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The Genome Specific Grain Proteins and the Phylogenetic Interrelation Between *Triticum L., Elytrigia Desf., Elymus L.* and *Agropyron Gaertn*

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Summary. Immunochemical protein studies show that nongliadins of the ethanol-soluble fraction (EF) provide the best biochemical information on genome interrelations in cereals. After electrophoresis (pH 3.2) these genome specific proteins are shown to fall within the category of α-prolamines and albumins where inhibitors against α-amylases are to be found. Although these genome specific proteins of the EF exhibit identical serological properties, they may differ in electrophoretic mobility. They may very well be controlled by different chromosomes within a certain, characteristic genome. Electrophoretic analyses will thus likely reveal interrelations between individual chromosomes or even chromosome segments while the precipitation spectrum of the EF will function as a marker of the whole genomes or at least of the major part of their genetic material. This advantage of the immunological technique has been explored in a study of the phylogenetic relations between different genomes belonging to *Triticum* L., *Elytrigia* Desf., *Elymus* L. and *Agropyron* Gaertn. Serological markers for the main genomes of *Triticum* were checked against species and genomes of the other three genera.

More than 80 species belonging to these three genera were proved to be immunologically distinctive from the wheat genomes. Antigens produced against diploid *Elytrigia* species were not only checked with other species belonging to *Elytrigia* but also to *Elymus* and *Agropyron*. This inventory of interrelationships confirmed previous knowledge and also added some new information on the phylogeny of the polyploid representatives of *Elytrigia* and *Elymus*.

Key words: Genome specific proteins – Nongliadins – Phylogenetic interrelation – Polyploidy – *Triticum* – *Elytrigia* – *Elymus* – *Agropyron*

Introduction

The origin and interrelation of genomes belonging to the genera *Triticum, Elytrigia, Elymus,* and *Agropyron* have been studied by many scientists. The information thus gathered has proved very useful in attempts to improve wheat by hybrid introgression. Along with the traditional approach using cytogenetics, biochemical methods are now often being applied in genome interrelation studies. Generally, these biochemical analyses concentrate on the proteins of the grain (Johnson 1972; Jaaska 1972; Aniol 1976).

In our laboratory, immunochemical methods have been preferred. They have proved very successful in analyzing the interrelation of genomes belonging to genera such as *Triticum, Aegilops* L. and *Secale* L. (Konarev et al. 1971, 1974, 1976; Konarev and Peneva 1977). The studies focused on the nongliadin proteins of the ethanol-soluble fraction (EF) because of their high antigenic activity and genome specificity (Peneva and Migushova 1973; Konarev et al. 1976; Konarev and Peneva 1977). The analyses were generally set up in such a way that serological markers for special genomes from diploids were checked against other presumably related genomes. The precipitation spectrum for the grain proteins of such a diploid will reveal components which are highly specific as well as those having a wider distribution. Inventories of this kind will add to our understanding of phylogenetic interrelationships.

This report will especially demonstrate the usefulness in phylogenetic studies of electrophoretic and immunochemical analyses of the nongliadin EF and add to the knowledge on evolutionary differentiation within the genus *Triticum* and some of its related genera.

Material and Methods

The present investigations are almost entirely based on seed samples from the N.I. Vavilov Institute. One sample of an *Elytrigia* species and one of *Elymus* were obtained from Utah State University by the kind assistance of Dr D. Dewey. In addition, a complete set of addition lines and disomic substitution lines with wheat cv. 'Chinese Spring' and diploid *Agropyron elongatum* (Host) P.B./

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Elytrigia elongata (Host) Nevski were supplied by Dr J. Dvorak, USA. Dr Y. Cauderon (France) furnished samples of *E. elongata* (2n = 14 and 28).

The immunochemical analysis has been described earlier (cf. Gavriljuk et al. 1973). Double immunodiffusion was carried out on 1% agar-agarose gels with a 1:5 proportion of agar to agarose. Antisera were produced against proteins of the ethanol fraction from *Triticum, Elytrigia, Elymus,* and *Agropyron* species (Konarev et al. 1979 a).

Different components of the ethanol-soluble fraction of the protein were separated by electrophoretic technique. The zones corresponding to these components were cut out from the polyacrylamide gel and the proteins from these zones were eluted by a second electrophoretic process (Konarev 1978). The components thus separated were studied with regards their antigenic properties by means of the immunochemical method.

