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ORIGINAL PAPER

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Development and validation of a PCR-based marker assay for negative selection of the HMW glutenin allele *Glu-B1-1d* (*Bx-6*) in wheat

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Abstract Polymorphisms between the coding sequences of high-molecular-weight (HMW) glutenin x-type genes at the *Glu-1* locus were used to amplify *Glu-1B* x-type-specific PCR fragments. PCR analysis in a wheat cultivar subset carrying different *Glu-1B* x-type alleles resulted in PCR fragments that differed in size for *Glu-B1-1d* (*B-x6*) and non-*Glu-B1-1d* (*B-x6*) genotypes. Subsequent sequencing analysis revealed a 15-bp in-frame insertion in the coding regions of all *Glu-B1-1d* (*B-x6*) genotypes which allowed the development of a *B-x6*-specific PCR assay for high-throughput allele sizing by ion-pair reversed-phase high-performance liquid chromatography. The assay was validated in a set of 86 German wheat cultivars, and genotyping data unequivocally verified the presence of HMW glutenin subunits GLU-B1-1D (*Bx-6*) + GLU-B1-2A (*By-8*) by means of sodium dodecyl sulphate-polyacrylamide gel electrophoresis. These results demonstrate that the PCR assay can be applied for the detection and negative selection of the 'poor breadmaking quality' *Glu-B1-1d* (*B-x6*) alleles in wheat breeding programs.

Introduction

The flour quality in common wheat depends on the composition of two major seed storage protein fractions, the glutenins and the gliadins. Of these, the high-molecular-weight (HMW) glutenins significantly influence dough strength and elasticity (Payne et al. 1981). The HMW glutenin subunits (GS) are encoded by six genes located at complex *Glu-1* loci on the long arms of homologous chromosomes 1A, 1B and 1D. Two tightly linked genes are present at each *Glu-1* locus, each of which encodes two types of HMW subunits: one of a higher molecular weight, designated as the x-type subunit, and the other of a lower molecular weight, designated as the y-type subunit (Harberd et al. 1986). These subunits display extensive variation, and their allelic composition is firmly associated with breadmaking quality. In particular, the GLU-D1-1D (*Dx-5*) + GLU-D1-2B (*Dy-10*) subunit pair at the *Glu-D1* locus contributes to good breadmaking quality, while the GLU-A1-1B (*Ax-1*) and GLU-B1-1A (*Bx-7*) subunits at the *Glu-A1* and *Glu-B1* loci, respectively, have been found to influence the breadmaking properties of wheat (Payne et al. 1987; Weegels et al. 1996).

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of seed protein is routinely used to analyse the composition of HMW glutenin subunits at the protein level (Bietz et al. 1975). This method is based on differences in molecular mass of the single protein subunits and allows the simultaneous assessment of complete HMW glutenin profiles. However, SDS-PAGE can only be carried out with extracted protein fractions from seeds. Furthermore, SDS gels are not well suited for automated high-throughput allele genotyping due to the need of manual interventions. The application of DNA markers for discriminating HMW glutenin subunits offers several advantages over the traditional testing method. The latter are detectable at all stages of plant growth and are not affected by the environment. PCR-based molecular markers in particular provide a powerful tool for high-throughput and cost-efficient genotyping due to their high potential for automation.

The availability of nucleotide sequences of different HMW glutenin alleles has enabled PCR-based methods to be used for assessing allelic variation at the *Glu-1* loci.

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With respect to *Glu-D1* and *Glu-A1*, several PCR assays have already been developed to detect the 'good-quality' HMW glutenin subunit alleles *Glu-D1-1d* (*Dx-5*; D'Ovidio and Anderson 1994; Smith et al. 1994; Ahmad 2000; De Bustos et al. 2000, 2001; Radovanovic and Cloutier 2003; Schwarz et al. 2003), *Glu-A1-1b* and *Glu-A1-1c* (*Ax-1* and*Ax-2**; Lafiandra et al. 1997; De Bustos et al. 2000, 2001; Radovanovic and Cloutier 2003), respectively. In the case of the *Glu-B1* locus, the HMW subunit pairs GLU-B1-1A (*Bx-7*) + GLU-B1-2A (*By-8*), GLU-B1-1A (*Bx-7*) + GLU-B1-2B (*By-9*) and GLU-B1-1H (*Bx-17*) + GLU-B1-2F (*By-18*) were found to be correlated with good breadmaking quality, whereas the combination GLU-B1-1D (*Bx-6*) + GLU-B1-2A (*By-8*) is associated with poor breadmaking properties (Branlard and Dardevet 1985; Payne et al. 1987; Lawrence et al. 1988). PCR-based assays have recently been developed to discriminate between chromosome 1B-encoded subunits (Ahmad 2000; Ma et al. 2003; Butow et al. 2003), but these are not aimed at identifying 'poor quality' GLU-B1-1D (*Bx-6*) subunits.

