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Identification of a High-Molecular-Weight Subunit of Glutenin Whose Presence Correlates with Bread-making Quality in Wheats of Related Pedigree

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Summary. The subunit composition of glutenin was analysed by SDS-polyacrylamide-gel electrophoresis using two varieties of contrasting pedigrees. 'Maris Widgeon', a variety of good bread-making quality, was shown to contain 2 glutenin subunits not present in 'Maris Ranger', a much higher yielding variety that is unsuitable for making bread. A third subunit was only found in 'Maris Ranger' glutenin. To determine if any of these subunits are directly related to bread-making quality, 60 randomly-derived F_2 progeny from a 'Maris Widgeon' × 'Maris Ranger' cross were analysed for bread-making quality and for glutenin subunit composition. A strong correlation was demonstrated between the presence of one of the two subunits inherited from 'Maris Widgeon', and quality. This subunit (termed subunit 1 glutenin) had an approx. mol. wt. of 145,000. It was also found in 'Maris Freeman', a bread-making variety selected from the same cross previously made in 1962. In further crosses involving 'Maris Widgeon' or its descendants, more bread-making varieties have been produced in the last decade at the Plant Breeding Institute, Cambridge and all but one have inherited glutenin subunit 1. The subunit has been traced back through 'Holdfast' to 'White Fife', a Canadian hard spring wheat of excellent bread-making quality. Some 67 varieties were screened for the presence of glutenin subunit 1 and it was found in 31% of them. Several unrelated varieties of good bread-making quality did not contain subunit 1 glutenin.

Key words: Glutenin – *Triticum* – Bread-making – SDS-polyacrylamide-gel-electrophoresis

Introduction

It is generally accepted that the properties of the proteins in a flour govern its suitability for processing into bread. This 'protein quality' has been linked to the glutenin group of proteins; the bread-making quality of a flour is directly proportional to (1) the amount of glutenin insoluble in 3M urea (Pomeranz 1965) or dilute acetic acid (Orth and Bushuk 1972; Mecham, Cole and Ng 1972) and (2) the mean molecular weight of total glutenin (Huebner and Wall 1976). Glutenin forms about 45% of the total endosperm protein and consists of several different subunits (Huebner and Wall 1976) which are connected together to form high-molecular-weight complexes (for review, see Kasarda, Bernardin and Nimmo 1976). The subunit composition of glutenin varies according to the wheat variety selected (Bietz, Shepherd and Wall 1975) so different combinations of subunits could account for the different properties of glutenin, which, in turn, could cause the differences in bread-making quality of flours.

To determine if this were so, Huebner (1970) prepared glutenin from 10 varieties of hexaploid wheat; the glutenin was reduced, alkylated and fractionated by starch-gel electrophoresis. Differences in subunits were observed between varieties but no obvious correlations with bread-making quality could be made. Orth and Bushuk (1973) came to a similar conclusion after they had fractionated the glutenin subunits of 24 varieties by SDS-polyacrylamide-gel electrophoresis.

In this communication, we have also searched for a relationship between glutenin subunit composition and bread-making quality by analysing glutenin from the progeny of a cross between good and poor bread-making varieties. We found that the presence of a subunit of glutenin, whose molecular weight is about 145,000, correlated with bread-making quality.

Materials and Methods

Wheat Varieties

Varieties were taken from the collection of wheats maintained at the Plant Breeding Institute, Cambridge. The only exception was

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'Scout 66', kindly provided by Dr. D.D. Kasarda. The pedigrees of some of the varieties studied are given in Figure 5.

The 'Maris Widgeon', 'Maris Ranger' Cross

In the breeding programme at the Plant Breeding Institute, Cambridge, 'Maris Widgeon', a low yielding variety of good bread-making quality was crossed with 'Maris Ranger', the highest yielding variety of the time but of poor bread-making quality. Progeny were selected during the subsequent generations of inbreeding for a combination of high yield and bread-making quality. In 1962, 'Maris Freeman' was the final selection from this cross.

