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PROTEIN COMPOSITION — QUALITY ASSOCIATIONS IN HIGH PROTEIN HEXAPLOID BREAD WHEAT DERIVATIVES OF WILD EMMER WHEAT

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ABSTRACT

Twenty-three hexaploid wheat lines, derived from bread wheats and a wild emmer wheat selection (G-25), were developed at the Volcani Centre in Israel where they exhibited improved disease resistance and increased grain protein content. They were grown in New Zealand during the 1984/85 growing season and tested for a range of quality parameters and for gliadin and high molecular weight glutenin composition.

The emmer derivatives exhibited superior protein content to the standards in all but two cases and superior loaf volume in about half the lines.

The derivatives were classified on the basis of gliadin and glutenin composition and these classifications tested to determine if they related to quality. Certain protein components were found to be consistently associated with superior loaf volume, work input requirements, SDS sedimentation volume and flour protein content. The protein component-quality associations identified are in agreement with those determined for European winter wheats.

KEYWORDS

Triticum dicoccoides, polyacrylamide gel electrophoresis, gliadin, HMW glutenin subunits.

INTRODUCTION

Wheat proteins are nutritionally important, as they constitute one of the major protein sources for many population groups. They are also functionally important as only the gluten protein complex has the viscoelastic properties necessary for the production of bread and a range of other cereal based products.

Two novel techniques for increasing both the amount of, and the technological quality resulting from, these proteins have been gaining interest recently. The first of these involves the introduction of alien genetic material into hexaploid bread wheats to incorporate specific disease resistance and increase protein content. Sources of these alien genes which have been exploited include *Agropyron elongatum, Aegilops umbellulata, Secale cereale* (rye), *Triticum speltoides* and *Triticum dicoccoides* (wild emmer). The second technique involves the identification of specific components of the protein complex, which have been shown to be associated with superior breadmaking quality (Sozinov and Poperelya 1980, Payne *et al.* 1984, Moonen *et al.* 1983). This technique offers the possibility of screening parental and early generation material for quality using test methods which require only half a wheat grain. Particular attention has been payed to the high molecular weight components of glutenin, the least soluble of the gluten fractions.

In this study we have attempted to examine the potential interaction of these two breeding techniques.

Workers at the Volcani Centre, Israel, have transferred genes which enhance grain protein levels from tetraploid wild emmer wheat (*Triticum dicoccoides* Koern) selection G-25 into hexaploid bread wheats (*Triticum aestivum* L. em. Thell.). The resulting derivatives have increased disease resistance (Grama *et al.* 1974) and higher grain protein content (Grama *et al.* 1984) than the parent bread wheats.

The present study was designed to determine whether these derivatives would exhibit a protein content and quality advantage under New Zealand conditions and, by analysis of the protein composition, determine how the theories of protein component — quality relationships, developed in European winter wheats, relate to material of this sort. Establishing the generality of these theories in a novel genetic base would increase their acceptability as a generally applicable plant breeding tool.

MATERIAL AND METHODS

The trial contained twenty-three hexaploid wheat lines derived from the wild emmer selection G25, plus the high quality bread wheats BTL (Israel) and Oroua (New Zealand).

The trial was sown in the spring on a Templeton silt loam near Lincoln. The sowing rate was designed to sow 60 seeds per metre of row. The rows were 150 mm apart and each plot was of three rows 12 m long. A basal fertiliser dressing of 150 kg/ha of diammonium phosphate was applied (27 kgN/ha) just before sowing. Soil tests indicated that sulphur levels were adequate.

The derivatives were selections from four crosses:

Cross	Pedigree	No. of selections
V761	BTL /3/ N163 / G25 // M708	5
V763	BTL /3/ N163 / G25 // Merav	12
V766	BTL /3/ Merav// N163 / G25	5
V882	N163 / G25 //Merav /3/ V778-751	1

An initial cross to durum wheat (Nursit 163) was found to be necessary to maintain fertility in the derived lines. BTL is a high protein, high quality Israeli bread wheat. Merav and M708 both have Kenya and Gabo in their parentages and were included in the crosses to improve the yield potential of the derived lines. V778-751 was unavailable for study, so any protein components in the V882 selection not accounted for by the other three parents were ascribed to this bread wheat.