In the investigation reported here, we looked for DNA polymorphisms between the HMW glutenin alleles *Glu-B1-1d* (*Bx-6*), *Glu-B1-1a* (*Bx-7*) and *Glu-B1-1 h* (*Bx-17*). We found an in-frame insertion in the coding regions of *Glu-B1-1d* (*Bx-6*) alleles that enabled us to develop a *Bx-6*-specific PCR assay for high-throughput allele sizing by ion-pair reversed-phase high-performance liquid chromatography (IP-RP-HPLC). The assay was validated in a set of 86 German wheat cultivars.

Materials and methods

Plant material

The nullitetrasomics N1AT1B, N1BT1A and N1DT1A of *Triticum aestivum* cv. Chinese Spring were used for chromosome assignment of the PCR products. Absence of a band in a particular nullitetrasomic line indicates the chromosome harbouring the corresponding sequence. IP-RP-HPLC allele sizing was carried out in a set of 86 *T. aestivum* common wheat and three *T. turgidum* durum wheat cultivars (Table 1). Data for HMW glutenin subunit constitutions were obtained from the Section for Seed Certification of the Bavarian State Research Center for Agronomy and the GrainGenes database (http://wheat.pw.usda.gov).

Table 1 HMW glutenin subunits at the *Glu-B1* locus of selected German wheat cultivars

	Cultivar	*Glu-B1* subunits		Cutivar	*Glu-B1* subunits		Cultivar	*Glu-B1* subunits
1	Agent	6+8	30	Corvus	6+8	59	Naxos	7+9
2	Alidos	17+18	31	Darwin	6+8	60	Olivin	7+9
3	Altos	17+18	32	Dekan	6+8	61	Ortler	7+9
4	Andros	6+8	33	Devon	7+9	62	Pagode	7+9
5	Anemos	7+9	34	Dream	7+9	63	Pegassos	7+9
6	Apollo	6+8	35	Drifter	7+8	64	Petrus	7+9
7	Applaus	7+9	36	Ebi	7+8	65	Piko	6+8
8	Ares	6+8	37	Estica	6+8	66	Previa	6+8
9	Aristos	7+9	38	Eta	7+9	67	Quattro	7+9
10	Armada	6+8	39	Flair	6+8	68	Ramiro	7+9
11	Aron	7+9	40	Florida	6+8	69	Rektor	7+9
12	Astron	7+9	41	Futur	7+8	70	Ritmo	6+8
13	Atlantis	6+8	42	Glockner	7+9	71	Romanus	6+8
14	Batis	7+9	43	Gorbi	6+8	72	Semper	7+9
15	Benno	6+8	44	Granada	6+8	73	Skater	6+8
16	Biscay	6+8	45	Habicht	6+8	74	Sperber	7+9
17	Bold	6+8	46	Herzog	7+9	75	Tambor	7+9
18	Borenos	7+9	47	Ibis	7+9	76	Tarso	7+9
19	Borneo	7+9	48	Jubilar	6+8	77	Terrier	6+8
20	Bovictus	7+9	49	Kanzler	7+8	78	Thasos	7+9
21	Bussard	7+9	50	Kontrast	17+18	79	Toni	6+8
22	Caesar	7+9	51	Kornett	7+9	80	Toronto	7+9
23	Cardos	7+9	52	Kris	7+9	81	Tower	6+8
24	Certo	6+8	53	Kronjuwel	6+8	82	Trakos	7+9
25	Clan	6+8	54	Maverick	17+18	83	Vergas	6+8
26	Combi	7+9	55	Melon	7+8	84	Winni	7+9
27	Contra	6+8	56	Mikon	7+9	85	Xanthos	6+8
28	Convent	6+8	57	Monopol	7+9	86	Zentos	7+9
29	Cortez	7+8	58	Munk	7+9			

DNA isolation and PCR amplification

Genomic DNA from kernels was extracted and purified as described by Schwarz et al. (2003). PCR was carried out on a PRIMUS 96 advanced thermocycler (Peqlab) in 20-µl reaction volumes containing 50 ng genomic DNA, 1.5 m*M* $MgCl_2$, 0.2 µ*M* of each of primer bx7-f (5′-cactgagatggc-taagcgcc-3′) and bx7-r (5′-gccttggacggcaccacagg-3′), 0.2 m*M* dNTP and 0.5 U thermostable proofreading polymerase Optimase (Transgenomic) in 1× PCR reaction buffer supplied by the manufacturer. PCR products were amplified using following conditions: an initial denaturation for 2 min at 95°C; 35 cycles of 30 s at 95°C, 30 s at 50°C and 1 min at 72°C; a final extension for 5 min at 72°C.