More recently, sixty F_2 plants were selected at random from a cross between 'Maris Widgeon' and 'Maris Ranger' and grown for 2 further generations as F_2 progenies without selection. F_5 bulks of these progenies were used in this study.

SDS-sedimentation Test

Flour samples were tested for bread-making quality by the SDS-sedimentation test of Axford, McDermott and Redman (1978). In this method, the volume of material which sediments after mixing flour with a solution of SDS and lactic acid is measured. Work in this Institute has confirmed a reliable, positive correlation between sedimentation volume and bread-making quality of flours (Blackman and Bingham, unpublished data).

Preparation of Glutenin

Meal (4g) was defatted with 100 ml of n-hexane. After drying, the residue was stirred with 200 ml of 0.5 M NaCl for 90 min at 4°C. The suspension was centrifuged at 38,000 × g_{max} for 15 min at 4°C and the pellet resuspended in cold water and centrifuged again. The pellet obtained was suspended in 70% (v/v) ethanol at 4°C and stirred for 4h at 4°C. The mixture was centrifuged as before, and the pellet dispersed in 70% (v/v) ethanol and recentrifuged. The final pellet was dried under vacuum and stored at −20°C.

SDS-polyacrylamide Gel Electrophoresis

Isolated glutenin, flour or milled grain (4 mg) was suspended in 100 µl of a medium containing 2% (w/v) sodium dodecyl sulphate (SDS), 5% (w/v) 2-mercaptoethanol, 0.001% (w/v) pyronin Y, 10% (v/v) glycerol and 0.063M Tris HCl, pH 6.8. The samples were left at room temp. for about 2 h and occasionally shaken. They were then placed in a boiling waterbath for 2 min, allowed to cool and then 50 µl of each sample was loaded onto a 17% polyacrylamide gel which measured 16 cm × 13 cm × 1.5 mm. The gel, which usually contained 13 samples, was then subjected to electrophoresis at about 8 mA for about 18 h when the tracking dye, pyronin Y, had reached within 1 cm of the bottom of the gel. The gel was stained with Coomassie Brilliant Blue R and destained as described previously (Payne and Corfield 1979).

In experiments where quantification of stained bands was required, the gel was cut into longitudinal strips of approx. 1 cm wide so that each strip contained the fractionated products of one sample. The strip was scanned at 560 nm using a modified Hilger-Gilford spectrophotometer.

Results and Discussion

The 'Maris Widgeon', 'Maris Ranger' Cross

The banding patterns of glutenin subunits from 'Maris Freeman' and its two parents ('Maris Widgeon' and 'Maris Ranger') are shown in Figure 1. Some differences were detected; subunits 1 and 2 (molecular weights 145,000 and 71,500 respectively) occurred in the glutenin of 'Maris Widgeon' and 'Maris Freeman' but not 'Maris Ranger', and subunit 3 (molecular weight 51,000) was found only in 'Maris Ranger' glutenin. Many of the minor protein bands (Fig. 1) were probably due to contaminating albumins, globulins and gliadins (Payne and Corfield 1979).

We conclude from this experiment that subunits 1 and 2 have been inherited by 'Maris Freeman' from 'Maris Widgeon' along with bread-making quality whereas subunit 3, which is present in the low-quality parent, has not. To try and determine if the presence of subunits 1 and 2 in glutenin actually impart bread-making quality to a flour, a more detailed analysis of the 'Maris Widgeon' × 'Maris Ranger' cross was made.

