Identification and Characterisation of a Novel Glutenin Subunit in Bread Wheat (*Triticum aestivum* L.)

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List of Abbreviations

AACC	American Association of Cereal Chemists
AGT	Australian Grain Technologies
A-PAGE	Acid polyacrylamide gel electrophoresis
AWCC	Australian Winter Cereals Collection
CSIRO	Australian Commonwealth Scientific and Research Organisation
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
EBI	European Bioinformatics Institute
EPP	SDS-extractable polymeric protein
GRIS	Genetic Resources Information System
HMW-GS	High molecular weight glutenin subunit
HPLC	High performance liquid chromatography
HRM	High resolution melting
IPTG	Isopropyl-β-*D*-thiogalactopyranoside
ISBP	Insertion site-based polymorphism
LB	Lysogeny broth
LC-MS	Liquid chromatography-mass spectrometry
LMW-GS	Low molecular weight glutenin subunit
LTR	Long terminal repeat
MAR	Matrix attachment region
MAS	Molecular assisted selection
M_r	Molecular weight

NCBI	National Center for Biotechnology Information
NIRS	Near- infrared spectrophotometry
PCR	Polymerase chain reaction
PEB	Phosphate extraction buffer
PPK	Putative protein kinase
PVDF	Polyvinylidine difluoride
R_{max}	Extensograph maximum resistance
RP-HPLC	Reverse-phase high performance liquid chromatography
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SE-HPLC	Size-exclusion high performance liquid chromatography
SNP	Single nucleotide polymorphism
TCA	Trichloroacetic acid
TFA	Trifluoroacetic acid
TPP	Total polymeric protein
UPP	SDS-unextractable polymeric protein in total polymeric protein
UV	Ultraviolet
4-VP	4-vinylpyridine

Table of Contents

List of Tables

List of Figures

List of Appendices

Abstract

Bread is one of the major constituents of the human diet and wheat (*Triticum aestivum* L.) is the most important cereal for bread making. The gluten proteins (glutenins and gliadins) are recognised as important components affecting the processing quality of wheat flour. *Glu-B1al* is an allele that includes a duplication of a gene encoding an x-type high-molecular-weight glutenin subunit, and is thought to increase dough strength through overexpression of that subunit. In this research, a particular glutenin subunit in an Australian cultivar, H45, was investigated. H45 seemed to carry *Glu-B1al*, but it has relatively low unextractable polymeric protein (UPP, an indicator of weak dough). Two Bx genes from H45 were cloned and sequenced. Their sequences differ from each other, and each differ by four single nucleotide polymorphisms (SNPs) from the sequence of the Bx genes of *Glu-B1al* in the Canadian wheat cultivar Glenlea. One of the SNPs leads to an extra cysteine residue in one of the subunits. The *Glu-B1* allele of H45 was designated *Glu-B1br*.

With a restriction digest assay designed to distinguish the *Glu-B1br* allele from other overexpression alleles, it was demonstrated that *Glu-B1br* is co-inherited with low UPP. Among accessions present in the pedigree of H45 and accessions carrying overexpression alleles, *Glu-B1br* was detected only in H45.

Efforts were made to develop alternative markers for *Glu-B1br*. Potential polymorphic regions within or close to *Glu-B1* locus were investigated, but no closely linked polymorphisms were found that could be targeted for marker design.

Individual glutenin subunits encoded by overexpression alleles and a mutant gene (*MutBx7.1*)

derived from the first gene (*Bx7.1*) of *Glu-B1br* were obtained by heterologous expression. Flour incorporation tests showed that the glutenin subunit with the extra cysteine residue (Bx7.1) affects flour and dough mixing properties differently from MutBx7.1 and from the Bx subunits encoded by other overexpression alleles. Given that Bx7.1 and MutBx7.1 differ only with respect to the additional cysteine in Bx7.1, the effects of Bx7.1 on the dough properties of H45 can be attributed directly to that cysteine, which may act by impeding polymerisation.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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