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Aneuploid Analysis of Low Molecular Weight Gliadins from Wheat

G. Salcedo, J. Prada, R. Sánchez-Monge and C. Aragoncillo
Departamento de Bioquímica, E.T.S. Ingenieros Agrónomos, Madrid (Spain)

Summary. The chromosomal location of genes affecting five components from the low molecular weight gliadin (LMWG) fraction from wheat endosperm has been investigated by aneuploid analysis. Genes controlling these proteins were assigned to chromosomes 4B, 7A and 7D. Chromosomes from homoeologous groups 1, 2 and 6, where genes controlling classical gliadins are located, are not involved in the control of LMWG.

Key words: Wheat – Gliadins – Chromosomal control – Aneuploid analysis

Introduction

The aneuploid series of hexaploid wheat, *Triticum aestivum* L., which were first developed by Sears (1954, 1966a, 1966b), have enabled the assignment of the chromosomal location of genes that control many enzyme systems (Barber et al. 1968; Hart 1970; Hart and Langston 1977) and endosperm proteins (Wrigley and Shepherd 1973; Orth and Bushuk 1974; Bietz et al. 1975; Aragoncillo et al. 1975). These assignments have been periodically catalogued by R. Morris (1974, 1975, 1977, 1978) and recently reviewed by Konzak (1977) and García-Olmedo et al. (1978).

Gliadins, the reserve prolamins from wheat, have received special attention in the above context. It has been repeatedly established that genes presumably encoding gliadin components are located in homoeologous chromosome groups 1 and 6 (Boyd and Lee 1967; Shepherd 1968; Wrigley and Shepherd 1973; Kasarda et al. 1976; Sasek and Kosner 1977); whereas group 2 chromosomes have been implicated in their regulation (Shepherd 1968; Solari and Favret 1970; Waines 1973).

We have recently described a group of endosperm prolamins, designated low molecular weight gliadins (LMWG), which are present in classical gliadin preparations and overlap with them during electrophoresis at pH 3,2 (Salcedo et al. 1979). We now present evidence that LMWG are controlled by different chromosomes to those affecting classical gliadins and they are less variable.

Materials and Methods

Compensated nulli-tetrasomics (except those nullisomic for chromosomes 2A and 4A), ditelosomics 4Aα, 7AL, 7AS, 7BL and 7DS, and nullisomic 7D from *T. aestivum* cv. 'Chinese Spring' were obtained from E.R. Sears (Columbia, Missouri, USA). Blau-Korn, a 4A/5R wheat/rye substitution, was the gift of F.J. Zeller (Munich, D.F. Germany). Tetra-Rescue, obtained by D genome extraction from hexaploid Rescue (Kaltsikes et al. 1968) was donated by P.J. Kaltsikes (Manitoba, Winnipeg, Canada). *T. aestivum* and *T. turgidum* cultivars listed in Tables 1 and 2 were from our own collection.

Wheat kernels were crushed with a hammer between two metal plates and then transferred to a small tube. Lipids were extracted with petroleum ether (b.p. 40°-70°; 10 v/w) and discarded. Proteins were extracted at room temperature with 70% ethanol (10 + 10 v/w) and the solvent vacuum-evaporated. Two-dimensional electrophoresis was performed as previously described (Salcedo et al. 1979): 1. dimension at pH 9.0 in 2 × 70 mm polyacrylamide (10%) gel columns and the 2. dimension at pH 3.2 in 2 mm thick starch gel slabs. Staining was carried out with 0.05% nigrosine for 16 hours. Non-identity of map components was checked, when necessary, by joint electrophoresis. Densitometry was performed in a Chromoscan (Joyce and Loebl) with the 620 nm filter.

Results and Discussion

Variability of LMWG in T. aestivum L. and T. turgidum Desf.