The bread-making qualities of 60 F_2 progenies from this cross were determined by the SDS-sedimentation test and the results are shown in Figure 2. A Gaussian distribution of bread-making quality was obtained, the majority of samples being intermediate between the values obtained for the two parents. The same samples were also

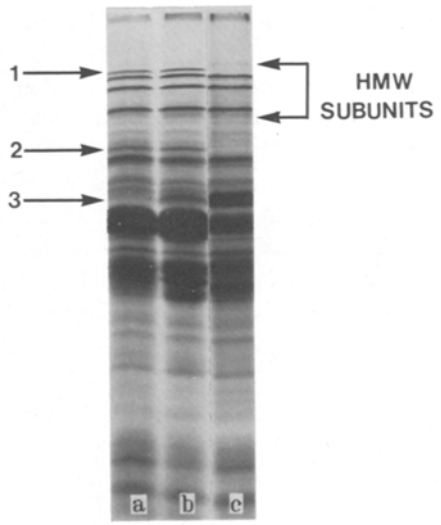


Fig. 1a-c. SDS-polyacrylamide-gel electrophoresis of glutenin subunits from a 'Maris Freeman', b 'Maris Widgeon' and c 'Maris Ranger'. The gels were calibrated with respect to molecular weight using the following standards: *E. coli* RNA polymerase, α subunit (160,000), bovine serum albumin (68,000), RNA polymerase β subunit (39,000) and soybean trypsin inhibitor (21,500)

analysed by gel electrophoresis and scored for the presence of subunits 1, 2 and 3. Because of the number of samples involved, total protein rather than isolated glutenin was analysed. A strong correlation existed between the presence of subunit 1 and the bread-making quality (Fig. 3a). Thus, 21 samples scored 1, 2 or 3 in the SDS-sedimentation test and all possessed subunit 1. Eleven samples fell into the two worst categories of the test and only 4 of them were shown to contain this subunit. In contrast to subunit 1, the presence of subunit 2 did not correlate with breadmaking quality (Fig. 3b). There is some suggestion of a weak inverse correlation between subunit 3 and quality (Fig. 3c).

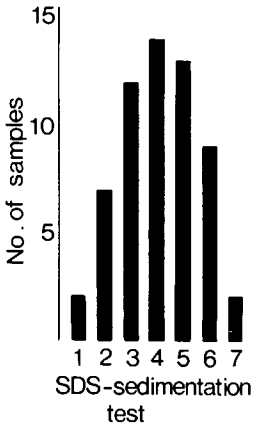


Fig. 2. Assessment of the bread-making quality of F_5-progeny from a 'Maris Widgeon', 'Maris Ranger' cross by the SDS-sedimentation test. The volume of sedimented material for each sample was measured and given a score from 1 to 7, as follows: 1) 81-86 ml; 2) 76-80 ml; 3) 71-75 ml; 4) 66-70 ml; 5) 61-65 ml; 6) 56-60 ml; 7) 51-55 ml. 'Maris Widgeon' scored 1 (7 samples) or 2 (1 sample) whereas 'Maris Ranger' scored 7 (6 samples) or 6 (2 samples)

The simple, positive/negative scoring for subunit 1, 2 and 3 has, unfortunately, an important disadvantage. The grains milled to produce a sample of flour were likely to be of mixed genotype as they had been bulked from the F_2 generation. Thus, a sample may have been prepared from some grains which contained subunit 1 and others which did not. Unless the proportion of subunit 1 in the final mixture was very low, then the sample would be scored positive in the same way as a pure sample would. The sedimentation value for bread-making quality would, on the other hand, be representative of the whole sample. To overcome this disadvantage, the three polyacrylamide gels which had the best resolution of polypeptides were selected and the separations of each of the 39 samples were quantified spectrophotometrically. The results, when plotted against sedimentation volume (Fig. 4) are somewhat scattered, but there is nevertheless a significant correlation between the relative amount of subunit 1 in a flour and its bread-making quality ($r = 0.72$; $P < 0.001$).

