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Chromosomal Control of Non-gliadin Proteins from the 70% Ethanol Extract of Wheat Endosperm

C. ARAGONCILLO, M. A. RODRIGUEZ LOPERENA, PILAR CARBONERO and F. GARCIA-OLMEDO

Department of Biochemistry, Universidad Politécnica de Madrid, E.T.S. Ingenieros Agronomos, Madrid (Spain)

Summary. The non-gliadin fraction of the 70% ethanol extracts of compensated nulli-tetrasomics and ditelosomics of *Triticum aestivum* cv. Chinese Spring has been analyzed by combined electrofocusing and electrophoresis. Seventeen of the 21 protein map components of the euploid have been ascribed to eight chromosomes: 4Aβ, 3BS, 6BS, 7BS, 3Dβ, 4D, 5D and 7DS.

The relationship of the different map components with other proteins previously associated with the same chromosomes is discussed.

Introduction

The central role played by wheat kernel protein in the nutrition of mankind justifies the vast amount of research carried out on many aspects of its production and utilization.

Although the study of the genetic control of this protein has long been of great interest to plant breeders, significant advances in this field have taken place only recently. These have been made possible by the development of methods of ever increasing resolution for the analytical fractionation of proteins.

In particular, research on the chromosomal location of genes coding for kernel proteins has received great impetus with the production of different kinds of aneuploids, especially the ditelosomics and the compensated nulli-tetrasomics of *Triticum aestivum* cv. Chinese Spring developed by Sears (1954, 1966).

Some of the studies have dealt with chromosomal effects on total kernel protein (Eastin *et al.*, 1967; Riley and Ewart, 1970; Jha *et al.*, 1971; Siddiqui, 1972; Morris *et al.*, 1973) but most have focused on specific protein fractions.

The gliadins are the group that has been more extensively investigated, both because of their possible technological significance, as a major fraction of gluten, and because of their impact on the nutritional value of wheat, as the fraction with the lowest lysine content. In earlier work (Boyd and Lee, 1967; Solari and Favret, 1967 and 1970; Shepherd, 1968; Boyd *et al.*, 1969), chromosome assignments were based on the one-dimensional electrophoretic patterns of gliadins, but in some cases the products of genes from different chromosomes were superimposed on the electrophoretic pattern and the genes could not be unequivocally located. This problem was recently resolved by Wrigley and Shepherd (1973) using combined electrofocusing and electrophoresis. Thus, it was confirmed that genes coding for gliadins are located in homoeologous chromosome groups 1 and 6.

Glutenin, probably the fraction of greater technological importance, has been less investigated because of analytical problems. The first chromosome-glutenin component associations have been reported only recently by Orth and Bushuk (1974).

Chromosomal location of components of other protein fractions has also been investigated. Components of chloroform-methanol extract have been associated with chromosomes 7D and 7B (García-Olmedo and Carbonero, 1970). Bozzini *et al.* (1971) assigned two albumins to chromosomes 3D and 4D respectively and Noda and Tsunewaki (1972) have located genes for buffer soluble proteins in homoeologous chromosome group 3 by electrofocusing. We have recently reported the location of purothionin genes in group 1 (García-Olmedo *et al.* 1974).

Proteins extracted with 70% ethanol from wheat endosperm are mostly gliadins, which are in the 40,000—80,000 molecular weight (MW) range, but also include some glutenins, which are of higher molecular weight, and a significant amount of proteins under 40,000 MW, which migrate ahead of gliadins in electrophoresis at pH 3.2 and have been often considered as contaminant albumins and globulins. The importance of the latter fraction is twofold: they are of great phylogenetic and taxonomic significance, as shown by the work of Johnson and coworkers (Johnson and Hall, 1965, Hall *et al.*, 1966; Waines, 1969; Johnson, 1972), and they are richer in lysine than gluten. An attempt has been made by Waines (1973) to locate genes for components of this fraction by one-dimensional electrophoresis. Although some bands disappeared when particular chromosomes were absent, others could not be properly assigned because of lack of resolution. We

have recently mapped this fraction by combined electrofocusing and electrophoresis and have shown that it is constituted of components extractable with chloroform-methanol but not with water and components extractable with water but not with chloroform-methanol (Rodriguez-Loperena *et al.*, submitted). We report here the unequivocal chromosomal location of genes for the majority of the map components.