Of the components that can be observed in the LMWG fraction obtained by gel filtration of *T. aestivum* 70% ethanol extracts, only 7 can be easily detected by two-dimensional electrophoresis of crude extracts from small samples (50-100 mg). The remaining components are

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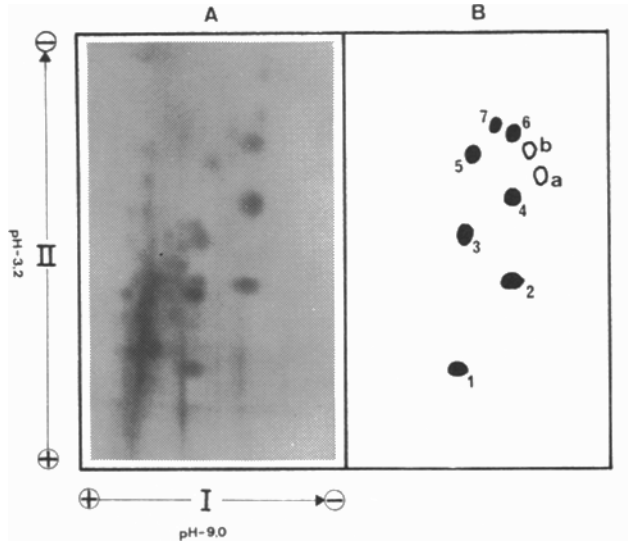

Fig. 1 A and B. A Two-dimensional electrophoretic map of 70% ethanol extract from *T. aestivum* cv. 'Chinese Spring', B Diagram indicating LMWG in the above map (black spots) and variants found in other cultivars (open spots)

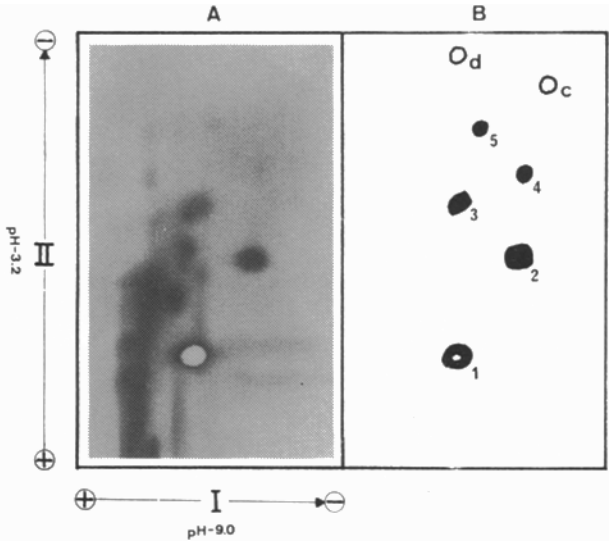

Fig. 2 A and B. A Two-dimensional electrophoretic map of 70% ethanol extract from *T. turgidum* cv. 'Senatore Capelli', B Diagram indicating LMWG in the above map (black spots) and variants found in other cultivars (open spots)

Table 1. Distribution of low molecular weight gliadins in cultivars of *T. aestivum*

	Components[a]								
Phenotypes[b]	1	2	3	4	5	6	7	a	b
I	+	+	+	+	+	+	+	–	–
II	+	+	+	+	+	+	–	–	–
III	+	+	+	+	+	–	+	–	–
IV	–	+	+	+	+	–	+	+	–
V	+	+	+	–	+	–	+	–	+

[a] + = present; – = absent

[b] I = cvs. 'Candeal de Castilla', 'Chinese Spring' and 'Yactana'; II = cvs. 'Estrella', 'Pané 247' and 'Rescue'; III = cvs. 'Aragón 03', 'Ariana', 'Calatrava', 'Campeador', 'Cascón', 'Florence Aurore', 'Gredos', 'Impeto', 'Libero', 'Mara' and 'Traquejos'; IV = cvs. 'Cabezorro' and 'Rieti'. V: cv. 'Canaleja'

Table 2. Distribution of low molecular weight gliadins in cultivars of *T. turgidum*

	Components[a]							
Phenotypes[b]	1	2	3	4	5	6	c	d
I	+	+	+	+	+	–	–	–
II	+	–	+	–	+	–	+	+
III	+	+	+	+	–	+	–	–
IV	+	+	–	+	–	+	–	–