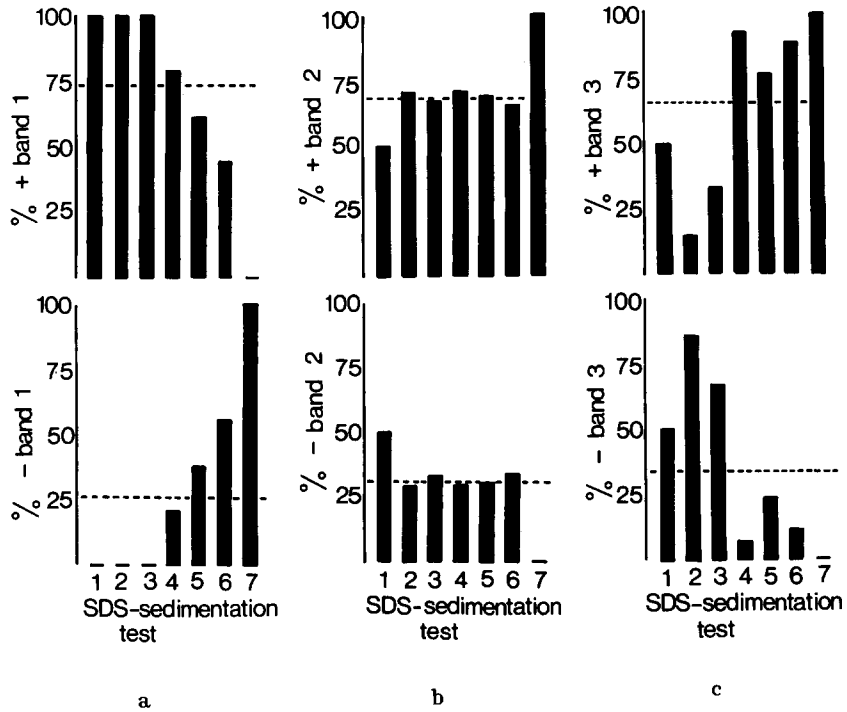


Fig. 3a-c. The relationship between bread-making quality and the presence in glutenin of **a** subunit 1, **b** subunit 2 and **c** subunit 3. The dotted, horizontal lines represent the percentage of the total number of samples which contain or lack each of the 3 subunits

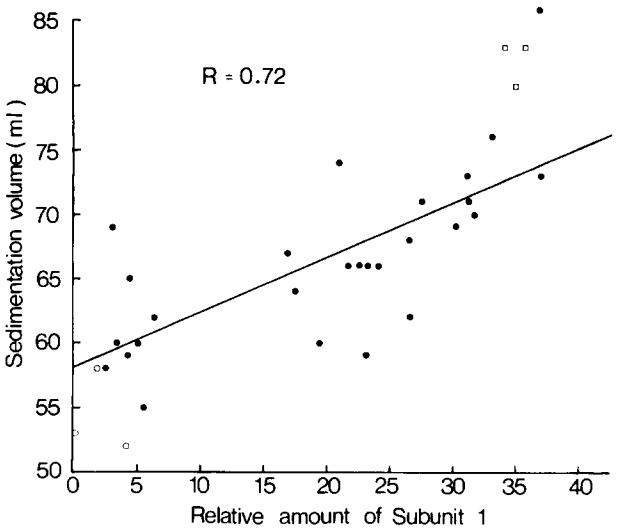


Fig. 4. Relationship between sedimentation volume of samples in the SDS-sedimentation test and the relative amount of subunit 1 glutenin. Solid circles are for progeny of the 'Maris Ranger', 'Maris Widgeon' cross; open circles are for 'Maris Ranger' and open squares for 'Maris Widgeon'. A regression line was calculated for the solid circles only. The correlation coeficient, 0.72, was shown to be significant at $P < 0.001$. The relative amounts of subunit 1 glutenin in flour samples were calculated from spectrophotometric measurements of gel strips as described in the methods. The results are expressed as (area under glutenin subunit 1 peak ÷ area under peak of the standard; the next major subunit of lower molecular weight) X 100