Materials and Methods

The compensated nulli-tetrasomics and the ditelosomics of *Triticum aestivum* cv. Chinese Spring used in this study, which are mentioned in Results, were kindly given by E. R. Sears (Columbia, Missouri, USA), T. Mello-Sampayo (Oeiras, Portugal) and J. R. Lacadena (Madrid, Spain).

Kernels and half-kernels were extracted with 70% ethanol (10 + 10 + 10 v/w), the combined extracts evaporated in vacuo, redissolved in 9M urea and incorporated into the electrofocusing polymerization mixture.

Protein maps of the extracts were obtained by combined electrofocusing and electrophoresis following a method based on that of Wrigley (1970), which has been described elsewhere (Rodriguez-Loperena *et al.*, submitted). Electrofocusing (pH range 5–8) was carried out in 2×140 mm polyacrylamide gel columns in all cases and electrophoresis (pH 3.2) in the second dimension was performed in 1 mm thin starch gels for wheat samples weighing less than 25 mg and in 2 mm starch gels for bigger samples, usually 35–50 mg to detect minor components.

Results

The complete non-gliadin protein map of the 70% ethanol extract of *Triticum aestivum* cv. Chinese Spring kernels is shown in Fig. 1. Also included are partial maps of one ditelosomic and 7 nulli-tetrasomic lines with different spots missing, which account for 17 out of the 21 components of the euploid map.

Table 1 summarizes the results obtained with all the available nulli-tetrasomics and ditelosomics of homoeologous groups 3, 4, 6 and 7.

Component 1 was absent in nulli 5D tetra 5A and nulli 5D tetra 5B, enhanced in nulli 5B tetra 5D, and normal in nulli 5A tetra 5D, nulli 5B tetra 5A and nulli 5A tetra 5B. Component 21 was enhanced in nulli 1B tetra 1A and normal in all other nullitetrasomics of group 1. The available nullitetrasomics of group 2 (nulli 2B tetra 2A, nulli 2B tetra 2D, nulli 2D tetra 2A and nulli 2D tetra 2B) showed the euploid phenotype.

It should be mentioned that component 9 can interfere with the observation of component 10 in some gels. For this reason, the results for homoeologous group 6 were confirmed by mapping water extracts, which we have previously shown to include component 10 but not component 9.

Although components 12 and 13 have been listed as absent in ditelo 4A α (Table 1), faint spots can be observed in their position which probably correspond to components contributed by the D genome, because *Ae. squarrosa* maps do show such spots (Rodriguez-Loperena *et al.*, submitted).

Table 1. *Deviations with respect to euploid Chinese Spring of protein maps of nulli-tetrasomics and ditelosomics of homoeologous chromosome groups* 3, 4, 6 and 7

Samples*	Components number**				
	5	6	7	14	15
n3A t3B	+	++	++	++	++
n3A t3D	++	+	+	+	+
n3B t3A	+	−	−	−	−
n3B t3D	++	−	−	−	−
n3D t3A	−	+	+	+	+
n3D t3B	−	++	++	++	++
dt 3B-L	+	−	−	−	−
dt 3D-α	−	+	+	+	+

Samples	Components number			
	12	13	16	17
n4B t4A	++	++	++	+
n4B t4D	+	+	+	++
n4D t4A	++	++	++	−
n4D t4B	+	+	+	−
dt 4A-α	−	−	−	+

Samples	Components number	
	2	10
n6A t6B	++	++
n6A t6D	+	+
n6B t6A	−	−
n6B t6D	−	−
n6D t6A	+	+
n6D t6B	++	++
dt 6B-S	+	+

Samples	Components number					
	2	3	4	8	9	11
n7A t7B	+	+	+	++	++	+
n7A t7D	+	++	++	+	+	+
n7B t7A	+	+	+	−	−	+
n7B t7D	+	++	++	−	−	++
n7D t7A	+	−	−	+	+	−
n7D t7B	−	−	−	++	++	−
dt 7A-L	+	+	+	+	+	+
dt 7A-S	+	+	+	+	+	+
dt 7B-L	+	+	+	−	−	+
dt 7B-S	+	+	+	+	+	+
dt 7D-S	+	+	+	+	+	+

* n-nulli; t-tetra; dt-ditelosomic

** + as in the euploid; − absent; ++ enhancement; components not listed, as in the euploid

Discussion

It can be concluded from these results that the gene (s) for component 5 are located in chromosome arm 3Dβ and those for components 6, 7, 14 and 15 in 3BS. Working with the same extract, Waines

