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Glutenin Subunits of Genetically Related European Hexaploid Wheat Cultivars: Their Relation to Bread-making Quality

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Summary. The subunit composition of glutenin from 47 European wheat cultivars was studied using SDS-polyacrylamide gel electrophoresis. These cultivars are genetically related since they originate from the same stock. Moreover, the diversity of sample, containing cultivars with very different French bread-making qualities, makes it possible to investigate the relationship between glutenin subunit composition and bread-making quality. 16 electrophoretic types of glutenin subunits could be distinguished: these were grouped into four classes. Depending on the cultivar, six to eight glutenin subunits with MW more than or equal to 62,000 were detected. Subunits 3 and 5, with an approximate MW of 122,000 and 108,000 respectively, seem to play a prominent role on bread-making quality; they were found in cultivars of good quality and were absent in those unsuitable for making French bread. Two other subunits (9 and 10; MW: 71,000 and 66,000, respectively) have a less defined influence but may be needed in some types of glutenin structure. Aneuploid analysis shows that in 'Chinese Spring', subunit 5 is coded by a gene on the long arm of chromosome 1B. The location of genes coding for subunits 3, 9 and 10 could not be determined.

Key words: Glutenin – Wheat – Bread-making – Chromosomal control

Introduction

Many workers have tried to explain the bad bread-making quality of some hexaploid wheat varieties. They considered glutenin as the prime factor governing the strength and elasticity of dough. This high molecular weight (HMW) protein fraction, characterised by its insolubility in ethanol 70%, is comprised of subunits of different MW linked by disulphide bonds (Gueguen et al. 1974; Wall 1979).

The highest MW subunits of the HMW glutenin fraction (Huebner and Wall 1976) seem to play a prominent role in controlling wheat quality (Payne and Corfield 1979).

Comparisons between tetraploid and hexaploid wheats show that the absence of the D genome results in a loss of the HMW glutenin subunits considered essential for bread-making (Orth and Bushuk 1973b). Huebner (1970), Orth and Bushuk (1973a), Bietz et al. (1975) observed that electrophoretic patterns of reduced glutenin indicate some variations among a wide range of hexaploid varieties; however, no obvious relation between quality and glutenin subunits appeared. More recently, Payne, Corfield and Blackman (1979) found that a HMW glutenin subunit present in 'Maris Widgeon', a variety of excellent English bread-making quality, is absent in 'Maris Ranger', unsuitable for making bread. Analysis of 60 randomly-derived F_2 progeny from a cross between 'Maris Widgeon' and 'Maris Ranger' showed a correlation between the presence of this subunit and bread-making quality.

In order to explain the possible disagreement between those results, it remains to be seen whether such a relation between electrophoretic patterns of glutenin subunits and bread-making quality is linked to the poor genetic variability of studied varieties. That is why we have tried to determine the subunit composition of glutenin from mainly French European hexaploid cultivars of diverse bread-making qualities. Their peculiarity is related to their genetic relationship since all these cultivars come from the same stock (Joudrier 1974). However, although related, those cultivars have a larger genetic diversity than the strains studied by Payne et al. (1979). We believe that they represent cultivars having diverse bread-making qualities found in Europe.

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Material and Methods

Wheat Cultivars

We have studied: a) 39 French cultivars related to cultivars 'Cappelle-Desprez', 'Etoile de Choisy', 'Heine VII', 'Petit Quinquin', 'Vilmorin 27', 'Vilmorin 29', 'Yga', etc. which are genetically related and originated from the local variety 'Noe' (Zeven and Zeven-Hissink 1976). b) 7 English and 1 Dutch cultivars originating from 'Cappelle-D'. or 'Heine VII'. c) 10 North American cultivars to compare with European cultivars: 'Camus', 'Coronation', 'Garnet', 'Laval', 'Marquis', 'Mexique 50', 'Park', 'Regent', 'Reward' and 'Thatcher'. d) Compensating nulli-tetrasomic stocks (except N2AT2B, N4AT4B, N4DT4B) and 21 ditelocentric strains of *Triticum aestivum* cv. 'Chinese Spring' (kindly provided by Dr. C.N. Law, Plant Breeding Institute, Cambridge, UK), in order to determine chromosomal location of genes coding HMW subunits of glutenin.