Results

Results indicate a close interrelation between genome A in *T. araraticum* Jakubz. and the *timopheevii* group, and that of *I. boeoticum* Boiss. (Konarev et al. 1974, 1976). All wheats belonging to the *araraticum-timopheevii* complex were found to carry the antigen-markers characteristic of the A^b genome of *T. boeoticum*. Immunochemical analyses of the protein components eluted from the separate zones obtained for *T. araraticum* (genome formula $A^bA^bB^{sp}B^{sp}$) showed the antigen-markers of the A^b genome to be located in the 2nd to the 5th zones of the gel (Fig. 1a).

Antigenic properties identical to those of the A^b genome could not to be found among $\alpha - \omega$ gliadins nor among the components located between the 6th and 7th zones. The sixth zone of the ethanol fraction spectrum contains albumins which act as inhibitors to α-amylases.

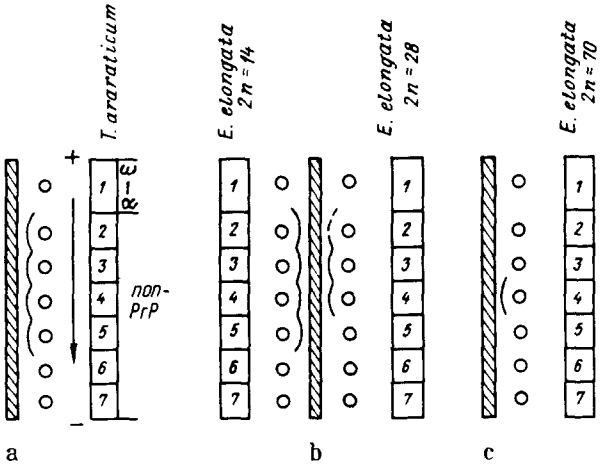

Fig. 1a-c. Immunodiffusion analysis of ethanol-soluble protein components (α-ω prolamines and nonprolamine proteins – non-PrP) of *T. araraticum* and *E. elongata*. Zones designated 1-7 were cut out of gels after electrophoresis (pH 3.2). Antisera against antigens specific for A^b-genome of *T. boeoticum* (a); against antigens specific for E-genome of *E. elongata* (b, c)

Among these proteins only one distinguishing marker was discovered: a specific albumin of bread wheat (Konarev 1978). So far no reliable genome marker has been found among the $\alpha - \omega$ prolamines. It can thus be concluded that within the four zones representing the nongliadin protein spectrum and containing proteins of different electrophoretic mobility there exists components reliably demonstrating identical antigenic properties between the A genome of *T. boeoticum* and the *araraticum-timopheevii* complex (Fig. 1a). Since there are no visible differences in the intensity of the A^b genome antigen-markers in the precipitation spectra, the degree of identity can be evaluated as being high. The interpretation is such that the A^b genome apparently has changed very little over time in the *araraticum-timopheevii* complex as well as in the diploid *boeoticum*. Morphological and cytogenetic criteria support this impression. In the emmer group, which carries the A^u genome from *T. urartu* Thum. ex Gandil. and the B^l genome from *Ae. longissima* Schw. et Mush, the immunochemical reactions indicating homology are usually weaker (Konarev et al. 1976), indicating some tendency of differentiation.

A similar trend can be observed in the *Elytrigia elongata* complex. An immunochemical identity control with reference to the diploid (2n = 14) *elongata* E genome has the same 2nd to 5th zones of the precipitation spectrum as the critical ones (Fig. 1b). The tetraploid *E. elongata* carries identical antigen-markers in the 3rd and 4th zones only, and a weak immunochemical reaction in the 2nd zone. Such a result will again indicate a differentiation process. The second genome of the 4X *elongata* is apparently another genome closely related to the genome characteristic for *Aegilops squarrosa* auct. non L. syn. *Ae. tauschii* Coss. (Konarev 1979a).

This process of differentiation has evidently developed even further in the decaploid *E. elongata* (2n = 70). Only one or two genomes of this type are related to the E genome of the diploid representative. The interrelation is here so weakened that only zone 4 of the decaploid *elongata* spectrum shows immunochemical identity with genome E (Fig. 1c). A similar increased tendency of genomic differentiation in connection with polyploidy can also be observed when studying the proteins specific of genome S of *E. stipifolia* (Czern. ex Nevski) Nevski (Vasiljeva 1979).

