BIOCHEMICAL EVIDENCE OF CHROMOSOME HOMOEOLOGY AMONG RELATED PLANT GENERA

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SUMMARY

Biochemical markers associated with homoeologous chromosome groups 3 and 7 of *Triticum aestivum* L. have been investigated in genetic stocks carrying chromosomes or chromosomal segments of the same homoeology groups from *Agropyron elongatum* and *Secale cereale.*

Chromosomes 3Ag of Agropyron and 3R of Secale control proteins a_3 and b_3 with the same properties as proteins 5, 6 and 7 associated with 3B and 3D of Triticum. It is concluded that genes for proteins 5, a_3 and b_3 are located in segments proximal to the centromere in the β arms of chromosomes 3D and 3Ag, respectively. Proteins 3, 4 and 11, controlled by 7D-short arm of Triticum, are replaced by proteins a_7, b_7 and c_7, when that chromosome is replaced by 7Ag. Genes for these proteins are located proximal to the centromere in the short arms of chromosomes 7D and 7Ag. Finally, a gene that controls sterol esterification is similarly located in the short arms of chromosomes 7D and 7Ag.

INTRODUCTION

Chromosomes from related taxa are considered homoeologous if they have evolved from a common ancestral chromosome and remained genetically similar, although losing the ability to pair in meiosis under normal conditions.

Chromosome homoeology has often been assessed by estimation of genetic compensation by one chromosome of loss of phenotypic traits, such as vigor and fertility, caused by the absence of its homoeologue. In other instances, the approach has been to test meiotic pairing ability between homoeologues under certain experimental conditions.

An investigation of biochemical systems associated with the different chromosomes affords a more direct proof of homoeology down to the level of chromosome segments.

Bread wheat, *Triticum aestivum* L. (2n = 42), although a functional diploid, is really an allohexaploid composed of three duplicated homoeologous genomes (AA, BB, DD), each consisting of seven different chromosomes [1]. The distribution of genes controlling isozymes and other proteins, as well as metabolic products, have amply confirmed the homoeologies established by cytogenetical methods [2].

Less biochemical data are available concerning homoeology between chromosomes of different genera. Barber et al. [3] tentatively concluded that chromosome G of rye (*Secale cereale*) was homoeologous of 3A of Triticum on the basis of the esterase isozyme patterns. More recently, Shepherd [4] has shown that genes controlling prolamines, which are located in homoeologous chromosome groups 1 and 6 of Triticum, are similarly located in their homoeologues of *Aegilops umbellulata*, chromosomes B and A respectively, and in chromosome E of *Secale cereale*, which is homoeologous with group 1.

We present here biochemical evidence of intergeneric homoeology between homoeologous group 3 of Triticum and chromosomes 3Ag of *Agropyron elongatum* and 3R of *Secale cereale* and between group 7 of Triticum and chromosome 7Ag of *Ag. elongatum.*

MATERIAL AND METHODS

Biological material

Ditelosomics and compensated nulli-tetrasomics of *Triticum aestivum* L. cv. Chinese Spring, the 3D/3Ag wheat/Agropyron substitution line and homoeologous transfer lines carrying segments of chromosomes 3Ag or 7Ag were provided by E.R. Sears. The latter were obtained taking advantage of homoeologous meiotic pairing in the absence of a diploidizing gene, located in chromosome 5B of Triticum, and carry genes for resistance to the leaf rust *Puccinia recondita* [5,6]. Agrus wheat, which is a 7D/7Ag substitution, and the equivalent Chinese Spring substitution were the gift of C.J. Driscoll [7]. The Kharkov wheat/Dakold rye 3D/3R substitution was obtained by Jenkins [8].

Methods

Single kernels were crushed between two metal plates, the lipid extracted and the sterol ester patterns analysed as previously described [9,10]. The delipidated material was extracted with 70 % ethanol and the non-gliadin fraction of the extract was analysed by combined electrofocusing (pH range 5—8) and electrophoresis (pH 3.2) as previously described [11]. Sequential extractions of protein controlled by the alien chromosomes were performed with 70 % ethanol, chloroform—methanol (2:1) and water as previously described [11].