French cultivars are classified according to the criteria of French bread-making quality: 1) 'Améliorant' (term used in France for cultivars which yield flours too strong to make bread but are used in mixture with other flours): 'Rex'. 2) Very good and good bread-making qualities: 'Alto', 'Arcole', 'Bocquiau', 'Capitole', 'Castan', 'Copain', 'Courtot', 'Ducat', 'Eloi', 'Glanor', 'Hardi', 'Lutin', 'Marengo', 'Moisson', 'Protinal', 'Rudi', 'Top' and '32-7-4'. 3) Medium bread-making qualities: 'Arminda', 'Axel', 'Blason', 'Cappelle-D.', 'Fleurus', 'Gamin', 'Marne', 'Nicam', 'Roazon' and 'Trio'. 4) Mediocre bread-making qualities: 'Braco', 'Champlein', 'Gaillard', 'Heima', 'Joss', 'Réso', 'Rivoli', 'Talent' and 'Wattines'. 5) Unsuitable for bread-making: 'Corin'. According to the same criteria, English and Dutch cultivars are considered as mediocre ('Maris Fundin' and 'Maris Hobbit') or unsuitable for making French bread ('Clement', 'Maris Huntsman', 'Maris Hustler', 'Maris Kinsman', 'Maris Mardler' and 'Maris Nimrod').

Dough strength was tested by measuring 'W' obtained by an instrument called 'Alvéographe Chopin' (this instrument is an essential tool in France). It is very strong for 'Rex' (mean W = 300), strong for 'Courtot' (mean W = 200) or 'Hardi' (mean W = 180), moderate for 'Capitole' (mean W = 160), semi-moderate for 'Cappelle-D.' (mean W = 120), weak for 'Wattines', 'Heima', 'Champlein' or 'Joss' (mean W = 90-100) and very weak for 'Maris Huntsman' or 'Clement' (mean W = 50-60).

Preparation of Glutenin and SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Glutenin was isolated according to the method of Bietz et al. (1975) from 20 mg of undefatted meal. After lyophilisation, the extract was mixed with 5 mg of sodium dodecyl sulphate (SDS) and was dispersed in 100 µl of buffer containing 0.0625 M Tris HCl, pH 6.8, 5% 2-mercaptoethanol and 2% SDS. The samples were kept at room temperature for about 2 h, shaken several times and subsequently placed in boiling waterbath fro 4 min. 100 µl of the same buffer, containing 0.001% bromophenol blue, was then added. After centrifugation, the supernatant was analyzed by SDS-PAGE in gel tubes (length: 8,5 cm, diameter: 0.5 cm). The concentration of polyacrylamide was 5%. The electrode buffer was prepared as described by Laemmli (1970); the running gel was composed of 0.150 M Tris HCl, pH 8.8. Electrophoresis was performed at a constant current of 2 mA per gel until the bromophenol blue marker reached the bottom of the gel. Gels were stained with Coomassie Brilliant Blue R250 and destained according to Weber and Osborn (1969). The standard curve for the MW determinations was prepared with the following polypeptide chains: β galactosidase (MW: 133,000); bovine serum albumin (68,000); catalase (60,000); pyruvate kinase (57,000); ovalbumin (45,000); pepsin (35,000); trypsin (24,000) and ribonuclease (13,700).

Results

Variability of Glutenin Subunits Electrophoretic Patterns

The electrophoregrams of reduced glutenin from cultivars 'Rex' (A), 'Castan' (B), 'Protinal' (C) and 'Maris Huntsman' (D) (Fig. 1) show the number and approximate MWs of glutenin subunits detected in the 47 cultivars studied. The observed analogy of composition in low MW glutenin subunits led us to focus the study on subunits with MWs more than or equal to 62,000 (band 11). They range from the number six to eight, according to the cultivar. Several of them (bands 6, 7, 8, 11) are present in the subunit composition of glutenin from all the cultivars, sometimes in different relative concentrations. Other visible bands, differing in their number as well as in their mobility and intensity, show the variability of the cultivars; they make it possible to distinguish 16 types of electrophoretic patterns (Fig. 2-5) that can be grouped into the following classes:

1 The glutenin subunit 5 (108,000) is present in culti-

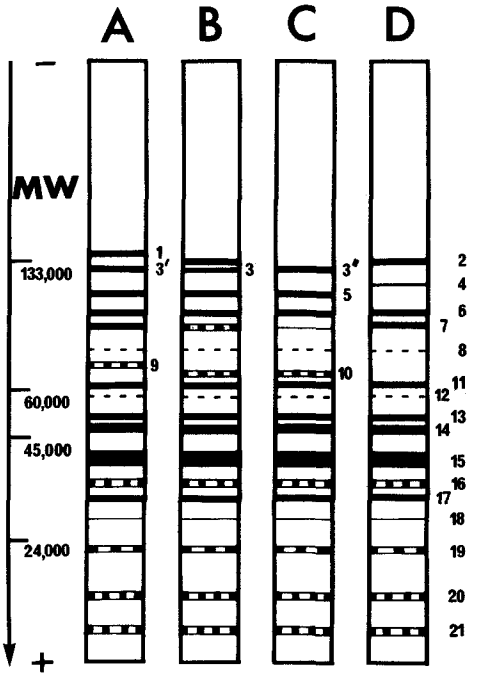

Fig. 1A-D. Diagram of SDS-PAGE of glutenin subunits from cultivars A 'Rex'; B 'Castan'; C 'Protinal'; D 'Maris Huntsman'. Approximative molecular weight (MW): 1 = 140,000; 2 = 133,000; 3,3' or 3" H = 122,000; 4 = 111,000; 5 = 108,000; 6 = 96,000; 7 = 92,000; 8 = 80,000; 9 = 71,000; 10 = 66,000; 11 = 62,000

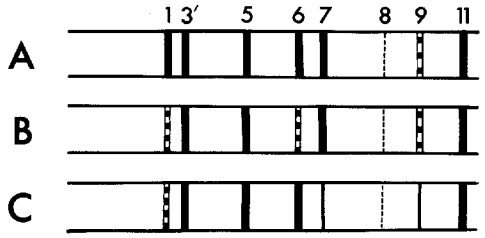

Fig. 2A-C. Class I: Diagram of HMW glutenin subunits from cultivars A 'Rex'; B 'Gamin'; C 'Ducat'

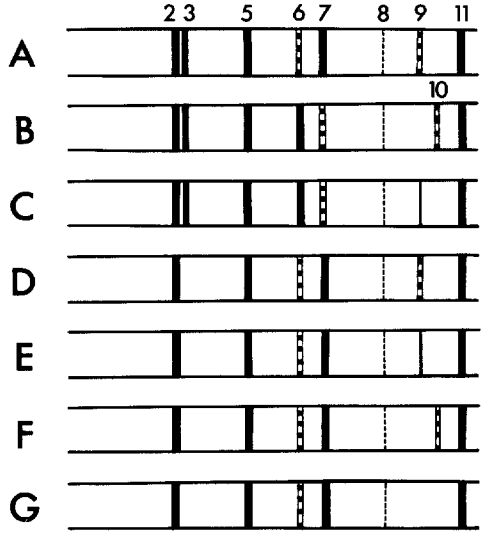

Fig. 3A-G. Class II: Diagram of HMW glutenin subunits from cultivars A 'Courtot', 'Hardi', 'Marengo'; B 'Castan'; C 'Top'; D 'Arcole', 'Capitole', 'Copain', 'Maris Hobbit', 'Maris Fundin', 'Moisson', 'Roazon', 'Rudi'; E 'Axel', 'Blason', 'Cappelle-D.', 'Fleurus', 'Marne'; F 'Arminda', 'Nicam', 'Trio'; G 'Braco', 'Champlein', 'Gaillard', 'Réso', 'Rivoli', 'Talent', 'Maris Kinsman'

vars of 3 classes (I, II and III) (Fig. 2-4) which are different in MW of their highest band:

- Class I = 140,000 (subunit 1) (Fig 2)
- Class II = 133,000 (subunit 2) (Fig. 3)
- Class III = 122,000 (subunit 3") (Fig. 4)

2 The glutenin subunit 5 is absent in cultivars of class IV. (Fig. 5).

Relations of Patterns to Bread-making Quality

'Rex' (Fig. 2A), which produces a very strong flour, shows a more complex electrophoretic pattern. Eight subunits with MWs more than or equal to 62,000 were detected, five of which, intensively coloured, have MWs ranging from 92,000 to 140,000. In the same way, many cultivars of very good and good bread-making qualities ('Hardi', 'Courtot', 'Marengo', 'Castan', 'Top', 'Capitole', 'Rudi', 'Copain', etc. ...) (Fig. 3A-D) have a complex glutenin sub-