Sequence analysis

PCR products were sequenced in both directions with the bx7-f and bx7-r primers on an ABI377 platform (Applied Biosystems, Foster City, Calif.) using standard dye terminator chemistry (Amersham-Pharmacia Biotech, Piscataway, N.J.). Editing of DNA sequences was performed with SEQUENCE NAVIGATOR software (Applied Biosystems), and the multiple sequence alignment tool CLUSTALW (Thompson et al. 1994; http://www.ebi.ac.uk/clustalw) was used for evaluating polymorphisms. Similarity searches were carried out using BLAST algorithms (Gish and States 1993).

Agarose gel analysis

PCR products were separated by electrophoresis on 3.0% Metaphor agarose gels (FMC BioProducts) in TAE buffer containing ethidium bromide.

IP-RP-HPLC analysis

IP-RP-HPLC analysis was performed on a fully automated WAVE Nucleic Acid Fragment Analysis System (Transgenomic). Unpurified PCR reactions were automatically loaded on the column with an autosampler. The stationary phase consisted of a DNA SEP Column System (Transgenomic) filled with alkylated nonporous poly (styrene divinylbenzene) particles. The column's mobile phase consisted of a mixture of 0.1 *M* triethylamine acetate (pH 7.0) with (buffer A) or without 25% acetonitrile (buffer B). DNA fragments were eluted with a linear acetonitrile gradient of 0.5% per minute at a flow rate of 0.75 ml/min and detected by 260 nm absorbance. A column temperature of 50°C was used for sizing the PCR fragments. At non-denaturing conditions the DNA separation is based solely on fragment size and is independent of sequence. The application of an increasing acetonitrile gradient releases the sample in order of increasing length. The analysis took 8 min, including column regeneration and re-equilibration to the starting conditions. IP-RP-HPLC data were managed with NAVIGATOR SOFTWARE ver. 1.5.2 (Transgenomic).

Results and discussion

Amplification and characterisation of *Glu-1B* x-type-specific PCR fragments

The coding sequences of *Glu-A1-1b* (*Ax-1;* Halford et al. 1992; EMBL Accession No. X61009), *Glu-A1-1c* (*Ax-2** ; Anderson and Greene 1989; M22208), *Glu-B1-1cs* (*Bx-1* ; Schlumbaum et al. 1998; Y10954), *Glu-B1-1a* (*Bx-7* ; Anderson and Greene 1989; X13927), *Glu-D1-1a* (*Dx-2* ; Sugiyama et al. 1985; X03346) and *Glu-D1-1d* (*Dx-5* ; Anderson et al. 1989; X12928) were compared with each other. The CLUSTALW alignment revealed polymorphisms between the HMW glutenin x-type genes from the A, B and D genomes, which allowed the selection of *Glu-1B* x-type-specific PCR primers bx7-f and bx7-r exhibiting allele-specific mismatch 3′-residues (Fig. 1). The amplification of HMW glutenin x-type gene sequences from the A and D genomes was successfully suppressed, as demonstrated by PCR analysis of nullitetrasomic lines (Fig. 2). The lack of amplification of the expected 321-bp PCR fragment in N1BT1A of *T. aestivum* cv. Chinese

