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Molecular characterisation of the inactive allele of the gene *Glu-A1* and the development of a set of AS-PCR markers for HMW glutenins of wheat

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Abstract The present work reports new PCR markers that amplify the complete coding sequence of the specific alleles of the high molecular weight (HMW) glutenin genes. A set of AS-PCR molecular markers was designed which use primers from nucleotide sequences of the *Glu-A1* and *Glu-D1* genes, making use of the minor diffeences between the sequences of the *x1*, *x2** of *Glu-A1*, and the *x5* and *y10* of *Glu-D1*. These primers were able to distinguish between *x2** and the *x1* or *xNull* of *Glu-A1*. Also *x5* was distinguishable from *x2*, and *y10* from *y12*. The primers amplified the complete coding regions and corresponded to the upstream and downstream flanking positions of *Glu-A1* and *Glu-D1*. Primers designed to amplify the *Glu-A1* gene amplified a single product when used with genomic DNA of common wheats and the *xNull* allele of this gene. This work also describes the cloning and characterisation of the nucleotide sequence of this allele. It possesses the same general structure as *x2** and *x1* (previously determined) and differs from these alleles in the extension of the coding sequence for a presumptive mature protein with only 384 residues. This is due to the presence of a stop codon (TAA) 1215-bp downstream from the start codon. A further stop codon (TAG), 2280-bp downstream from the starting codon is also found. The open reading frame of *xNull* and *x1* alleles has the same size in bp. Both are larger than *x2** which shows two small deletions. The reduced size of the presumptive mature protein encoded by *xNull* could explain the negative effect of this allele on grain quality.

Key words Wheat · High-molecular-weight glutenin · AS-PCR · *Glu-A1* locus · Null allele · *Triticum aestivum*

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Introduction

The properties of wheat flour have been related to the allelic combination of the genes coding for the high-molecular-weight (HMW) subunits of glutenin (Payne 1987; Shewry et al. 1992). Common wheats contain three *Glu-1* loci, *Glu-A1*, *Glu-B1* and *Glu-D1*, located on the long arms of homoeologous group-1 chromosomes (Payne et al. 1982). The proteins encoded by these genes are easily extracted from individual grains and can be separated by SDS-PAGE electrophoresis (Payne et al. 1981). Molecular studies have shown that each locus contains two tightly linked genes (*x* and *y*) coding for protein subunits of different molecular weights (Harberd et al. 1986). Considerable differences in the SDS-PAGE patterns can be seen for the HMW glutenin subunits of different wheat varieties. Accordingly, different alleles for the complex *Glu-1* genes have been reported. A numbering system for the HMW glutenin subunits and a system for allele classification at the *Glu-1* loci have been proposed by Payne and Lawrence (1983).

Payne (1987) demonstrated that breadmaking quality is firmly associated with the presence of HMW subunits: *x5+y10* providing good quality and *x2+y12* poor quality. Moreover, Odenbach and Mahgoub (1988) investigated the influence of individual HMW glutenin subunits on baking quality and found significant differences depending on allelic composition. *Glu-A1 ×2** and *Glu-D1 x5+y10* were associated with large sedimentation volumes, whereas their allelic variants *Glu-A1 x-Null* and *Glu-D1 x2+y12* gave small sedimentation volumes.

Molecular analyses have demonstrated that the *Glu-A1* locus encodes for either a single subunit or no subunit at all. If only one subunit is present it is invariably the product of the *x*-type (D'Ovidio et al. 1996). The *Glu-A1* locus is characterised by three main *x*-type alleles, *a*, *b* and *c* (Payne and Lawrence 1983), which code respectively for HMW glutenins 1, 2* and an apparently inactive product (null allele) (Thompson et al. 1983). The nucleotide sequences of the alleles *Glu-A1 x1*

and *x2** have been previously reported (Anderson and Greene 1989; Halford et al. 1992).