RESULTS

A composite two-dimensional map of non-gliadin endosperm proteins from 70 % ethanol extracts of *Triticum aestivum* L. and chromosome substitutions 3D/3Ag, 3D/3R and 7D/7Ag is presented in Fig. 1. Map positions of components controlled by the alien chromosomes have been determined by joint mapping of each substitution line with the euploid wheat. Components of this group of wheat proteins have been previously characterized in part and assigned to specific chromosomes [11—16 and our unpublished results].

Protein(s) 5 is controlled by the β arm of chromosome 3D and proteins 6 and 7 by the S arm of chromosome 3B [15]. These proteins can be readily distinguished from other map components because they are extracted equally well with 70 % ethanol and with water, while they are practically not extracted by chloroform—methanol (2:1) [11]. Two minor components, also controlled by 3B-L, are of lower molecular weight (unpublished results). Both, chromosome 3Ag from Agropyron and chromosome 3R from Secale, control two components, designated a_3 and b_3 (Fig. 1, Table I), with lower isoelectric points and electrophoretic mobilities, but otherwise identical properties, than components 5, 6 and 7. That the two 3Ag proteins map at the same positions as those controlled by 3R has been ascertained by joint mapping of both substitution lines.

The distribution of a_3 and b_3 in substitution and homoeologous transfer lines is summarized in Table I. Fourteen 3D/Ag transfers (Nos. 1—6, 8, 11,

TABLE I

DISTRIBUTION OF BIOCHEMICAL MARKERS IN SUBSTITUTION AND TRANSFER LINES INVOLVING HOMOEOLOGOUS CHROMOSOME GROUP 3

Markers are shown in Fig. 1 and described in the text. Transfer 3D/Ag-12 has also a segment from 3B [17].

Line	Triticum			Agropyron and Secale	
	5	6	7	a_3	b_3
Euploid	+	+	+	—	—
Nulli 3B tetra 3A	+	—	—	—	—
Ditelo 3B-L	+	—	—	—	—
Nulli 3D tetra 3A	—	+	+	—	—
Ditelo 3D-α	—	+	+	—	—
Substitution 3D/3R	—	+	+	+	+
Substitution 3D/3Ag	—	+	+	+	+
Transfer 3D/Ag-12	—	+	+	+	+
Transfers 3D/Ag-1—6, 8, 11, 14—16, 18, 19, 21	+	+	+	—	—
Transfers 3B/Ag-10, 13	+	+	+	—	—