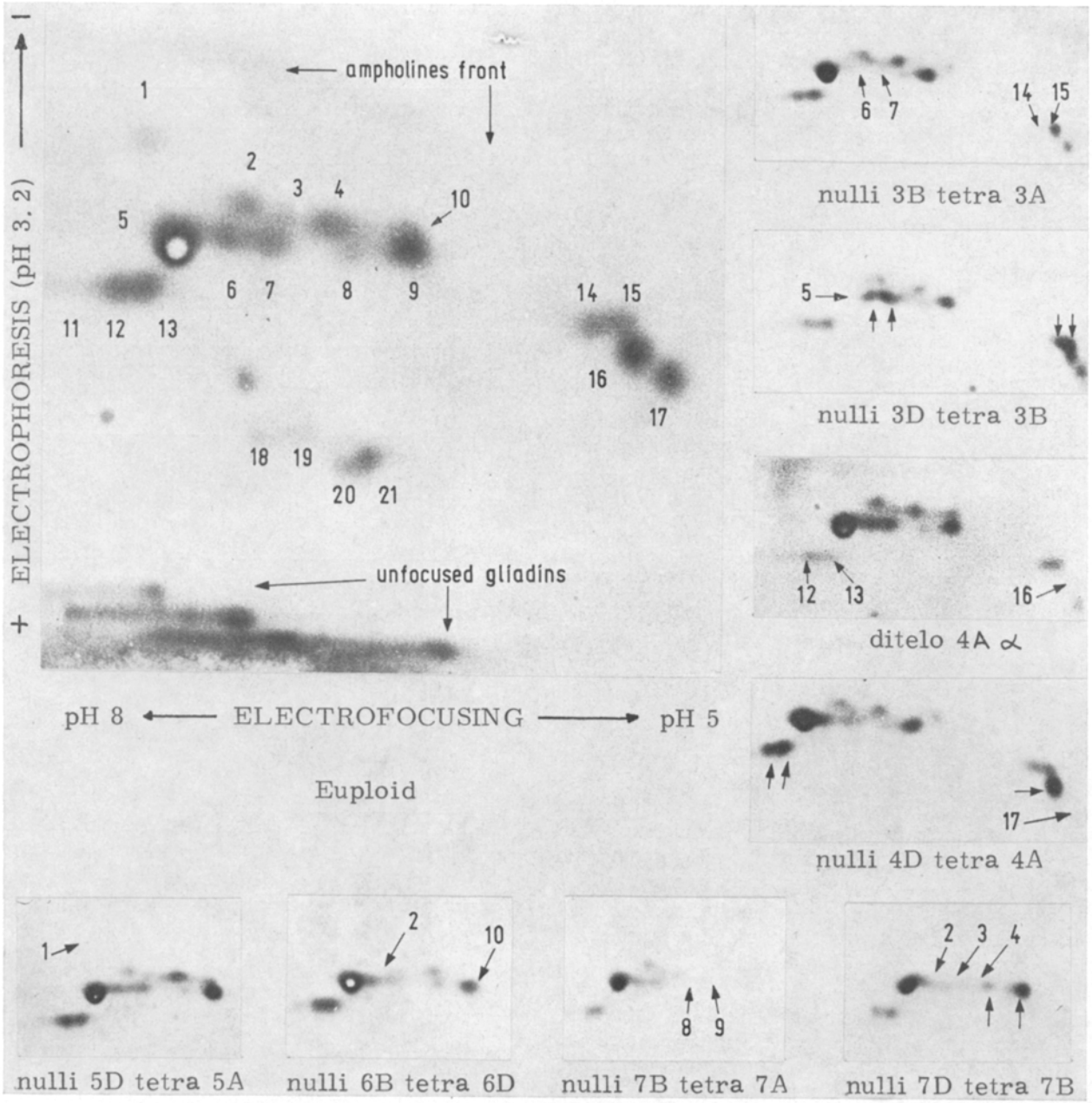

Fig. 1. Protein maps obtained by combined electrofocusing and electrophoresis of non-gliadin proteins of the 70% ethanol extracts of the euploid, a ditelosomic and 7 nullitetrasomics of *Triticum aestivum* cv. Chinese Spring. Only the section of the map affected in each aneuploid is shown. Missing spots are indicated by their map number and an arrow. Unnumbered arrows indicate enhancement of the spots pointed to

