Characterisation of glutenin subunits in spelt wheat (*Triticum aestivum* ssp. *spelta* L)

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INTRODUCTION

The allelic variants of the loci controlling the high and low molecular weight glutenins are the most important determinants of the genetic differences in various quality attributes, especially dough strength, extensibility and dough development time, in common wheat. Since its introduction, the Payne-score has become an essential tool to predict the genetic potential of quality attributes. Recently, methods involving both the high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) alleles have been developed with improved predicting strength and underlining the importance of the contribution of both kinds of glutenin subunits and their interactions in the final quality [1, 4, 7, 14]. However, their role in spelt wheat (*Triticum aestivum ssp. spelta* genome: AABBSS) has not been well established. Spelt flour is used mainly for speciality bread, pasta, muesli, flakes and other baked products. In order to improve its dough characteristics, it is important to understand the extent of genetic variability in both HMW-GS and LMW-GS [6]. These subunits have been traditionally profiled in wheat using various methods such as SDS-polyacrylamide gel electrophoresis (SDS-PAGE), capillary electrophoresis and HPLC [5]. Recently, a new powerful methodology, Matrix-Assisted Laser Desorption/Ionization Time-of-Flight mass spectrometry (MALDI-TOF) has been introduced with significantly better resolution, throughput and reproducibility [12, 16].

MATERIAL AND METHODS

A collection of 98 spelt accessions representing various parts of the world, were procured mainly from the Australian Winter Cereals Collections, (NSW DPI Tamworth) and were analysed for their glutenin allele composition using two independent methodologies: (1) traditional SDS- polyacrylamide gel electrophoresis and (2) MALDI-TOF analysis. Standard varieties of common wheat such as Chinese Spring, Cheyenne, Rosella, Diamondbird, Gabo and of durum (*Triticum turgidum* ssp. durum, cultivar Bellaroi) were included as standard checks for HMW and LMW subunits.

The total polymeric proteins were extracted from 10 mg ground seed, according to the method described previously [8]. LMW subunits were resolved after reduction and alkylation as described previously ([9, 17]. Allele composition of glutenin subunits were determined using SDS-PAGE method [11]. All the glutenin alleles were scored according to the nomenclature described [8]. For MALDI-TOF analysis, the extraction of glutenin protein, reduction/alkylation, separating HMW and LMW GS and initial samples preparation were carried-out according to the methods [10, 13, 15], respectively. MALDI-TOF mass spectrometric experiments were carried out on a Voyager DE-PRO TOF mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with UV nitrogen laser (337 nm). The instrument was used with the following parameters: laser intensity 2 500, mass range 50-100 kDa, acceleration voltage 25 kV, grid voltage 93%, guide-wire 0.2%, delay time 850 ns. Spectra were obtained in positive linear ion mode and were averaged from 50 laser shots to improve the signal to noise level. All the samples were automatically accumulated in a random pattern over the sample spot to provide the final spectrum. A purpose-made computer program has been developed to automatically identify HMW and LMW GS alleles from the spectra [2].

RESULTS AND DISCUSSION

Glutenin subunit composition among the 98 lines showed extensive polymorphism, covering 64 different allelic combinations of the six loci. The maximum glutenin polymorphism was observed at *GluB1* locus (Table 1). A number of unique alleles: *Glu-A1d, Glu-A1e, Glu-B1l, Glu-B1m, Glu-B1n, Glu-D1h, Glu-D1I* and *Glu-D1j* were identified (Table 1). The most frequent alleles within spelt germplasm were *GluA1a* (72.4%) and GluD1a (68.4%) ,Table 1. MALDI-TOF analysis of the spelt samples also allowed characterisation of the spelt germplasm for HMW-GS

and LMW-GS. Based on the MALDI-TOF profiles, the population clustered into four distinct subgroups. At *Glu-A1*, *Glu-B1* and *Glu-D1* loci five, ten and six alleles were observed, respectively. LMW-GS displayed similar polymorphism, as five alleles were identified at both the *Glu-A3* and *Glu-B3* loci. Four alleles were observed at the *Glu-D3* locus. An almost perfect correspondence was seen between alleles identified with SDS-PAGE and MALDI-TOF.