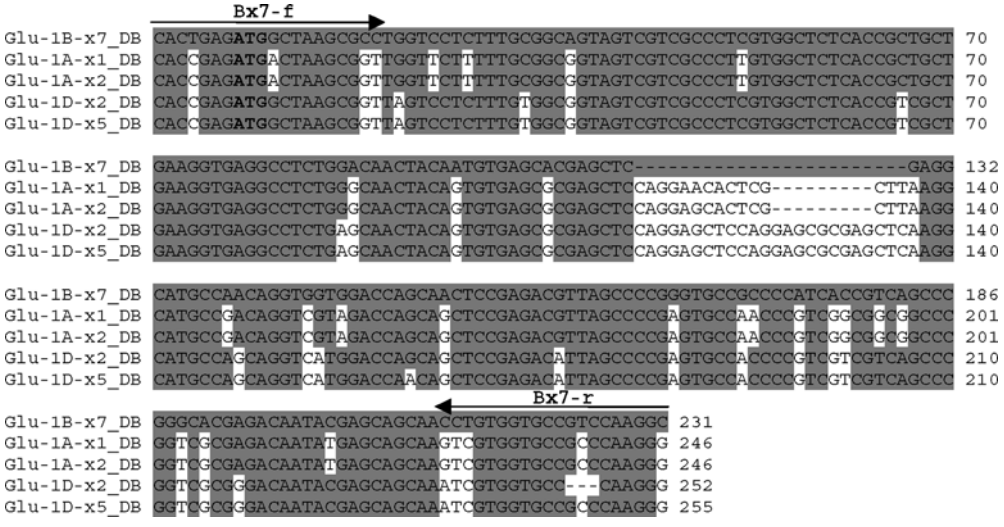

Fig. 1 Comparative alignment of the coding sequence of HMW glutenin x-type alleles at the *Glu-1* locus from public databases (*DB*). The primer binding sites for *Glu-B1* x-type-specific amplification are indicated by *arrows*. The start codon is indicated in *bold*

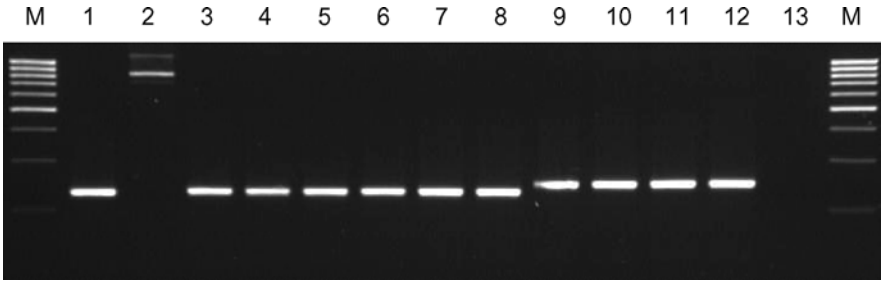

Fig. 2 Chromosomal assignment and allele-specific analysis of the *Glu-B1* x-type amplicons. *Lanes*: *1* Nullitetrasomic line N1AT1B, *2* nullitetrasomic line N1BT1D, *3* nullitetrasomic line N1DT1B, *4* Chinese Spring, *5–7 Glu-B1-1a* (*Bx-7*) wheat varieties, *8 Glu-B1-1 h* (*Bx-17*) wheat variety, *9–12 Glu-B1-1d* (*Bx-6*) wheat varieties, *13* control (H_2O), *M* 100-bp ladder

Spring proved its allocation to chromosome 1B. PCR analysis in a wheat cultivar subset carrying *Glu-B1-1d* (*Bx-6*), *Glu-B1-1a* (*Bx-7*) and *Glu-B1-1 h* (*Bx-17*) alleles, respectively, resulted in different PCR fragment sizes for the *Glu-B1-1d* (*Bx-6*) and non-*Glu-B1-1d* (*Bx-6*) genotypes (Fig. 2). Subsequent sequencing and comparative analysis of three *Glu-B1-1a* (*Bx-7*) PCR fragments (amplified with DNA from wheat cvs. Monopol, Bussard and Combi), one *Glu-B1-1 h* (*Bx-17*) PCR fragment (wheat cv. Alidos) and four *Glu-B1-1d* (*Bx-6*) PCR fragments (wheat cvs. Florida, Bold, Flair and Apollo) confirmed a 15-bp insertion in the coding regions of all *Glu-B1-1d* (*Bx-6*) genotypes (Fig. 3). The inserted nucleotide sequence is a duplication of the adjacent 5′ sequence plus some additional bases to create a short palindrome at the 5′ end of the insertion/duplication. The insertion was in-frame and encodes for the deduced amino acid sequence, RKREL. A basic sequence similarity search (BLASTP) was conducted with the full length of the *Glu-B1-1d* (*Bx-6*) PCR fragment. The deduced amino acid sequence exhibited highest identities with several partial HMW glutenin subunit protein sequences of two wheat subspecies: two GLU-BX subunits of *T. turgidum* (SWALL Accession No. AAQ93629, Q8RVX0) and several GLU-B1-1 subunits of *T. spelta* genotypes (Q7XAI0–Q7XAI910, Q7XAJ0–Q7XAJ5, Q7X8G5, Q7X8G6, Q7XZH9), all of them featuring the RKREL insertion. Therefore, PCR analysis was carried out with DNA from *T. turgidum* cvs. Creso, Mondur and Combo. All three cultivars showed the RKREL insertion of the in durum wheat quite common *Glu-B1-1d (Bx-6)* allele. Since the in-frame insertion might change the three-dimensional structure of the glutenin subunit proteins, a functional association with baking quality properties can be assumed. In addition to the insertion, two single nucleotide polymorphisms (SNP) were found in the *Glu-B1-1d* (*Bx-6*) fragment sequence (Fig. 2). The polymorphism at nucleotide position 105 leads to an amino acid exchange from Arg to His, whereas a SNP at nucleotide position 211 is a silent third base substitution. No sequence variations were found between genotypes carrying the *Glu-B1-1a* (*Bx-7*) and *Glu-B1-1 h* (*Bx-17*) alleles, respectively, and the corresponding *Glu-B1-1a* (*Bx-7*) sequence from the public database.