[a] + = present; – = absent

[b] I = cvs. 'Andalucía', 'Granja de Badajoz', 'Híbrido D', 'Ledesma', 'Morisco de Tenerife', 'Mollá', 'Ruso', 'Senatore Capelli', 'Solacambre' and 'Tetra Rescue'; II = cvs. 'Alcalá la Real', 'Alonso', 'Enano de Jaen', 'Fartó Blanco', 'Fartó Rojo', 'Jerez 36', 'Recio de Málaga', 'R. Iznalloz' and 'Zoco de Jebel Hebil'; III = cv. 'Rubial'; IV = cv. 'Blanco de Corella'

either there at a low level or are eclipsed in the map by classical gliadins and would require a preliminary larger scale gel filtration step before the electrophoretic analysis.

The two-dimensional LMWG map of *T. aestivum* cv 'Chinese Spring' is represented in Figure 1 (A; and B, black spots). Results of a survey of *T. aestivum* cultivars are reported in Table 1. The relative position of the observed variant components is shown in Figure 1-B (open spots). Only 3 out of 20 cultivars displayed variant components, while all the others had the 'Chinese Spring' phenotype, either complete or with one component missing.

The LMWG map of *T. turgidum* cv. 'Senatore Capelli' is shown in Figure 2 (A; and B, black spots). This map is identical to that of the Tetra-Rescue line obtained by D genome extraction from the hexaploid cultivar 'Rescue'. Variants with respect to this map are represented as open spots (Fig. 2 B). Phenotypes found in a survey of *T. turgidum* cultivars are described in Table 2. Only component 7 is consistently missing from all cultivars analysed and only two cultivars had component 6. Component 4 is at a much lower level here than in *T. aestivum* (10%-15%, determined densitometrically).

Classical gliadins seem to present a greater variability (Doekes 1968; Autran and Bourdet 1975) than LMWG. However LMWG are more variable than CM proteins (García-Olmedo and García-Faure 1969; Rodriguez-Loperena

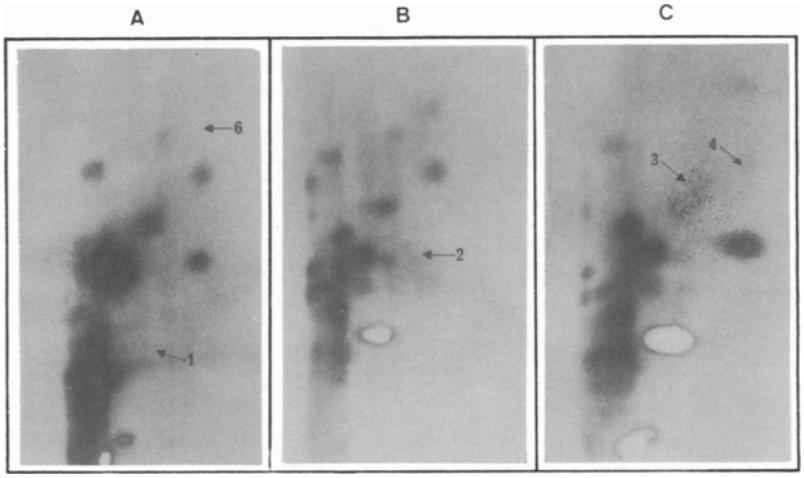

Fig. 3 A-C. Two-dimensional electrophoretic maps of the following cv. 'Chinese Spring' aneuploids: A nulli 4B tetra 4D B ditelosomic 7AL, C nulli 7D tetra 7B. Missing or decreased spots are indicated by their map number and an arrow

et al. 1975), another group of prolamins present in classical gliadin preparations. These LMWG proteins could be used both in cultivar identification and in phylogenetic studies.

Chromosomal Location of Genes Controlling LMWG

Qualitative or quantitative changes in the LMWG map were associated only with the aneuploids of groups 4 and 7. Map variations in aneuploids of these two homoeologous chromosome groups are summarized in Table 3 and illustrated in Figure 3.