Quality tests

Flour protein content

Flour protein content was determined on a Technicon Infraalyser 400 near infra-red reflectance spectrophotometer, calibrated against Kjeldahl protein. Protein content is expressed as a weight percent on a 14% moisture basis.

Sodium dodecyl sulphate (SDS) sedimentation volume

SDS sedimentation volumes for all flours were determined according to the methods of Axford *et al.* (1978). This test measures the amount of SDS-insoluble glutenin protein; a parameter which has been shown to be related to breadmaking quality.

Work input requirement

Work input requirements of all flours were determined on a 125 g Mitchell mixer. Work input is a measure of the amount of mechanical mixing required to develop a dough to optimum consistency and is expressed as watt-hours per kilogram of dough. Work input is related to the rheological strength of the dough.

Mechanical dough development (MDD) baking test

Breadmaking quality was assessed by baking all flours on the Wheat Research Institute 125 g MDD test bake. In this method doughs are produced from 125 g of flour using optimum water absorption and work input requirements determined previously, proofed for 10 minutes at 32°C, moulded, proofed again for 40 minutes at 40°C, then baked for 30 minutes at 220°C. The resulting loaves are assessed for volume, by rape seed displacement.

Electrophoresis of grain proteins

Acidic polyacrylamide gel electrophoresis (apage)

Gliadin proteins were extracted from ground or crushed grain with 1M urea and separated on 2.5 — 27% polyacrylamide gels (Gradient Laboratories Ltd., Pyrmont, NSW, Australia) in sodium lactate buffer (pH 3.1) for 750 volt-hours (Du Cros and Wrigley 1979). The gels were fixed in 12% trichloroacetic acid and stained with Coomassie Brilliant Blue G 250 (0.025% w/v).

SDS polyacrylamide gel electrophoresis (SDS— page)

Total proteins were extracted from ground or crushed grain according to the methods of Lawrence and Shepherd (1980) and separated on 17% polyacrylamide gels according to the method of Payne *et al.* (1980). Gel dimensions were 70*70*3 mm, and bromophenol blue was used as the tracking dye. Gels were stained and destained as described by Lawrence and Shepherd (1980). The HMW glutenin subunits are visualised as a distinct group of 3-5 bands at the low mobility end of the gel.

The nomenclature given by Payne was used to identify the high molecular weight (HMW) glutenin subunits (Payne *et al.* 1980, 1984).

RESULTS AND DISCUSSION

High grain weights for all but four lines indicated that the grain was well filled and suitable for milling. This was not at the expense of protein accumulation, as all but two of the derivatives had protein contents higher than the standards.

Work input requirements ranged from very low (5.2) to very high (32.1). Most New Zealand bakeries would not be able to handle these very high work inputs due to plant limitations. However, the high correlation between work input and loaf volume ($r = 0.88$) suggests that dough strength, indicated by high work input is the basis for the excellent baking quality of these lines. It is probably unreasonable to expect that selections could be made that retained the same high loaf volumes at a lower work input level.

All but two of the derivatives had protein contents higher than the standards. This confirms that genes which influence protein content have been successfully transferred from the tetraploid emmer wheat, G-25, into the hexaploid wheats. The high correlation between protein content and loaf volume ($r = 0.73$) suggests that the increased grain nitrogen has maintained functionally important protein. This conclusion is supported by the high correlation between protein content and SDS sedimentation volume ($r = 0.70$). SDS volume indicates the amount of highly polymeric glutenin, which appears to be the most functionally important fraction of the gluten proteins.

Table 1. Gliadin protein composition groups for 23 wild emmer derivatives and average quality values for each group. (Figures in parentheses represent the number of derivatives in each group.)

Gliadin group	GLI-A1	GLI-B1	GLI-B2	WI	PROT	SDS	LV
A (5)	2	1	1	24.4	13.1	89	947
B (4)	1	1	1	28.7	14.6	88	941
C (3)	2	1	2	22.7	12.9	89	918
D (1)	1	2	1	16.4	12.9	72	882
E (2)	2	2	2	11.7	13.6	69	873
F (8)	1	1	2	7.8	11.8	51	767
OROUA	NA	1	NA	14.9	11.2	72	828
BTL	2	1	2	17.0	11.5	74	900
M708	1	1	2				
MERAV	1	1	2				
G-25	NA	2	1				
N-163	NA	NA	NA				

(WI = work input, PROT = protein content, SDS = SDS sedimentation volume, LV = loaf volume, NA = refers to allelic variants which do not occur in the derivatives being analysed.)