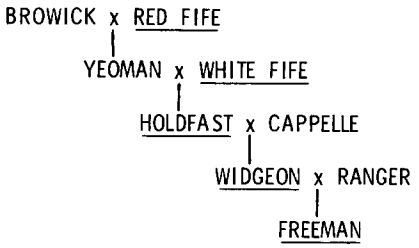
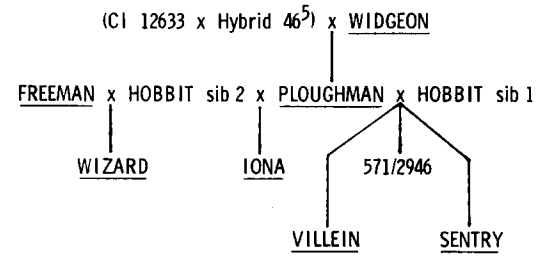


Fig. 5. Pedigree of 'Maris Freeman'. Varieties possessing subunit 1 glutenin are underlined



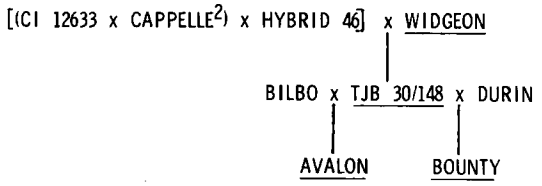


Fig. 6. Pedigrees of some recent varieties of bread-making quality from the Plant Breeding Institute, Cambridge. Varieties which contain subunit 1 glutenin are underlined

Occurrence of Glutenin Subunit 1 in Varieties Closely Related to 'Maris Widgeon'

The pedigree of 'Maris Widgeon' and 'Maris Freeman' is shown in Figure 5. 'Maris Widgeon' was selected in 1964 from a cross between the high yielding 'Cappelle-Desprez' and the good bread-making variety 'Holdfast'. The subunit 1 glutenin was inherited from 'Holdfast'. A study of the ancestors of 'Holdfast' is difficult because some of these wheats are landraces i.e., lines which are of uncertain parentage and may be heterogeneous. However, it is probable that subunit 1 glutenin originated from 'White Fife', a Canadian hard red spring wheat of excellent bread-making quality that was selected from 'Red Fife' towards the end of the last century.

More recently, 'Maris Widgeon' and certain of its offspring, such as 'Maris Freeman' and 'Ploughman', have been used extensively in breeding programmes to produce new varieties which are suitable for making bread (Fig. 6). In all cases, these good quality wheats were crossed with biscuit-making or feed wheats which had other desired qualities such as high yield or disease resistance but lacked glutenin subunit 1. Seven of the 8 new varieties developed from these crosses contained glutenin subunit 1 (Fig. 6)

In summary, glutenin subunit 1 has been successively inherited through a long, ancestral line of varieties that were selected by the plant breeders for bread-making quality.

Distribution of Glutenin Subunit 1 in Wheat Varieties of Diverse Origins

Sixty-seven varieties were screened for the presence of subunit 1 glutenin. The subunit (including any polypeptides of identical mobility) is a somewhat uncommon constituent of glutenin, being present in just 31% of all the varieties tested (Tables 1 and 2). It has a greater molecular weight than any other subunit of glutenin found in any of the varieties analysed.

The bread-making properties of many of the varieties tested are known so some general statements on the relationship between quality and the presence or absence of subunit 1 can be made. Most of the varieties which contain the subunit are of good bread-making quality, such

as 'Monopol' from Germany and 'Selkirk' from Canada. There are two major exceptions: 'Peko', a biscuit wheat which has a very weak dough, and 'Red River 68' which produces a dough that is too strong for making bread. All the high yielding winter wheat varieties bred at the Institute for biscuit making or animal feeding lack glutenin subunit 1 (Table 2). So do several other varieties of poor bread-making quality, such as 'Clement' from Holland and 'Atlas 66' from U.S.A. In contrast, several varieties regarded as highly suitable for bread-making lack subunit 1 glutenin. Examples are 'Adam' from Austria and 'Lancota' from USA.