The nongliadin proteins' composition are controlled by several of the chromosomes of a genome (Aragoncillo et al. 1975; Mitrophanova 1979). This offers a certain degree of increased reliability in evaluating identity but it also fits with an idea of a stepwise differentiation revealed by a successive weakening of the immunochemical precipitation reaction.

The fact that the characteristic nongliadin pattern is governed by several, or even a majority, of the chromo-

somes of a genome can amply be demonstrated by examining the individual chromosomes and adding them one by one to an alien genetic background. Such addition lines with wheat cv. 'Chinese Spring' as receptor and the genome E of *Elytrigia elongata* as donor show the immunochemical identity reaction with the E antiserum for five of the seven chromosome pairs. Chromosome 1E and 4E caused a stronger reaction, while 5E, 6E, and 7E had a weaker identity reaction (Konarev 1979a and b). The precipitation did, however, in no case reach the strength found, for example, in zone 4, in connection with the analyses of the 10X *elongata* type. This circumstance is not unexpected since the strength of a precipitation reaction is dependent on the number of protein components involved. This number is likely to be lower only when one chromosome, rather than the whole genome, is represented. Conversely, if only one chromosome pair is lacking, the weakening effect should be less conspicuous. In fact, when checking a complete series of wheat nulli-tetrasomics, each with absence of an individual pair of genome D chromosome, against the genome D antiserum, the precipitation never showed any noticeable difference in strength.

The dependence of the nongliadin EF protein spectrum on several chromosomes of a genome implies not only a broader check on biochemical interrelations, it also implies a possible technique of identifying individual chromosomes by the use of electrophoretic and/or immunochemical markers (Konarev and Peneva 1977; Konarev 1979b).

As to individual components of such a marker system it is to be expected that an intraspecific variation can occur, an expectation which has been verified (Konarev et al. 1976; Konarev 1979a). Species characterized by a weak precipitation reaction in a specific zone for a specific antiserum will also tend more often to include types entirely missing precipitation capacity in that situation. Such a trend towards a differentiation process has been found in emmer and bread wheats checked against the genome B^l antiserum, in polyploid *Elytrigia elongata* (2n = 56, 70) compared against genome E antiserum, in *E. repens* L. (Nevski) against genome S antiserum, and so on.

On the other hand, a more conservative evolution can be anticipated whenever no such intraspecific differentiation as to strength of the immunochemical precipitation can be found. Such a situation was observed for the A^b and B^{sp} antisera checked against representatives of the *araraticum-timopheevii* group, for the genome D antiserum checked against all forms and biotypes of the hexaploid *aestivum* wheat, and so on.

The number of zones reacting with precipitation and the strength of that reaction can evidently be used as guidelines as to degree of genome interrelations. It should, however, be observed that absence of identity reactions should not necessarily be taken as proof of separate origin. This must be borne in mind when absence of antigen reactions against genome E or S is found among samples of polyploid *E. elongata* and *E. repens*. A similar situation can also be observed within the emmer wheat group indicating a certain limitation to the biochemical method here applied. This limitation makes it important to check as many representatives as possible of a certain taxon and to use as many different protein markers as possible (Konarev et al. 1976).

From this viewpoint it might be of value to observe that some albumins which are inhibitors of α-amylases, also show different mobility characteristics in electrophoresis as well as differences in immunochemical properties (Konarev 1978). They were found to be governed by chromosomes belonging to the genomes B and D in the bread wheat (Pace et al. 1978).

Discussion

The results reported above are given as experimental confirmations on the applicability of the immunochemical method in the study of plant interrelations. Analyses of experimental material at our laboratory indicate that the serological approach can be as informative and reliable as a cytogenetical one. It is of special value that they furnish different types of information, thus complementing each other in a very interesting way.

During the period of 1968-1979, antisera against a large number of proteins from different plants have been produced at out institute: E.g., antisera have been obtained against proteins from all more important representatives of *Triticum* and *Aegilops*. They have made it possible to thoroughly study the serological characteristics of wheat genomes and to arrive at convincing ideas about interrelationships and origin (Konarev et al. 1976; Konarev et al. 1979b). Together with antisera produced against proteins from species belonging to the related genera *Elytrigia, Elymus,* and *Agropyron*, about eighty species altogether have been examined (Konarev 1977, 1979a, b; Konarev et al. 1979a; Konarev and Vasiljeva 1979).