Protein composition and quality associations

Gliadin composition

The twenty-three derivatives can be classified into 6 groups on the basis of their gliadin composition. Synthesis of the gliadin proteins is controlled by six independent gene loci on the short arms of the group one and six chromosomes (Sozinov and Poperelya 1980). The six types of patterns observed in the emmer derivatives can be explained in terms of the variants at each of these six loci.

These derivatives only exhibit variation at three of the loci: Gli-A1, Gli-B1 and one of the group six loci, probably Gli-B2, and only two allelic variants were observed for each of these three gene loci. The third pair of alleles is difficult to positively assign as the region of the electrophoregram in which it occurs contains components coded for by all three group six gene loci. The composition of these six groups of derivatives is summarised in Table 1, along with average quality parameters for each of the groups.

1 and 2 are arbitrary designations for the observed variants. None of the gliadin protein components observed in the derivatives were inherited from the durum parent. Gli-B1(2) and Gli-B2(1) were inherited from the emmer parent G-25. The remaining alleles listed here and those from the three loci which exhibit no variation originate from the bread wheat parents, which were all very similar in protein composition.

The best ranking of gliadin components which is consistent with the quality data given is:

Gli-A1 (2) > Gli-A1 (1)
Gli-B1 (1) > Gli-B1 (2)
Gli-B2 (1) > Gli-B2 (2)

The order given here for the B1 alleles is in agreement with the results of Sozinov and Poperelya (1980), which show a B1 allele equivalent to our (1) to be associated with superior quality. The other components we have identified are difficult to exactly match to any of those identified by Sozinov and Poperelya (1980), probably due to the fact that they were characterised on quite different electrophoretic systems.

Protein composition groups can be ranked on the basis of these component rankings and according to their relative performance for the different quality parameters. These two ranks can be correlated to produce a Spearman's correlation coefficient (Kendall 1955). The results are:

Quality measure	Work input	Protein	SDS	Loaf volume
Spearman's coefficient	0.84ns	0.41ns	0.90*	0.93*

(ns — not significant, * — $P<0.05$, ** — $P<0.01$).

None of these coefficients could be increased by changing the rankings of the gliadin components, therefore this represents the best average relationship between gliadin composition and quality as determined by a range of test methods.

Glutenin composition

Eight types of HMW glutenin electrophoregrams were observed in the derivatives. Synthesis of the HMW glutenin polypeptides is controlled by three independent gene loci situated on the long arms of the homoeologous group one chromosomes and HMW glutenin composition can best be represented by considering the allelic variants expressed at each of these loci (Payne *et al.*, 1980). The composition of the derivative groups is summarised in Table 2, with relevant average quality data.

The durum polypeptides designated 13 + 16 appeared, upon close comparison with established bread wheat standards, to have slightly different mobilities to the usual hexaploid wheat 13 + 16, however, this designation is the best approximation under the present system of nomenclature. Subunit 1 in the derivatives may have been

Table 2. HMW glutenin composition groups for 23 wild emmer derivatives and average quality values for each group. (Figures in parenthesis represent the number of derivatives in each group.)

Glutenin Group	GLU-A1	GLU-B1	GLU-D1	WI	PROT	SDS	LV
A (8)	2*	7+8	5+10	26.6	13.4	89	945
B (1)	2*	13+16	5+10	13.4	13.6	67	936
C (1)	2*	7+8	2+12	28.1	15.9	90	936
D (3)	1	7+8	5+10	21.2	13.2	88	918
E (1)	2*	17+18	5+10	16.4	12.9	72	882
F (6)	2*	13+16	2+12	8.9	12.3	57	792
G (1)	1	13+16	2+12	7.2	12.4	50	756
H (2)	1	7+9	2+12	5.8	11.0	45	720
OROUA	2*	NA	5+10	14.9	11.2	72	828
BTL	2*	7+8	5+10	17.0	11.5	74	900
M708	2*	17+18	2+12				
MERAV	1	7+9	2+12				
G-25	1	NA	-				
N163	null	13+16	-				

(WI = work input, PROT = protein content, SDS = SDS sedimentation volume, LV = loaf volume, NA refers to subunits not present in any of the derivatives being studied.)

inherited from the wild emmer, G-25, or the bread wheat, Merav. There is some evidence to suggest that the emmer subunit has marginally lower mobility than the Merav subunit, however, at this stage the distinction cannot be made with any certainty. The other subunit apparent in G-25 could not be matched to any established bread wheat subunit and was not present in any of the derivatives studied.