General Discussion

We can think of two reasons why the presence of subunit 1 glutenin correlates with the bread-making quality of winter wheat bred at the Institute: (1), subunit 1 improves the properties of glutenin for making bread; (2), the cistron(s) which code for subunit 1 are adjacent, and thus genetically linked, to the cistrons that are actually controlling bread-making quality. Unfortunately with present evidence, it is not possible to firmly conclude which reason is valid. However, as bread-making quality has already been linked to the glutenin proteins (Orth and Bushuk 1972), we tend to favour reason (1).

Subunit 1 has a similar molecular weight to several other subunits of glutenin, three in the case of 'Maris Widgeon' (Fig. 1). Other workers, using different varieties of wheat, have isolated these high-molecular-weight (HMW) subunits as a group by gel-filtration chromatography (Huebner and Wall 1974; Hamauzu, Kamazuka, Kanazawa and Yonezawa 1975). They have different properties to the other, smaller, subunits of glutenin. For instance, the HMW subunits are insoluble in aqueous ethanol whereas the majority of the other subunits are soluble (Bietz

Table 1. Varieties which contain glutenin subunit 1 or have protein of identical mobility

	Variety	Country of origin	Type
1.	Avalon[a]	UK	Winter
2.	Bounty	UK	Winter
3.	Hira	India	Spring
4.	Holdfast	UK	Winter
5.	Iona[a]	UK	Winter
6.	Maris Freeman	UK	Winter
7.	Maris Widgeon	UK	Winter
8.	Monopol	Germany	Winter
9.	Norin 10	Japan	Spring
10.	Peko	France	Spring
11.	Ploughman	UK	Winter
12.	Red Fife	Canada	Spring
13.	Red River 68	USA	Spring
14.	Redman	Canada	Spring
15.	Selkirk	Canada	Spring
16.	Sentry	UK	Winter
17.	Sicco	Holland	Spring
18.	Sirius	Germany	Spring
19.	Villein[a]	UK	Winter
20.	White Fife	Canada	Spring
21.	Wizard[a]	UK	Winter

[a] These varieties are currently under trial

Table 2. Varieties which lack subunit 1 glutenin

	Variety	Country of origin	Type
1.	Adam	Austria	Winter
2.	Aobakomughi	Japan	Spring
3.	Armada	UK	Winter
4.	Atlas 66	USA	Winter
5.	Atou	France	Winter
6.	Besostaja	USSR	Winter
7.	Blue Boy	USA	Winter
8.	Bouquet	France	Winter
9.	Brigand	UK	Winter
10.	Browick	UK	Winter
11.	Browick old true	UK	Winter
12.	Cappelle-Desprez	France	Winter
13.	Chinese Spring	China	Spring
14.	Clement	Holland	Winter
15.	Durin	UK	Winter
16.	Era	USA	Spring
17.	Flanders	France	Winter
18.	Gabo	Australia	Spring
19.	Hilgendorf	New Zealand	Spring
20.	Highbury	UK	Spring
21.	Hobbit	UK	Winter
22.	Hustler	UK	Winter
23.	Kharkov	USSR	Winter
24.	Lancota	USA	Winter
25.	Little Joss	UK	Winter
26.	Mardler	UK	Winter
27.	Maris Bilbo	UK	Winter
28.	Maris Fundin	UK	Winter
29.	Maris Huntsman	UK	Winter
30.	Maris Nimrod	UK	Winter
31.	Maris Ranger	UK	Winter
32.	Marquis[a]	Canada	Spring
33.	Marksman	UK	Winter
34.	Mexifen	Chile	Spring
35.	Nap Hal	India	Spring
36.	Nong Da	China	Winter
37.	Partizanka	USSR	Winter
38.	Prelude	Canada	Spring
39.	Red Dantzig	Poland	Winter
40.	Reward	Canada	Spring
41.	Sappo	Sweden	Spring
42.	Scout 66	USA	Winter
43.	Sportsman	UK	Winter
44.	Thatcher[a]	Canada	Spring
45.	Virtue	UK	Winter
46.	Yeoman	UK	Winter