The molecular characterisation of the loci *Glu-A1* and *Glu-D1* from genomic clones of the cultivars 'Hope' and 'Cheyenne' has been performed by different authors (Forde et al. 1985; Sugiyama et al. 1985; Thompson et. al 1985; Greene et al. 1988; Anderson and Greene 1989). These studies have revealed that all glutenin genes are organised with a large central region containing repeated short motifs coding for the same amino acids, flanked by unique N- and C-terminus domains. The sequences of genes *Glu-A1 ×2** and *Glu-D1 ×5* were analysed comparatively by Anderson and Greene (1989) who showed that they have a comparable structure and high similarty. More recently, other authors have demonstrated the usefulness of PCR-based analysis for distinguishing between cultivars with different HMW glutenin subunits (D'Ovidio and Anderson 1994; D'Ovidio et al. 1994, 1995; Varghese et al. 1996; Lafiandra et al. 1997).

The present paper reports the use of a set of AS-PCR molecular markers which employ primers designed from nucleotide sequences of the *Glu-A1* and *Glu-D1* genes. These primers were designed making use of the minor differences between the sequences of the alleles *x-1* vs *x-2** of *Glu-A1*, and *x5* vs *x12* and *y10* vs *y12* of *Glu-D1*, and represent the upstream and downstream positions of the coding regions of the genes. Primers designed to amplify the gene *Glu-A1* gave a single product using genomic DNAs of common wheats that have the inactive product of this gene. This work also describes the cloning and characterisation of the nucleotide sequence belonging to the *x-null* allele of this gene.

Materials and methods

Plant material

A series of common wheat lines were used as testers to study the allelic constitution of the genes *Glu-A1* and *Glu-D1* by SDS-PAGE and AS-PCR. These were: 'Chinese Spring' and 'Pané-247', which share the alleles *x-null* and *x2+y12*; 'Hope', which has *x1* and *x5+y10*; the cultivars 'Ablaca' and 'Bezostaya', and the lines '48–141', '88–31', '48–136' and '48–145' which have x2* and x5+y10. The lines '48–141', '88–31', '48–136' and '48–145' belong to a working collection of wheats derived from crosses involving the cultivars 'Anza, 'Amigo' and 'Glennson 81', and were kindly provided by Dr J.P. Gustafson.

The final task of the present project was the introduction of the best breadmaking genes into the Spanish wheat 'Pané 247'. As a step in this process it was necessary to perform crosses between it (female parent) and the lines '48–141', '88–31', '48–136' and '48–145' carrying the genes *Glu-A1 ×2** and *Glu-D1 x5+y10*. The F_2 progenies obtained by selfing each of the four hybrids were screened for HMW glutenin constitution by analysing the allelic segregation of the genes *Glu-A1* and *Glu-D1*. A double analysis using SDS-PAGE and AS-PCR markers was performed.

HMW glutenin analysis

In order to analyse endosperm proteins without interfering with germination, a piece of the endosperm was removed, making sure it contained no part of the embryo. This was ground and the proteins extracted as described by Singh and Shepherd (1991). Prolamins were analysed using vertical polyacrylamide gel-electrophoresis in the presence of 12% SDS (SDS-PAGE). Parentals, controls and segregant samples of the same progeny were included on the same gel. A molecular-weight standard (Sigma 6H) was used which contained myosin (205°000), β-galactosidase (116°000), phosphorylase B (97°000), bovine albumin (66°000), egg albumen (45°000) and carbonic anhydrase (29°000). Electrophoresis was performed at 16 mA/gel for 16–17 h at room temperature. Gels were stained with 1% Coomassie Brilliant Blue R-250 in ethanol.

DNA purification

Genomic DNA from wheat was extracted from leaves of single plants about 4–6 weeks old, as described by Sharp et al. (1988). The DNA of F_2 progenies was isolated from 100 mg of leaves using a DNeasy Plant Mini Kit (QIAGEN).