The best ranking of glutenin components which is consistent with this quality data is:

Glu-A1 (2*) > Glu-A1 (1)
Glu-D1 (5+10) > Glu-D1 (2+12)
Glu-B1 (7+8) > Glu-B1 (13+16) > Glu-B1 (17+18) > Glu-B1 (7+9)

These results are in agreement with those found by European workers (Payne *et al.* 1984, Moonen *et al.* 1983). The rankings determined above lead to the following Spearman's correlation coefficients:

Quality measure	Work input	Protein	SDS	Loaf volume
Spearman's coefficient	0.86*	0.84*	0.86*	0.97**

The coefficients for work input and SDS-sedimentation increase to r(s) = 0.93 if Glu-B1(17+18) is considered as greater in it's quality contribution than Glu-B1 (13+16), however, this inversion causes corresponding decreases in the coefficients for protein content and loaf volume (r(s) = 0.71 and 0.86 respectively). Considering the small populations being analysed it is probably safest to rate these two alleles as equivalent with regard to wheat quality. This is in agreement with the conclusions reached by Payne *et al.* (1984).

CONCLUSIONS

Despite the inclusion of genetic material from an alien gene pool, these derivatives appear to fit well into the current theories of protein composition — quality relationships proposed by a number of workers for European wheats. The same protein components associated with quality in European winter wheat appear to be associated with quality in Israeli wheats containing alien genes. The generality of these relationships support their usefulness as a tool for parental selection and as an aid in early generation screening by plant breeders.

The fact that most of the derivatives have higher protein contents than the high protein hexaploid parent (BTL) confirms the inclusion of genes from the tetraploid wheats which influence protein accumulation in the grain. However, a relatively wide range of protein contents still exists within the emmer derivatives and appears to be related to the presence of quality associated protein components, particularly HMW glutenin subunits derived mainly from the hexaploid wheats.

Two possible hypotheses exist. This could be due to additional protein enhancing genes which may be linked to specific protein components, such as the HMW glutenin subunits 2*, 5+10 and 7+8. Alternatively, it could be due to protein suppressing genes which may be linked to other protein components, such as the subunits 1, 2+12 and 7+9. Protein suppressing genes have been hypothesised as being associated with the D-genome of hexaploid wheats.

It appears possible, through this sort of breeding approach, to select simultaneously for increased protein content and desirable protein composition. In fact, there seems to be some degree of synergism associated with these dual breeding strategies.

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SYMPOSIUM DISCUSSION

Dr W. Bushuk, University of Manitoba

Are the high work of inputs of the landraces explained by a qualitative relationship with the glutenin sub-units or was there a quantitative relationship involved in terms of the amount of this protein or perhaps in terms of molecular weight distribution of this component.

Cressey

I cannot make any comment on the relative amounts of the protein components. As far as the molecular weight distribution goes, if the S.D.S. sedimentation test is taken to be an indication of the amount of high molecular weight glutenin polymer, then there was a strong relationship between work input and S.D.S. sedimentation, so molecular weight distribution could be explained.

Prof. D. von Wettstein, Carlsberg Laboratory

I read a very interesting paper recently by Dr Pyrav who studied isoenzymes among wheats — dicotyl, durum and aestivum wheats. The isoenzymes found in emmer wheats with regard to carbohydrate metabolism and coding the chromosomes, were not present in the aestivum or durum wheats. This would seem to intimate that either this gene has been changed since the genomes came together, or the old idea that emmer is one of the paired genomes is not correct. Have you got any idea by studying these high molecular weight glutenin patterns whether these isozymes are found in present day wheats.

Cressey

That is a very interesting question. Certainly I have found a lot of high molecular weight glutenin components in wild emmer populations which have never been recorded in cultivated hexaploid wheats. But on the other hand, that does not discount the idea that emmer may have been the progenitor of modern wheats. The number of emmer accessions which were involved in the initial hybridisation may have been relatively small and the components in hexaploid wheats came from those. The variations seen in wild emmer wheats may just represent accessions which never hybridised.