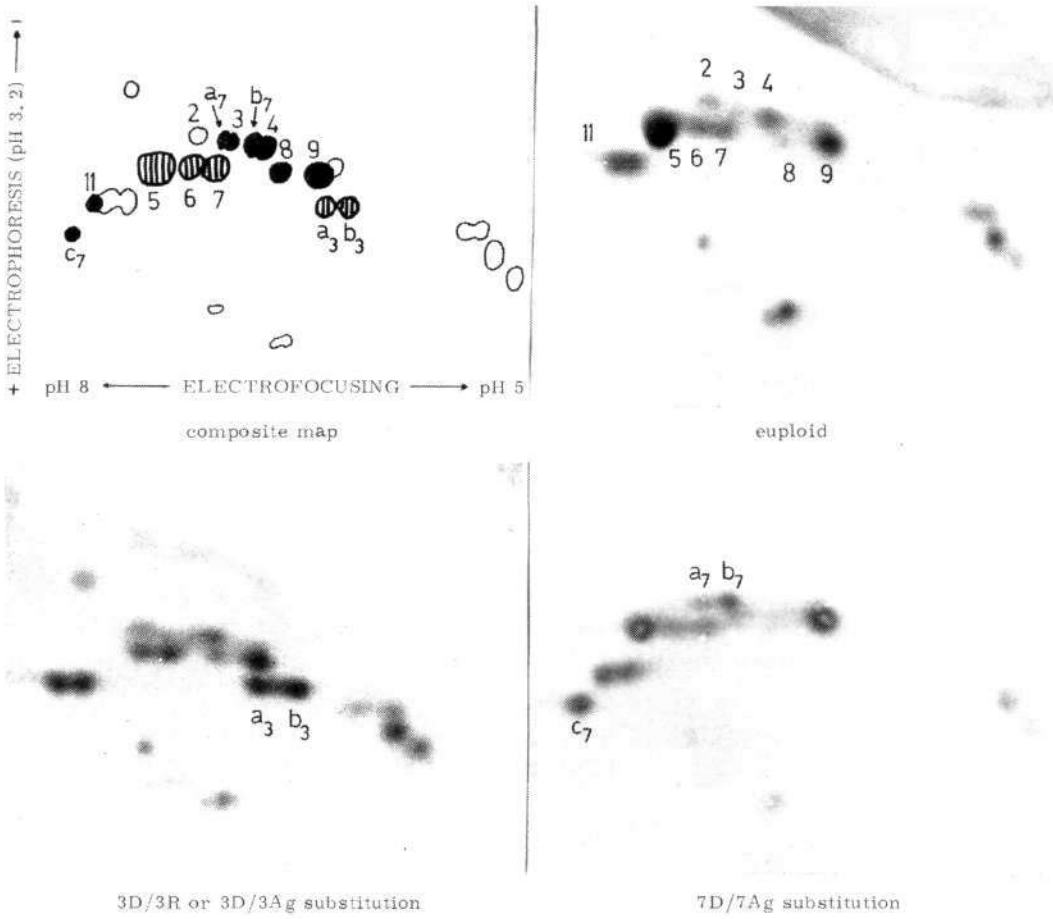

Fig. 1. Non-gliadin proteins of 70 % ethanol extracts of wheat kernels fractionated by combined electrofocusing (pH range 5—8) and electrophoresis (pH 3.2). A composite map of all proteins studied is shown together with stained gels of euploid Chinese Spring wheat, 3D/3R or 3D/3Ag substitution, and 7D/7Ag substitution.

14—16, 18, 19, 21), in which substantial segments of chromosome arm 3Dα have been replaced by homoeologous 3Ag segments (down to 0 % meiotic pairing with ditelo 3Dα [6]), do not show replacement of component 5 by a_3 and b_3. In transfer 3D/Ag-12, in which a 3Ag segment that presumably includes part of the β arm has replaced an homoeologous segment of 3D [17], the substitution of component 5 by a_3 and b_3 has taken place. In transfers 3B/Ag (Nos. 10, 13), components 6 and 7 are present while a_3 and b_3 are not.

Proteins 3, 4 and 11 are controlled by the short arm of chromosome 7D, and genes for proteins 8 and 9 are located in the short arm of 7B [12—15]. The most distinctive property of these proteins is their solubility in chloroform—methanol (2:1). Two components which are also soluble in this solvent mixture, are controlled by homoeologous group 4 and differ in molecular weight and

TABLE II

DISTRIBUTION OF BIOCHEMICAL MARKERS IN SUBSTITUTION AND TRANSFER LINES INVOLVING HOMOEOLOGOUS CHROMOSOME GROUP 7

Markers are shown in Fig. 1 and described in the text

Line	Triticum					Agropyron			Gene Pln
	3	4	8	9	11	a_7	b_7	c_7	
Euploid	+	+	+	+	+	—	—	—	+
Nulli 7B tetra 7A	+	+	—	—	+	—	—	—	+
Ditelo 7B-L	+	+	—	—	+	—	—	—	+
Nulli 7D tetra 7A	—	—	+	+	—	—	—	—	—
Ditelo 7D-S	+	+	+	+	+	—	—	—	+
Substitution 7D/7Ag	—	—	+	+	—	+	+	+	+
Agrus (subst. 7D/7Ag)	—	—	+	+	—	+	+	+	+
Transfers 7D/Ag-6, 11	—	—	+	+	—	+	+	+	+
Transfers 7D/Ag-1—5, 7—10	+	+	+	+	+	—	—	—	+

chloroform solubility [12,13, and unpublished results]. Proteins 8 and 9 are the counterparts of 3 and 4 [12,13]. In the 7D/7Ag substitutions, proteins 3, 4 and 11 are replaced by a_7, b_7 and c_7 which map very close to them (Fig. 1, Table II) and have the same solubility properties. These substitutions do not show component 2, controlled by gene(s) located in chromosome 6B [15].