These results were compared to the AACCI database covering the glutenin allele data of about 8000 bread-wheats from around the world [3]. Four of the 21 HMW-GS identified alleles were not present in any common bread wheats and can be characterised as specific for spelt. The level of polymorphism and the distribution of alleles at other loci, however, seem to be similar to common bread wheats, with one exception: the most frequent allele on *Glu-B*1 is the '*f*' allele (30.22%), followed by '*k'* (26.53%) and '*b'* (20.41%). These results suggest that the genetic variation of the HMW and LMW glutenin alleles within spelt wheat can be used further for improvement of spelt baking properties.

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Table 1: Frequency of HMW-GS alleles determined using SDS-PAGE in spelt accessions

Locus	Allele*	Glutenin Subunit	Allele frequency (%)	Accession Number
Glu-A1	*Glu-A1a*	1	72.4	
	Glu-A1b	2	0.3	
	Glu-A1c	Null	20.0	
	Glu-A1d	**1+2**	**2.0**	**10, 11**
	Glu-A1e	**>1+a**	**2.0**	**12, 16**
Glu-B1	*Glu-B1a*	7	5.1	
	Glu-B1b	7+8	19.4	
	Glu-B1c	7+9	11.2	
	Glu-B1d	6+8	5.1	
	Glu-B1f	13+16	27.5	
	Glu-B1j	21	1.0	
	Glu-B1k	22	2.0	
	Glu-B1l	**6+22**	**26.5**	**18, 20, 21, 23, 30, 34, 36, 37, 61, 63, 65, 68, 69, 71, 76, 77, 78, 79, 80, 81, 82, 83. 84, 85**
	Glu-B1m	**6+9**	**1.0**	**27**
	Glu-B1n	***13+8***	**1.0**	**51**
Glu-D1	*Glu-D1a*	2+12	68.4	
	Glu-D1b	3+12	2.0	
	Glu-D1d	5+10	14.3	
	Glu-D1e	2+10	1.0	
	Glu-D1g	2+11	1.0	
	Glu-D1h	**>1+10**	**1.0**	**16**
	Glu-D1i	**6+12**	**4.1**	**56, 58, 60, 86**
	Glu-D1j	**Null**	**8.2**	**87, 89, 90, 91, 92, 93, 96, 98**

*Alleles in bold are unique allele found in spelt accessions.

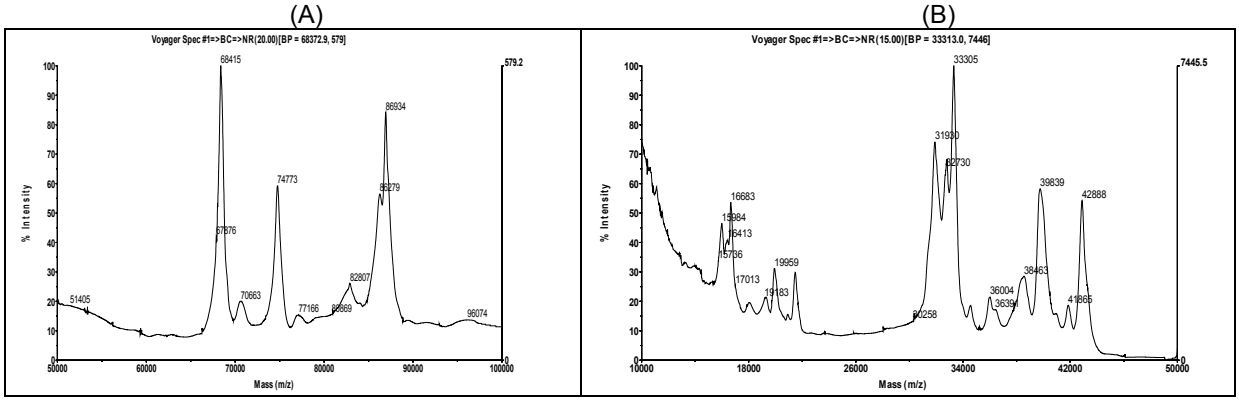

Figure 1. Typical MALDI-TOF separations of HMW (A) and LMW glutenins (B) in spelt wheat.