AS-PCR marker development: design of primers

DNA sequences have been reported for the genes and alleles *Glu-A1 x2** (Anderson and Greene 1989), *Glu-A1 x1* (Halford et al. 1992), *Glu-D1 x5* (Anderson et al. 1989), *Glu-D1 y10* (Anderson and Greene 1989), *Glu-D1 x2* (Sugiyama et al. 1985) and *Glu-D1 y12* (Thompson et al. 1985). The analysis of these sequences has allowed the recognition of the open reading frame for the coding sequence. Based on this information, four PCR primer sets were designed in the present work, corresponding to the polymorphic flanking regions of the coding sequence of the following alleles: *Glu-A1: x2** and either *x1* or *x Null*, and *Glu-D1: x5* and *y10*. After trying different primers, alleles *Glu-A1 x1* and *Glu-A1 x-null* were found to be indistinguishable. However, a common set of primers was useful in distinguishing these alleles from *x2**. The primers for *Glu-D1 ×5* and *Glu-D1 y10* revealed that both tightly linked genes invariably appear together in the same plants. The sequences of these primers are shown in Table 1.

PCR amplification

PCR amplifications were carried out using a Perkin Elmer 480 thermocycler. Reactions were performed in a final volume of 25 µl using 2 units of *Taq* DNA polymerase (Sigma) and a 10×*Taq* PCR buffer (Sigma).The quantities of other components of the reaction mixture and the PCR conditions were specific for each allele (Table 1). PCR products were analysed by electrophoresis using a 1% agarose gel in TAE buffer followed by staining with ethidium bromide.

DNA cloning and sequencing

The single band amplified by PCR using the primer *Glu-A1 x1* on template DNA from 'Pané 247' was purified from the agarose gels using the QIAquick Gel Extraction Kit (QIAGEN). The fragment was ligated into a Bluescript phagemid vector and used to transform competent cells of *Escherichia coli*. The clone was further subcloned for nucleotide sequencing using the Erase-a-base kit (Promega). For this purpose the clone was previously digested with *Kpn*I (to protect the primer binding site at the 3' end) and *Xho*I (to leave a 5' end adjacent to the insert from which deletions were made), and incubated at 35°C with exonuclease III with 1-min intervals between enzyme treatments. A series of different subclones was obtained and used for sequencing in an ABI Prism 377 sequencer (Applied Biosystem). The Sequence Navigator programme (Applied Biosystem) was used for sequence alignment.

Table 1 Design of forward and reverse primers, and PCR cycling conditions for the amplification of specific alleles of genes *Glu-A1* and *Glu-D1*

Genes and alleles	Forward and reverse PCR primers	PCR components	PCR cycling	
Glu-A1	L: 5´CCGATTTTGTTCTTCTCACAC 3´	100–200 ng DNA	1×	94° 2´
*x2**	R: 5´CACCAAGCGAGCTGCAGAT 3´	7.5 mM/dNTPs	40×	94° 1´–
		1 mM/primers		–60° 2´ –
				–72° 2´
			1×	72° 5´
Glu-A1	L: 5´ TCACCGACAGTCCACCGA 3´	100–200 ng DNA,	1×	94° 2´
x1 and *xNull*	R: 5´ ACCAAGCGAGCTGCAGAG 3´	5 mM/dNTPs	40x	94° 1´ –
		1 mM/primers		–64° 2´ –
				–72° 2´
			1×	72° 5´
Glu-D1	L: 5´ AGCCTAGCAACCTTCAC 3´	150–250 ng DNA,	1×	94° 2´
x5	R: 5´ AGACATGCAGCACATACC 3´	7.5 mM/dNTPs	40x	94° 1´ –
		5 mM/primers		–58° 2´
				–72° 2´
			1×	72° 5´
Glu-D1	L: 5´ CTAACTCGCCGTGCACA 3´	100–200 ng DNA,	1×	94° 2´
y10	R: 5´ AGCTAAGGTGCATGCATG 3´	7.5 mM/dNTPs	40×	94° 1´ –
		10 mM/primers		–66° 40´´ –
				–72° 2´
			1×	72° 5´