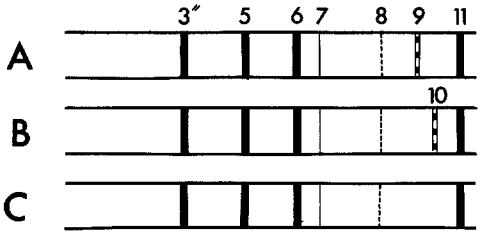

Fig. 4A-C. Class III: Diagram of HMW glutenin subunits from cultivars A '32-7-4'; B 'Protinal'; C 'Alto', 'Bocquiau', 'Glanor', 'Lutin'

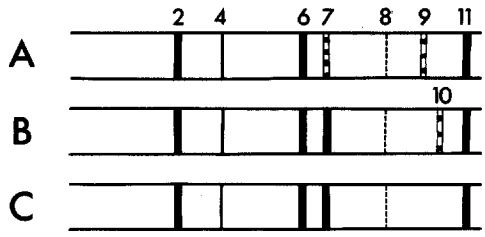

Fig. 5A-C. Class IV: Diagram of HMW glutenin subunits from cultivars A 'Eloi', 'Heima', 'Maris Mardler', 'Wattines'; B 'Corin', 'Joss'; C 'Clement', 'Maris Huntsman', 'Maris Hustler', 'Maris Nimrod'

unit composition (seven or eight glutenin subunits with MWs ranging from 62,000 to 133,000). On the other hand, cultivars of very poor mixing and bread-making qualities ('Clement', 'Maris Huntsman', 'Maris Hustler' and 'Maris Nimrod') lack several bands (n° 1, 3, 5, 9 or 10) (Fig. 5C).

The preceding classification suggests the possibility of the existence of a relatively good relationship of glutenin to quality. 'Rex', as well as 27 of the 28 cultivars classified from medium to very good qualities in French bread-making, have subunit 5. Three of the eleven mediocre and six of the seven unsuitable cultivars for French bread-making lack this subunit. Accordingly, glutenin subunit 5 may play positive role in raising bread-making quality.

The study of French cultivars from class II, originating either from 'Cappelle-D.' or from its parents ('Vilmorin 27' or 'Hybride du Joncquois'), suggests the possible influence of other subunits. Absence of band 3, dilution occurred in band 9 and, then, absence of bands 9 and 10, are correlated with a progressive decrease in quality. Thus, cultivars with mediocre bread-making qualities ('Braco', 'Champlein', 'Gaillard', 'Réso', 'Rivoli' and 'Talent') (Fig. 3G) lack these three bands. They yield flours of weak strenth, but, however, better than that of 'Maris Huntsman' or 'Clement' wherein subunit 5 is absent. Figure 6 specifies the characteristics of subunit composition of glutenin from five cultivars of class II. It can be noted that the band 3 present in 'Hardi' is probably inherited from 'Thatcher', a variety of very good quality, in which also band 3 is present.

Analysis of classes I and III indicates the possible role

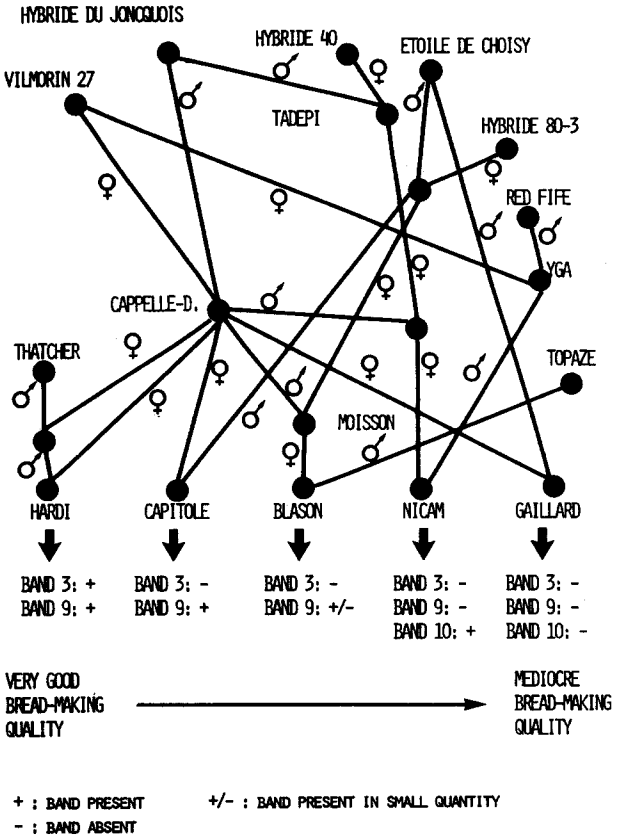

Fig. 6. Genetic relationship and some characteristics of subunit composition of glutenin from five cultivars of class II

of band 3. All the cultivars belonging to these two classes contain a glutenin subunit with an identical or quite similar MW to band 3 (bands 3' and 3''). Although glutenin subunit 2 is lacking, they are of good bread-making qualities, except for 'Gamin'.