[a] A very faint band with identical mobility to subunit 1 glutenin was detected for these varieties

and Wall 1973). They also have a different amino-acid composition, for example being much richer in glycine (Hamauzu et al. 1975; Khan and Bushuk 1978).

The HMW subunits of glutenin may play an important role in glutenin strength and flour quality. Arakawa and Yonezawa (1975) analysed the glutenin subunits of three flours, described as strong, medium and weak. The strong flour had the highest proportions of the HMW subunits and the weak flour, the lowest. The same group of workers also developed a procedure to measure the rate of aggregation of protein fractions from wheat in salt solutions (Arakawa, Matsumoto and Yonezawa 1974). They established that the rate of aggregation of glutenin correlated strongly with the bread-making quality of 4 wheat flours but no such correlation was found with the gliadins (Arakawa and Yonezawa 1975). In further studies (Arakawa, Yoshida, Morishita, Honda and Yonezawa 1977), the aggregation behaviour of glutenin subunits, separated into 3 groups by gel filtration, were tested using 10 varieties of wheat. The HMW subunits of glutenin were solely responsible for the differential aggregation observed with intact glutenin.

Huebner and Wall (1976) fractionated the endosperm proteins of many different wheat varieties in columns of Sepharose 4B and Sepharose 2B and in each case obtained 2 subfractions of glutenin, one of HMW and the other much smaller. Those flours, which had high ratios of large to small glutenin, produced the strongest doughs. More recently, Payne and Corfield (1979) have shown that glutenin of increasing molecular weight had increasing proportions of the HMW subunits. Presumably these subunits interact strongly with the other subunits and are important in stabilizing the HMW structures. Thus, this work combined with that of the Japanese workers on glutenin aggregation suggests that the HMW subunits of glutenin impart bread-making quality to a flour.

Additional evidence for the importance of the HMW subunits in breadmaking quality comes from the work of Bietz, Shepherd and Wall (1975). They analysed the glutenin subunits of 80 different varieties. One of them, 'Nap Hal', was unusual in lacking two of the principal HMW subunits, leaving it with only two. The variety has poor bread-making qualities. The two missing subunits are probably coded for by chromosome 1D (Bietz, Shepherd and Wall 1975) and the two remaining by chromosome 1B. It would be interesting in this context to determine which chromosome codes for subunit 1 glutenin.

If it is assumed that subunit 1 actually improves the properties of glutenin for bread-making, then it could do so in one of two ways. The first is that the unique structure of subunit 1 glutenin enables large and stable glutenin proteins to be formed and is much more efficient at doing this than the other HMW subunits present. If this is the case, then one would have to hypothesise that the varieties of quite different parentage from those studied here which lack subunit 1 glutenin yet have excellent bread-making qualities must have complementary subunits of different electrophoretic mobility. The second possible way of improvement is that the addition of genes which code for subunit 1 to a genome simply increases the amount of HMW subunits which are made. For instance, 'Maris Ranger' has only 3 distinguishable HMW subunits whereas 'Maris Widgeon' and the quality offspring of the 'Maris Ranger', 'Maris Widgeon' cross have 4. If this is so, then it follows that the properties of all the HMW subunits will be much the same.

To understand more of the issues raised here, we are currently preparing pure samples of several of the HMW subunits. We hope to determine if they have similar or different properties and structures. We also intend to determine the bread-making qualities and glutenin subunit composition of progeny from crosses between varieties which are unrelated to 'Maris Widgeon'.

Acknowledgement

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