IP-RP-HPLC genotyping

For automated fragment sizing of *Glu-1B* x-type-specific PCR fragments we used IP-RP-HPLC. In order to test the reproducibility of allele sizing, we calculated the average retention times and standard deviations of 20 repeated runs with *Glu-B1-1d* (*Bx-6*) and *Glu-B1-1a* (*Bx-7*) fragments. Highly reproducible averaged retention times of 4.000 min ($\sigma = 0.018$) and 4.157 min ($\sigma = 0.035$) were found for the larger *Glu-B1-1d* (*Bx-6*) and smaller *Glu-B1-1a* (*Bx-7*) fragments. Thus, a difference in length of only 15 bp (6%) resulted in an average difference in retention time of 0.157 min (Fig. 4), which allowed the unambiguous and automated discrimination of *Glu-B1-1d* (*Bx-6*) and *Glu-B1-1a* (*Bx-7*) alleles with the NAVIGATOR SOFTWARE ver. 1.5.2 (Transgenomic). Our observations were in agreement those of Huber et al. (1995) who thoroughly studied the precision and reproducibility of IP-RP-HPLC with respect to fragment sizing and were able to resolve DNA fragments differing only 2–3% in length.

Fig. 3 Comparative alignment of the *Glu-1B* x-type PCR fragment nucleotide sequences (*SQ*) and *Glu-B1-1a* (*Bx-7*) sequence from public databases (*DB*)

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Glu-1B-x7_DB   CACTGAGATGGCTAAGCGCCTGGTCCTCTTTGCGGCAGTAGTCGTCGCCCTCGTGGCTCTCACCGCCGCT 70
Glu-1B-x7_SQ   CACTGAGATGGCTAAGCGCCTGGTCCTCTTTGCGGCAGTAGTCGTCGCCCTCGTGGCTCTCACCGCCGCT 70
Glu-1B-x17_SQ  CACTGAGATGGCTAAGCGCCTGGTCCTCTTTGCGGCAGTAGTCGTCGCCCTCGTGGCTCTCACCGCCGCT 70
Glu-1B-x6_SQ   CACTGAGATGGCTAAGCGCCTGGTCCTCTTTGCGGCAGTAGTCGTCGCCCTCGTGGCTCTCACCGCCGCT 70

Glu-1B-x7_DB   GAAGGTGAGGCCTCTGGACAACTACAATGTGAGCACGAGCTC--------------GAGGCATGCCAAC 125
Glu-1B-x7_SQ   GAAGGTGAGGCCTCTGGACAACTACAATGTGAGCACGAGCTC--------------GAGGCATGCCAAC 125
Glu-1B-x17_SQ  GAAGGTGAGGCCTCTGGACAACTACAATGTGAGCACGAGCTC--------------GAGGCATGCCAAC 125
Glu-1B-x6_SQ   GAAGGTGAGGCCTCTGGACAACTACAATGTGAGCGCGAGCTCCGGAAGCGCGAGCTCGAGGCATGCCAAC 140

Glu-1B-x7_DB   AGGTGGTGGACCAGCAACTCCGAGACGTTAGCCCCGGGTGCCGCCCCATCACCGTCAGCCCGGGCACGAG 195
Glu-1B-x7_SQ   AGGTGGTGGACCAGCAACTCCGAGACGTTAGCCCCGGGTGCCGCCCCATCACCGTCAGCCCGGGCACGAG 195
Glu-1B-x17_SQ  AGGTGGTGGACCAGCAACTCCGAGACGTTAGCCCCGGGTGCCGCCCCATCACCGTCAGCCCGGGCACGAG 195
Glu-1B-x6_SQ   AGGTGGTGGACCAGCAACTCCGAGACGTTAGCCCCGGGTGCCGCCCCATCACCGTCAGCCCGGGCACGAG 210