Comparisons with North American Cultivars

The analysis of North American cultivars of excellent qualities indicates that they contain glutenin subunit 5. Electrophoretic patterns belong to class II (types A or D) or to class III, but never to class IV.

Analysis of Nulli-Tetrasomic and Ditelocentric Lines

The study of these lines show that in 'Chinese Spring' subunits 2 and 7 are coded by genes on the long arm of chromosome 1D and that subunit 5 is coded by a gene on the long arm of chromosome 1B. The intensity of coloration of band 6 decreases in N1BT1A and N1BT1D lines, showing that a gene on chromosome 1B codes a subunit in that band. We cannot precisely chromosomally locate the gene coding for glutenin subunit 9 (see also Orth and Bushuk 1974; Bietz et al. 1975), nor the bands 1, 3 and 10 which are absent in 'Chinese Spring'.

Discussion

A relationship between the rate of aggregation of separated gluten and mixing properties of flours has already been established (Arakawa et al. 1974). Further studies have permitted the determination of the role of glutenin (Arakawa et al. 1976) and the main importance of HMW glutenin subunits (Arakawa et al. 1977). According to these researchers, quantitative and qualitative differences in glutenin subunit composition may explain the properties of the glutenins extracted from wheats of diverse baking qualities. However, works concerning hexaploid varieties did not show such obvious relationships. Although it has been established that quantitatively the importance of some HMW glutenin subunits can differ from one variety to another (Bietz and Wall 1972), only the bad bread-making quality of 'Nap Hal' may be interpreted by the absence of two HMW glutenin subunits (Bietz et al. 1975). On the other hand, according to the findings of Orth and Bushuk (1973a), the variation from number five to three of detected HMW glutenin subunits from mainly American varieties cannot be related to differences in bread-making quality. Our work also reveals, to a slight extent, that the bread-making quality may be somewhat independent of the number of HMW subunits from European and North American cultivars. But among those, subunits 3 and 5 may, however, be useful. Furthermore, subunits 9 or 10 seem to have a less distinct role to play, though they may influence this quality in some types of glutenin structure (class II).

The specificity of cultivars studied here may be based on two facts: 1) these are mostly selected within France and originate from the same parents. The genetic relationship of these cultivars, consequently, gives a relative genetic homogeneity to the pattern studied and, as we think, may have helped determine the subunits linked to bread-making quality. 2) We are of the opinion that they constitute a good cross section of the wide range of bread-making quality of European cultivars. This enabled us to study wheats comparable with North American cultivars, and others, adapted to English bread-making. Therefore, the results obtained allow us to analyze the relationships existing between electrophoretic patterns of glutenin subunits and bread-making quality of flours. However, such relationships may also be influenced by extraction and electrophoretic processes (Bietz and Wall 1975; Khan and Bushuk 1977).

Payne et al. (1979) suggested that HMW glutenin subunits correlated with bread-making quality enable large

and stable glutenin proteins to be formed. We can, therefore, suggest that subunits 3 and 5 belong to this type of subunit and contribute to the improvement of the physical properties of gluten. This might also explain the role of acid-insoluble glutenin of wheats showing good bread-making quality (Legouar et al. 1979). However, these proteins can also be considered as genetic markers, coded by genes located close to others possibly controlling or regulating bread-making quality factors. This may be the reason why 'Eloi' does not contain subunits 5 and 3, although it has good mixing properties, and why 'Maris Kinsman', containing subunit 5, is unsuitable for bread-making.

Preceding works insisted on the role of glutenin subunits coded by D genome and specially chromosome 1D (Orth and Bushuk 1974; Bietz et al. 1975). Nonetheless, it would be interesting to find the genetic location of the gene coding glutenin subunit 3, which is absent in 'Chinese Spring'. It would not be out of place to point out here that, in 'Chinese Spring', subunit 5 is coded by a gene on chromosome 1B. Studies of glutenin subunits, as well as those of other wheat proteins (Branlard 1979; Kobrehel 1979) could become very useful in the early breeding of new European hexaploid wheat varieties.

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