(1973) found that chromosome 3D modified the intensity of a complex band which presumably included two proteins found in *Aegilops squarrosa*. Equally tentative was his assignment of a third *Ae. squarrosa* component probably present in the D genome of Chinese Spring. He also positively located gene(s) for one electrophoretic band in chromosome 3B. We have demonstrated that components 5, 6, 7, 14 and 15 are also extracted by water (Rodriguez-Loperena *et al.*, submitted). Noda and Tsunewaki (1972) assigned two components of the buffer soluble proteins to chromosome arms 3Dβ and 3BS respectively, using electrofocusing, and Bozzini *et al.* (1971) have located gene (s) for an albumin, designated PCS, in chromosome 3D by immunochemical methods. Taking into account isoelectric point, electrophoretic mobility and solubility, it seems likely that component 5 is identical with that assigned by Noda and

Tsunewaki (1972) to the same chromosome arm and also with one of the faster moving *Ae. squarrosa* components considered by Waines (1973). However, it seems different from PCS albumin because the isoelectric point of the latter (pH 9.1) is outside the pH range (5–8) of the ampholines used in our maps and would not be detected on extraction with 70% ethanol. Components 6 and 7 of our map probably correspond to band 4 of Noda and Tsunewaki (1972) and components 14 and 15 to band 3.

Data for homoeologous group 4 are consistent with the location of genes for components 12, 13 and 16 in chromosome arm 4Aβ and for component 17 in chromosome 4D. Components 12 and 13 are included in band CM3 of the chloroform-methanol extract, which was assigned to chromosome 4A by Aragoncillo (1973), and in all probability, in the band at 83 mm of Waines (1973), which he found to be enhanced in the tetra 4A's. Similarly, component 16 would be part of the band at 69 mm and component 17 of the one at 65 mm. We have not seen any effect of chromosome 4B. Since component 17 is hardly extracted with water and has a different isoleectric point from the Mb 0,19 albumin of Bozzini *et al.* (1971), which was assigned to 4D, their being identical is excluded.

Component 1 can be assigned to chromosome 5D, although it is not enhanced in nulli 5A tetra 5D, and component 10 to 6B. We are not aware of any report about proteins of their electrophoretic mobilities controlled by these chromosomes.

An especial case is that of component 2, which is not synthesized by nulli 6B tetra 6A, nulli 6B tetra 6D and nulli 7D tetra 7B, but is present in nulli 7D tetra 7A and in nulli 7A tetra 7B. Obviously the structural gene(s) for component 2 have to be located in chromosome 6B. The absence of this component in nulli 7D tetra 7B has to be ascribed to the inhibitory effect of four doses of chromosome 7B, which is not effective in the presence of 7D. Similar phenomena have been observed by Shepherd (1968) in the gliadins and by Orth and Bushuk (1974) in the glutenins.

Components 3 and 4 are controlled by chromosome arm 7DS and components 8 and 9 by 7BS. García-Olmedo and Carbonero (1970) located genes for two components of the chloroform-methanol extract, designated CM1 and CM2, in chromosomes 7D and 7B respectively. Rodriguez-Loperena *et al.* (submitted) have demonstrated that CM1 includes components 3 and 4 and CM2 is composed of 8 and 9. Furthermore, CM1 is identical with the band at 105 mm of Waines (1973) and CM2 is included in a very wide band of his pattern, between 90 and 100 mm, which may explain why he did not detect any effect of 7 B in that zone.

Component 11 is a faintly stained spot that seems to be associated with chromosome 7D and is included in band CM3 of the chloroform-methanol extract (Rodriguez-Loperena *et al.*, submitted).

Components 18, 19, 20 and 21 have not been assigned to any chromosome, probably because genes coding for them are located in more than one pair of homoeologous chromosomes. The fact that component 21 is affected by chromosome 1A suggests that group 1 might be involved.

These data about the chromosomal control of non-gliadin proteins extracted with 70% ethanol are essentially consistent with the genome assignment based on the protein maps of tetraploid and hexaploid wheats and of *Aegilops squarrosa* (Rodriguez-Loperena, submitted).

Because of their low intragenomic variability (Johnson, 1972; Aragoncillo, 1973; Rodriguez-Loperena *et al.*, submitted), the components of this group of proteins should be, at the present degree of resolution, excellent chromosome markers in phylogenetic studies.

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Dr. C. Aragoncillo
Dr. M. A. Rodriguez Loperena
Dr. Pilar Carbonero
Dr. F. García-Olmedo
Department of Biochemistry
Universidad Politécnica de Madrid
E.T.S. Ingenieros Agrónomos
Madrid – 3 (Spain)