Glu-1B-x7_DB   ACAATACGAGCAGCAACCTGTGGTGCCGTCCAAGGC 231
Glu-1B-x7_SQ   ACAATACGAGCAGCAACCTGTGGTGCCGTCCAAGGC 231
Glu-1B-x17_SQ  ACAATACGAGCAGCAACCTGTGGTGCCGTCCAAGGC 231
Glu-1B-x6_SQ   GCAATACGAGCAGCAACCTGTGGTGCCGTCCAAGGC 246
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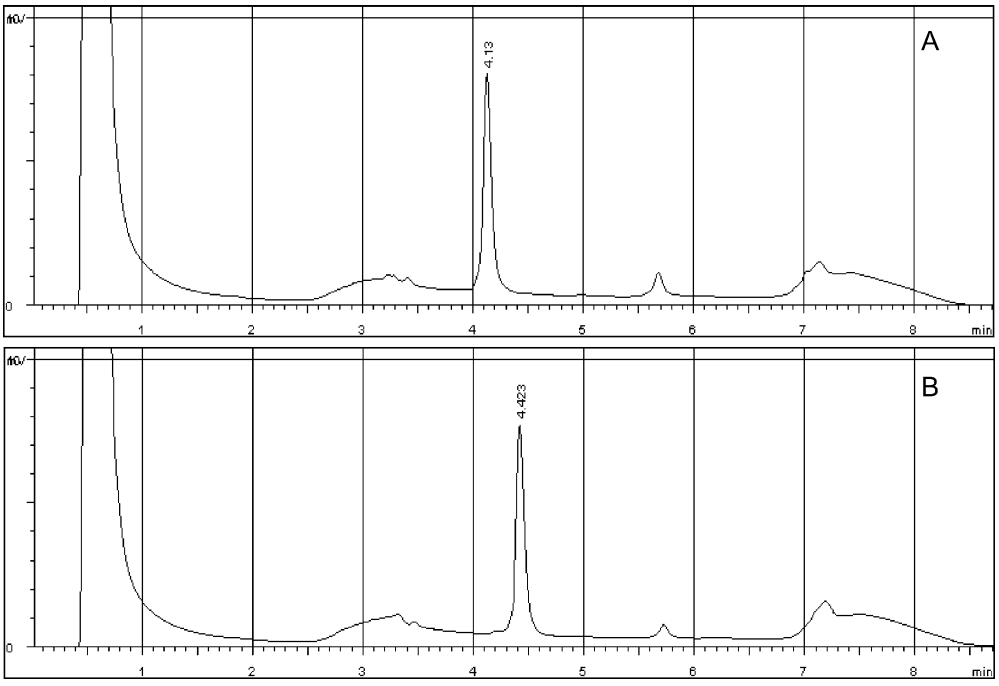

Fig. 4 IP-RC-HPLC elution profiles of PCR fragments generated with template DNA of wheat varieties containing the-*Glu-B1-1a* (*Bx-7*) (**A**) and *Glu-B1-1d* (*Bx-6*) (**B**) alleles, respectively

This excellent size-dependent separation efficiency makes IP-RP-HPLC also applicable for sizing DNA microsatellites. Devaney et al. (2000) optimised IP-RP-HPLC conditions (column temperature, flow rate, percentage organic modifier per minute) for genotyping short tandem repeats and achieved a resolution of fragments that differed as little as 1% in chain length. Alternative and frequently used methods for high-throughput allele sizing are multi-color fluorescence-based DNA analysis systems for example, the GeneScan/Genotyper platform of Applied Biosystems (Schwarz et al. 2000; Rampling et al. 2001). The use of fluorescently labelled primers or nucleotides with distinguishable wavelength emissions different PCR fragments to be amplified in one reaction (multiplexing) and different samples to be electrophoresed simultaneously (multimixing). Although multifluorophore genotyping systems provide the capability to analyse hundreds of samples per day, the costs per sample of this type of analysis are high compared to IP-RP-HPLC genotyping due to the requirements of PCR chemicals and instrumentation for the former. In our investigation we carried out IP-RP-HPLC genotyping on a set of 86 German bread wheat cultivars (Table 1). All of the *Glu-B1-1d* (*Bx-6*) genotypes exhibited the larger fragment size compared to the *Glu-B1-1a* (*Bx-7*) and*Glu-B1-1 h* (*Bx-17*) genotypes. Consequently, we concluded that the 15-bp in-frame insertion is diagnostic for the *Glu-B1-1d* (*Bx-6*) allele.