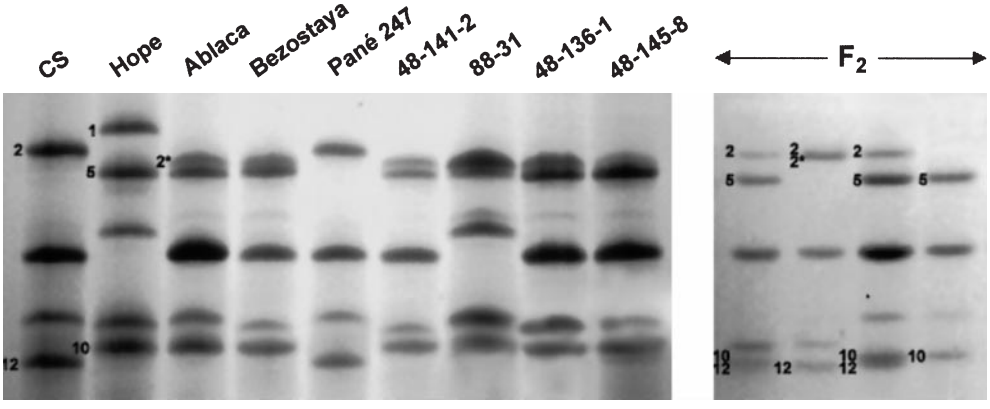


Fig. 1 HMW patterns of several wheats and parental lines (left) and F_2 segregant plants from the hybrid 'Pané-247'×'48–136–1' (right) studied by 12% SDS-PAGE

Results and discussion

Analysis of HMW: SDS-PAGE

Electrophoretic analyses of HMW glutenins in the materials employed were performed using wheat varieties of known allelic composition to identify the alleles of the gene markers located on the long arms of the chromosomes of homoeologous group 1. These included the genes *Glu-A1* and *Glu-D1*. Segregation of these genes in the F_2 offspring of each hybrid was further studied. These results were reported in a previous paper (Rubio et al. 1999). The genes *Glu-A1* and *Glu-D1* differentiated 'Pané-247' and the lines '48–141', '88–31', '48–136' and '48–145', due to the existence of null allelism in *Glu-A1*, and the *x2+y12* allele of *Glu-D1* (Fig. 1).

Analysis of HMW: AS-PCR

To test the capability of PCR technology to assist in the selection of HMW glutenin genes, specific primers were designed for the alleles that differentiate 'Pané-247' and the selected lines used as parentals. The value of PCR in obtaining useful molecular markers for plant breeding has been extensively demonstrated (D'Ovidio et al. 1990; Williams et al. 1990; Waugh and Powell 1992; Chen et al. 1994). Of particular interest are the characterisation techniques of HMW glutenin alleles reported by different authors (D'Ovidio and Anderson 1994; D'Ovidio et al. 1995; Tahir et al. 1996; Varghese et al. 1996; Lafiandra et al. 1997). Due to the high degree of similarity between the sequences of the known alleles of *Glu-A1*, in the present study it was necessary to design specific primers from the sequence of *x1* which could also amplify *xNull*. There were two objectives to this approach:

(1) To achieve, through independent systems, the amplification of either *xNull* or *x2**. This would distinguish heterozygous from homozygous plants in the segregating progeny, thus facilitating marker-assisted selection.
(2) The amplification of the xNull allele as a single band in agarose gels which would permit its isolation, cloning into adequate vectors, sequencing and characterisation, and reveal the differences between the active and inactive alleles. Knowledge of the sequence could then facilitate the design of specific primers to distinguish *xNull*.

PCR analyses were performed using allele-specific primers with the aim of amplifying the complete coding regions of the *x2**, *x1* or *xNull* (*Glu-A1*), *x5* and *y10* (*Glu-D1*) genes. A comparison of previously reported sequences shows the difficulties in designing specific pairs of primers with the capacity to distinguish between the highly homologous alleles of the complex multigene HMW family. This difficulty is greater for the coding sequence than for the polymorphic flanking regions due to the existence of a large repetitive domain in all HMW glutenin genes and because the N-terminal and C-terminal regions are highly conserved. These circumstances were also considered by D'Ovidio and Anderson (1994) who designed primers external to the coding region of the *Glu-D1x* alleles in order to distinguish wheat cultivars by the analysis of PCR products.