A gene that controls sterol esterification in endosperm is also located in the short arm of 7D [9,10]. *Agropyron elongatum* shows the same sterol ester pattern as *Triticum aestivum* L. cv. Chinese Spring, which is characterized by having palmitate as the main ester and corresponds to the allele designated *Pln* in the latter. Chromosome 7Ag restores the esterification activity lost in stocks lacking 7D.

In two of the 11 7D/Ag transfers, proteins 3, 4 and 11 have been replaced by a_7, b_7 and c_7. All the transfers show the sterol esterification pattern characteristic of Chinese Spring wheat and of the 7D/7Ag substitution line (Table II).

DISCUSSION

Previous homoeology assignments for the chromosomes implicated in our study, which were based on cytogenetic evidence [6,7], are consistent with the present finding that the same molecular systems are associated with chromosomes of the same intergeneric homoeologous group. Furthermore, the data on wheat/Agropyron transfers indicate that genes for the investigated biochemical markers are in homoeologous chromosomal segments.

Map components a_3 and b_3 from chromosomes 3Ag or 3R, and components 6 and 7 from 3B, seem to have only one counterpart in chromosome 3D (component 5). This can be interpreted either as loss of genetic information

for a second component or as coincidence in map position of the two 3D components. That the intensity of spot 5 is clearly greater than that of 6, 7, a_3 and b_3, is consistent with the second hypothesis but does not exclude the first one, because the greater intensity could be the result of a gene dosage compensation effect. Our unpublished results suggest that such type of phenomena affect the synthesis of these proteins (e.g., net synthesis of 6 and 7 is a direct function of 3B dosage and an inverse one of 3D dosage).

The expression of component 2, which structural gene is located in chromosome 6B, is affected by chromosomes of group 7 in some complex way; thus it is absent in nulli 7D tetra 7B but present in nulli 7D tetra 7A [15]. Chromosome 7Ag either suppresses or fails to mediate the synthesis of component 2. Otherwise, 7Ag controls 3 components which are exchanged with their 7D counterparts and is also able to replace 7D in mediating sterol ester synthesis in the final stages of endosperm development.

Genes for resistance to the leaf rust *Puccinia recondita* are located in the α arm of 3Ag and the short arm of 7Ag [6,17]. All transfers with 3Ag or 7Ag segments selected by Sears carry genes for resistance.

Component 5 is controlled by 3Dβ. All but one of the rust resistance transfers have component 5 and do not show a_3 and b_3. This can be explained in terms of the association of genes for a_3 and b_3 with 3Agβ. Transfer 3D/Ag-12 is rust resistant and has exchanged component 5 for a_3 and b_3, which would place genes for 5, a_3 and b_3 in segments proximal to the centromere of 3Dβ and 3Agβ, respectively.

In chromosome 7D, rust resistance and the biochemical markers are also controlled by different chromosome arms [6,10,15,17]. Segments from the long arm of 7Ag, which are presumably terminal from the meiotic pairing data [17], do not carry genes for a_7, b_7 and c_7, nor replace the segment that controls components 3, 4 and 11, while in two other transfers the exchange of protein markers has occurred. Since one of the latter has the terminal part of chromosome 7D [17], the genes for these proteins must be located in the proximal parts of the short arms of chromosome 7D and 7Ag respectively. The fact that none of the transfers affecting the short arm of chromosome 7D has been affected in the sterol ester pattern is consistent with an equivalent location of the gene involved in 7Ag and in 7D.

As pointed out by Sears [17], the information gained in the present study should be useful in connection with the practical goal of obtaining rust-resistant transfer lines with small internal segments of Agropyron chromosomes. It is also worth mentioning that the group of proteins implicated in this investigation, because of their low intragenomic variability, have been extremely useful in the confirmation of genome origins and the establishment of phylogenetic relationships [18,19].

Finally, these and the previously cited results are consistent with the idea that the lack of meiotic pairing between homoeologues is under the positive control of genes like the one in chromosome 5B [20], rather than being the result of extensive chromosome structural changes.

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