase I. Spezifischer Nachweis nach Disk-Electrophorese und Vorkommen von Pflanzen. Experientia 24:889.

LERCH, B., and G. WOLF 1972. Isolation of phosphodiesterase from sugar beet leaves. Biochim. Biophys. Acta 258:206-218.

MacDONALD, T., and J. BREWBAKER 1972. Isoenzyme polymorphism in flowering plants VIII. Genetic control and dimeric nature of transaminase hybrid maize isoenzymes. J. Hered. 63:11-14.

MITRA, R., and C. R. BHATIA 1971. Isoenzymes and polyploidy. I. Qualitative and quantitative isoenzymes studies in the Triticinae. Genet. Res., Camb., 18:57-69.

SEARS, E. R. 1969. Wheat cytogenetics. Ann. Rev. Genetics 3:451-468.

WOLF, G. 1968. Nachweis pflanzlicher Ribonukleasen in Polyacrylamid-Gelen nach Disk-Elektrophorese. Experimentia 24:890.