In studying about thirty different species of wheat grass it can be concluded that the octo- and decaploid representatives of *Elytrigia elongata* and the hexaploid species *E. intermedia* (Host) Nevski, *E. trichophora* (Link) Nevski and *E. juncea* (L.) Nevski originated by intergeneric genomic introgression (Konarev et al. 1979a). The serological spectra of their grain protein indicate some relationship to bread wheat. It is especially genome B^l and D of the hexaploid wheat that carries serological characteristics in common with the above-mentioned polyploid *Elytrigia* representatives. The primary, pivotal

genome A is more specific for the wheat group, but there are more similarities with *Elytrigia* in the genome A^b of *T. boeoticum* than in the genome A^u of *T. urartu*. It is the latter that is built in the emmer and dinkel wheats (Konarev et al. 1974, 1979b).

The distribution of serological characteristics for grain proteins of genome S of *Elytrigia stipifolia* and genome E of 2× *E. elongata* has been examined among species belonging to the *Elytrigia-Elymus-Agropyron* complex (Konarev et al. 1979a; Konarev and Vasiljeva 1979). The conclusions drawn are very much the same as those given out from cytogenetic studies reported by Dewey (1974, 1977a and b); the two approaches thus support each other.

By our immunochemical comparisons it was concluded that genome D of bread wheat is related to one of the genomes of the tetraploid species *Agropyron subsecundum* Link, *A. rechingeri*, *A. pringlei* Hitch., *A. striatum* and *E. elongata*. The same conclusion was also reached for some *Elymus* species which were earlier grouped into *Roegneria* C. Koch (Konarev and Vasiljeva 1979). As already reported above, the second genome of the 4× *Elytrigia elongata* is related to the genome E of the diploid representative of the species. Serological interrelations with this genome E were also found with grain proteins from *E. smithii* Rydb. and *A. littorale* (Host) Dum. and that from the tetraploid *Elymus* group consisting of *E. caninus* (L.) L., *E. canadensis* L., etc. (Konarev and Vasiljeva 1979). It appears quite possible that the large group of tetraploid species of the *Elytrigia-Elymus* complex is a hybrid swarm based on the genomes interrelated with genome E and S. The genome E of the 4× *Elytrigia elongata* appears to be that genome among the tetraploids which is most related to the E genome on the diploid level. The corresponding genome in *E. smithii*, *A. littorale*, and *A. pringlei* appears more differentiated.

From the serological comparative studies here reported it comes out that there must exist some sort of evolutionary, rather close interrelation between the *Triticum-Aegilops* cluster on the one side and the *Elytrigia-Agropyron* complex on the other. The introgression appears to be much lower from the latter to the former than vice versa, which may reflect their difference in reproduction system. In the last-mentioned group it appears to be fairly common to find serological markers interconnected with more basic genomes than could be expected from their level of ploidy. Thus, the two diploid species *Elytrigia stipifolia* and *E. libanoticum* Hack. ex Kneuck carry the main immunochemical characteristics of the S genome but have also antigen markers in common with genome B^l from *Aegilops longissima*. Antigen markers characteristic of the genome D, E, and S can be found among the tetraploid *E. caninus*, *E. canadensis*, and *E. glaucus* Regel. Such an intermixing may just reflect the common origin of the three basic genomes but it may also indicate a more vivid process of hybrid introgression going on in that group. Such processes must have been essential in the evolution of the cereals (Tzvelev 1976; Dewey 1977a).

It should be noted that the American species *A. ferganense* Drob. and *A. inerme* Rydb. do not have any other genome antigen markers than those connected with genome S. They have been isolated from the genomes A^b, B^l, and D which were geographically restricted to the Old World. This observation favours the idea of a hybrid introgression explaining the speciation processes in the latter region.

As indicated above, introgressed antigen markers will generally exhibit a weaker precipitation reaction than those interconnected with original genome(s). This may be due to genetic background effects but is more likely due to the circumstance that there are more cooperating nongliadin protein components involved in the precipitation the more a certain genome is represented (Konarev 1979b). Thus, the antigen reaction of *Elytrigia stipifolia* towards antiserum from genome D is weaker than that of *T. aestivum*, but it is stronger than any other species when checked against the genome S antiserum (Vasiljeva 1979).

The results reported testify to the great value of utilizing serological genome markers in the analyses of species interrelation in cereals. Studies of the antigen composition of ethanol-soluble proteins together with the data on immunochemical precipitation reactions of other proteins allows us to gain important information and reveal the complex nature of the speciation process in such plants as the cereals.

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