Components 1 and 6 are controlled by chromosome 4B. Chromosome 4A does not seem to be involved in the control of this group of proteins: Blau-Korn, a 4A/5R substitution, shows the hexaploid wheat phenotype but none of the clearly distinguishable rye LMWG. No effect has been associated with the absence of chromosome 4D. Possible increases of proteins 1 and 6 in tetra 4B stocks could not be ascertained because, in the first case, there was negative staining in the center of the spot, and, in the case of protein 6, the error of the densitometric measurements was too great. The results are compatible with location of the structural genes for these proteins in chromosome 4B.

Group 7 chromosomes control components 2, 3 and 4 in a less clearly defined way. Component 2 is greatly decreased in nulli 7A tetra 7B and nulli 7A tetra 7D, and apparently absent in ditelo 7AL, whereas it is not affected by the absence of other chromosomes of the same group. Component 3 is markedly decreased in stocks nullisomic for chromosome 7D but not affected by the absence of either chromosome 7A or 7B. Component 4 seems to be absent from all stocks nullisomic for chromosome 7D, although a faint spot can be detected in the same map position in nulli 7D tetra 7B. This component is also markedly decreased in nulli 7A tetra 7B but not in either of the 7A ditelosomic stocks and does not seem to increase in nulli 7B tetra 7A.

As previously discussed (García-Olmedo et al. 1978), aneuploid analysis does not always discriminate between structural genes and/or regulatory or modifier genes, due to the possible superposition of structural-gene-dosage ef-

Table 3. Low molecular weight gliadins in aneuploids of chromosome groups 4 and 7

Stocks[a]	Components number[b]		
	1	6	
n4B t4A	a	a	
n4B t4D	a	a	
n4D t4A	e	e	
n4D t4B	e-i	e-i	
Blau-Korn (subst. 4A/5R)	e	e	
dt 4Aα	e	e	
Stocks	Components number		
	2	3	4
n7A t7B	d	e	d
n7A t7D	d	i	i
n7B t7A	i	e	e
n7B t7D	e	i	i
n7D t7A	i	d	a
n7D t7B	e	d	a
dt 7A-L	a	e	e
dt 7A-S	e	e	e
dt 7B-L	e	e	e
dt 7D-S	e	e	e
n 7D	e	d	a

[a] n = nulli; t = tetra; dt = ditelosomic

[b] e = euploid value; d = decreased (10%-50% of euploid value); i = increased; a = absent

fects for proteins encoded by duplicate homoeologous genes, which may have the same or different levels of expression with regulatory or modifier genes located in the same or different chromosomes (Salcedo et al. 1978; Aragoncillo et al. 1978). This is clearly the case with the effects associated with group 7 chromosomes.

Component 5 was not affected in any of the stocks analysed and component 7 was too faint in cultivar 'Chinese Spring' to allow a proper analysis.

The LMWG are not controlled by chromosomes of groups 1, 2 and 6 where genes affecting classical gliadins have been located.

Although LMWG are present in classical gliadin preparations obtained by different procedures (Salcedo et al. 1979), they were not detected by previous workers because they stain poorly and are eclipsed by classical gliadins in one-dimensional electrophoresis. In addition, when electrofocusing (pH 5-9) x electrophoresis was used, these proteins were excluded from the map due to the fact that their isoelectric points are higher than 9.

It should be mentioned that reports indicating that some gliadin components were controlled by chromosomes 4A (Sasek and Kosner 1977) or 7D (Solari and Favret 1970) have not been confirmed by other workers and, in the latter case, the proteins were present in 'Thatcher' but not in 'Chinese Spring' wheat.

Purification of LMWG individual components is under way in order to ascertain possible structural relationships both among themselves and with respect to previously reported CM proteins, which are also controlled by chromosomes of groups 4 and 7 (García-Olmedo and Carbonero 1970; Aragoncillo et al. 1975).

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Dr. G. Salcedo
Dr. J. Prada
Dr. R. Sánchez-Monge
Dr. C. Aragoncillo
Departamento de Bioquímica
E.T.S. Ingenieros Agrónomos
Madrid (Spain)