Marker-assisted negative selection of *Glu-B1-1d* (*Bx-6*) alleles

Based on quality evaluations of a large numbers of cultivars, Payne et al. (1987) developed a scoring system for HMW-GS in which individual subunits are graded using numbers (1–4) according to their contribution to breadmaking quality. In the case of the *Glu-1B* locus, the subunit combinations of GLU-B1-1A (*Bx-7*) + GLU-B1-2A (*By-8*), GLU-B1-1A (*Bx-7*) + GLU-B1-2B (*By-9*) and GLU-B1-1H (*Bx-17*) + GLU-B1-2F (*By-18*), which are positively correlated with dough strength and breadmaking potential of flours, were assigned quality scores of 2 or 3, whereas GLU-B1-1D (*Bx-6*) + GLU-B1-2A (*By-8*) was assigned to the worst quality score 1. Several investigators have subsequently verified the validity of these quality scores for the chromosome 1B-encoded subunits (Preston et al. 1992; Dong et al. 1992; Khatkar et al. 1996). Moreover, it has also been observed that subunit GLU-B1-1E (*Bx-20*) has a detrimental effect on dough strength (Shewry et al. 2003), whereas the over-expression of GLU-B1-1A (*Bx-7*) was found to be associated with improved dough strength (Lukow et al. 1992). For the discrimination of *Glu-B1-1a* (*Bx-7*) alleles, which differ with respect to expression level, a marker has been developed based on an 18-bp insertion/deletion at the C-terminal end of the central repetitive domain of the *Glu-B1-1a* (*Bx-7*) coding sequence (Butow et al. 2003). To date, however, negative selection of *Glu-B1-1d* (*Bx-6*) alleles at the DNA level was not feasible, since the already developed PCR-based assays for *Glu-B1* subunits only allowed discrimination between the 'good quality' subunits GLU-B1-1A (*Bx-7*) and GLU-B1-1H (*Bx-17*) (Ahmad 2000; Ma et al. 2003). We describe here for the first time a highly reproducible and rapid PCR-based method for the automated genotyping of *Glu-B1-1d* (*Bx-6*)-specific fragments. The genotyping data we obtained enabled marker-assisted negative selection of 'poor quality' *Glu-B1-1d* (*Bx-6*) alleles. This is a major step forward in compiling PCR assays which allow comprehensive

scoring of *Glu-1* alleles, and our assay can be integrated in future baking quality breeding programmes.

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References

Ahmad M (2000) Molecular marker-assisted selection of HMW glutenin alleles related to wheat bread quality by PCR-generated DNA markers. Theor Appl Genet 101:892–896

Anderson OD, Greene FC (1989) The characterisation and comparative analysis of high-molecular-weight glutenin genes from genomes A and B of a hexaploid bread wheat. Theor Appl Genet 77:689–700

Anderson OD, Greene FC, Yip RE, Halford NG, Shewry PR, Malpica-Romero JM (1989) Nucleotide sequences of the two high-molecular-weight glutenin genes from the D-genome of a hexaploid bread wheat, *Triticum aestivum* L. cv. Cheyenne. Nucleic Acids Res 17:461–462

Bietz JA, Shepherd KW, Wall JS (1975) Single kernel analysis of glutenin: use in wheat genetics and breeding. Cereal Chem 52:513–532

Branlard G, Dardevet M (1985) Diversity of grain protein and bread wheat quality. 2. Correlation between high molecular-weight subunits of glutenin and flour quality characteristics. J Cereal Sci 3:345–354

Butow BJ, Ma W, Gale KR, Cornish GB, Rampling L, Larroque O, Morell MK, Békés F (2003) Molecular discrimination of Bx7 alleles demonstrates that a highly expressed high-molecular-weight glutenin allele has a major impact on wheat flour dough strength. Theor Appl Genet 107:1524–1532

De Bustos A, Rubio P, Jouve N (2000) Molecular characterization of the inactive allele of the gene Glu-A1 and the development of a set of AS-PCR markers for HMW glutenins of wheat. Theor Appl Genet 100:1085–1094

De Bustos A, Rubio P, Soler C, Jouve GN (2001) Marker assisted selection to improve HMW-glutenins in wheat. Euphytica 119:69–73

Devaney JM, Girard JE, Marino MA (2000) DNA microsatellite analysis using ion-pair reversed-phase high-performance liquid chromatography. Anal Chem 72:858–864

Dong H, Sears RG, Cox TS, Hoseney RC, Lookhart GL, Shogren MD (1992) Relationships between protein composition and mixograph and loaf characteristics in wheat. Cereal Chem 69:132–136

D'Ovidio R, Anderson OD (1994) PCR analysis to distinguish between alleles of a member of a multigene family correlated with wheat bread-making quality. Theor Appl Genet 88:759–763

Gish W, States DJ (1993) Identification of protein coding regions by database similarity search. Nat Genet 3:266–272

Halford NG, Field JM, Blair H, Urwin P, Moore K, Robert L, Thompson R, Flavell RB, Tatham AS, Shewry PR (1992) Analysis of HMW glutenin subunits encoded by chromosome 1A of bread wheat (*Triticum aestivum* L.) indicates quantitative effects on grain quality. Theor Appl Genet 83:373–378

Harberd NP, Bartels D, Thompson RD (1986) DNA restriction fragment variation in the gene family encoding high-molecular-weight (HMW) glutenin subunits of wheat. Biochem Genet 24:579–592

Huber CG, Oefner PJ, Bonn GK (1995) Rapid and accurate sizing of DNA fragments by ion-pair chromatography on alkylated nonporous poly(styrene divinylbenzene) particles. Anal Chem 67:578–585

Khatkar BS, Bell AE, Schofield JD (1996) A comparative study of the inter-relationships between mixograph parameter and bread making qualities of wheat fours and glutens. J Sci Food Agric 72:71–85

Lafiandra D, Tucci GF, Pavoni A, Turchetta T, Margiotta B (1997) PCR analysis of x- and y-type genes present at the complex *Glu-A1* locus in durum and bread wheat. Theor Appl Genet 94:235–240

Lawrence GJ, MacRitchie F, Wrigley CW (1988) Dough and baking quality of wheat lines deficient in glutenin subunits controlled by the *Glu-A1, Glu-B1* and *Glu-D1* loci. J Cereal Sci 7:109–112

Lukow OM, Forsyth SA, Payne PI (1992) Over-production of HMW glutenin subunits coded on chromosome 1B in common wheat, *Triticum aestivum*. J Genet Breed 46:187–192

Ma W, Zhang W, Gale KR (2003) Multiplex-PCR typing of high molecular weight glutenin alleles in wheat. Euphytica 134:51–60

Payne P, Corfield KG, Holt LM, Blackman JA (1981) Correlations between the inheritance of certain high-molecular-weight subunits of glutenin and breadmaking quality in progenies of six crosses of bread wheat. J Sci Food Agric 32:51–60

Payne PI, Nightingale MA, Krattiger AF, Holt LM (1987) The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. J Sci Food Agric 40:51–65

Preston KR, Lukow OM, Morgan B (1992) Analysis of relationship between flour properties and protein-fractions in a world wheat collection. Cereal Chem 69:560–567

Radovanovic N, Cloutier S (2003) Gene-assisted selection for high molecular weight glutenin subunits in wheat doubled haploid breeding programs. Mol Breed 12:51–59

Rampling LR, Harker N, Shariflou MR, Morell MK (2001) Detection and analysis systems for microsatellite markers in wheat. Aust J Agric Res 52:1131–1141

Schlumbaum A, Neuhaus JM, Jacomet S (1998) Coexistence of tetraploid and hexaploid naked wheat in a neolithic lake dwelling of central Europe: Evidence from morphology and ancient DNA. J Archaeol Sci 25:1111–1118

Schwarz G, Herz M, Huang XQ, Michalek W, Jahoor A, Wenzel G, Mohler V (2000) Application of fluorescence-based semi-automated AFLP analysis in barley and wheat. Theor Appl Genet 100:545–551

Schwarz G, Sift A, Wenzel G, Mohler V (2003) DHPLC scoring of a SNP between promoter sequences of HMW glutenin x-type alleles at the Glu-D1 locus in wheat. J Agric Food Chem 51:4263–4267

Shewry PR, Gilbert SM, Savage AWJ, Tatham AS, Wan YF, Belton PS, Wellner N, D'Ovidio R, Bekes F, Halford NG (2003) Sequence and properties of HMW subunit 1Bx20 from pasta wheat (*Triticum durum*) which is associated with poor end use properties. Theor Appl Genet 106:744–750

Smith RL, Schweder ME, Barnett RD (1994) Identification of glutenin alleles in wheat and triticale using PCR-generated DNA markers. Crop Sci 34:1373–1378

Sugiyama T, Rafalski A, Peterson D, Soll D (1985) A wheat HMW glutenin subunit gene reveals a highly repeated structure. Nucleic Acids Res 13:8729–8737

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Weegels PL, Hamer RJ, Schofield JD (1996) Functional properties of wheat glutenin. J Cereal Sci 23:1–18