The search for specific differences between the pairs of alleles focused on the immediate upstream and downstream non-coding flanking regions of the genes. The amplification of the complete sequence of the gene is of interest in verifying the correspondence between protein size, as deduced from the coding region of the gene, and that obtained by separating proteins using SDS-PAGE (D'Ovidio and Anderson 1994; D'Ovidio et al. 1994). This also presents the opportunity for isolating specific variants and the determination of the possible domains involved in variations in length between genes or alleles (D'Ovidio et al.1995). Finally, knowledge of the complete coding sequence allows the detection of the presence of inactive genes (D'Ovidio et al. 1994; Varghese et al. 1996) and offers information that could be of use in genetic transformation programmes in which transgenic wheats are desired.

Differences in base pairs, GC percentage requirements, limitations to the annealing of secondary structures during amplification, the concentration of template DNA and other reactants required, plus the cycling temperatures of the PCR reaction, were all investigated to design the primers. Figure 2 shows the sites of the sequences selected which allowed the synthesis of complementary primers in both non-repetitive flanking regions. The forward primers were at the following positions upstream of the 3′ end of the start codon (ATG): –160 (*x2**), –3 (*x1* and *xNull*), –69 (*x5*) and –124 (*y10*). The complementary sites of the reverse primers were at +5 (*x2**, *x1* and *xNull*), +161 (*x5*) and +16 (*y10*) downstream from the 5′ end of the stop codon. The final sets of primers and PCR conditions are given in Table 1. An example of their application is illustrated in Fig.°3.

Of particular difficulty was the design of primers and the setting of PCR conditions which would allow a distinction between the alleles of *Glu-A1*. After trying different primers and/or conditions, a system was established for distinguishing between *x2** and either the *x1* or *xNull* alleles. The PCR reactions were performed using two different forward primers and a partially common reverse primer (Fig. 2 and Table 1). The best PCR conditions were determined by varying the concentration of dNTPs and the PCR cycling temperature.

The reverse primers used to distinguish between *x2** and the *x1* or *xNull* alleles showed only two minor differences. That of *x2** contained one additional base at the 3′ end and one different base at the 5′ end with respect to the *x1* and *xNull* reverse primers. It is worthy of mention that the system used was able to distinguish between alleles based on primers that differ by one base only, as reported previously by other authors (D'Ovidio et al. 1995; Lafiandra et al. 1997; Anderson et al. 1998).

Electrophoretic analyses of the PCR reactions performed on the genomic DNA from cultivars, parentals and tester lines of common wheat gave the expected results in all cases. Thus, 'Chinese Spring' and 'Pané-247', which have the same allelic composition for the loci *Glu-A1* and *Glu-D1*, showed the same amplified products as assessed by SDS-PAGE (Fig. 3a, b). Both wheats presented a single band of about 2537 bp in the agarose gels after using the *Glu-A1 x1* set of primers. No bands were shown in the lanes of PCR experiments using the *x2** primer set with the above mentioned lines. The genomic DNA of the cultivar 'Hope', which has the *x1* of *Glu-A1*, showed the same results as 'Chinese Spring' and 'Pané-247'.

The cultivars 'Ablaca', and 'Bezostaya' and the parental lines '48–141', '88–31', '48–136' and '48–145', which all share the allele *Glu-A1 x2**, gave opposite results to the above cultivars. They showed only a single band of about 2652 bp in PCR reactions when the primer set for *x2** was employed, and no products when the *x1* or *xNull* set was used.

Finally, genomic DNA of individual plants of the F_2 progenies involving 'Pane-247' and the lines '48–141', '88–31', '48–136' and '48–145' was used as a template in PCR reactions using the four primer sets. Segregant plants heterozygous for the *Glu-A1* system, as assessed by SDS-PAGE, showed both products in the corresponding separated reactions (*x-Null* and *x2**). In all cases the alleles scored confirmed the original SDS-PAGE results (right lanes in Figs. 1 and 3a–b).

Similar efficiency was observed when analysing the PCR products amplified with specific primers designed to detect the presence or absence of the *x5* and *y10* alleles of *Glu-D1*. The lines 'Chinese Spring' and 'Pané-247' showed no amplification product in the corresponding reactions. However, 'Hope', 'Ablaca', 'Bezostaya' and the selected lines showed single bands

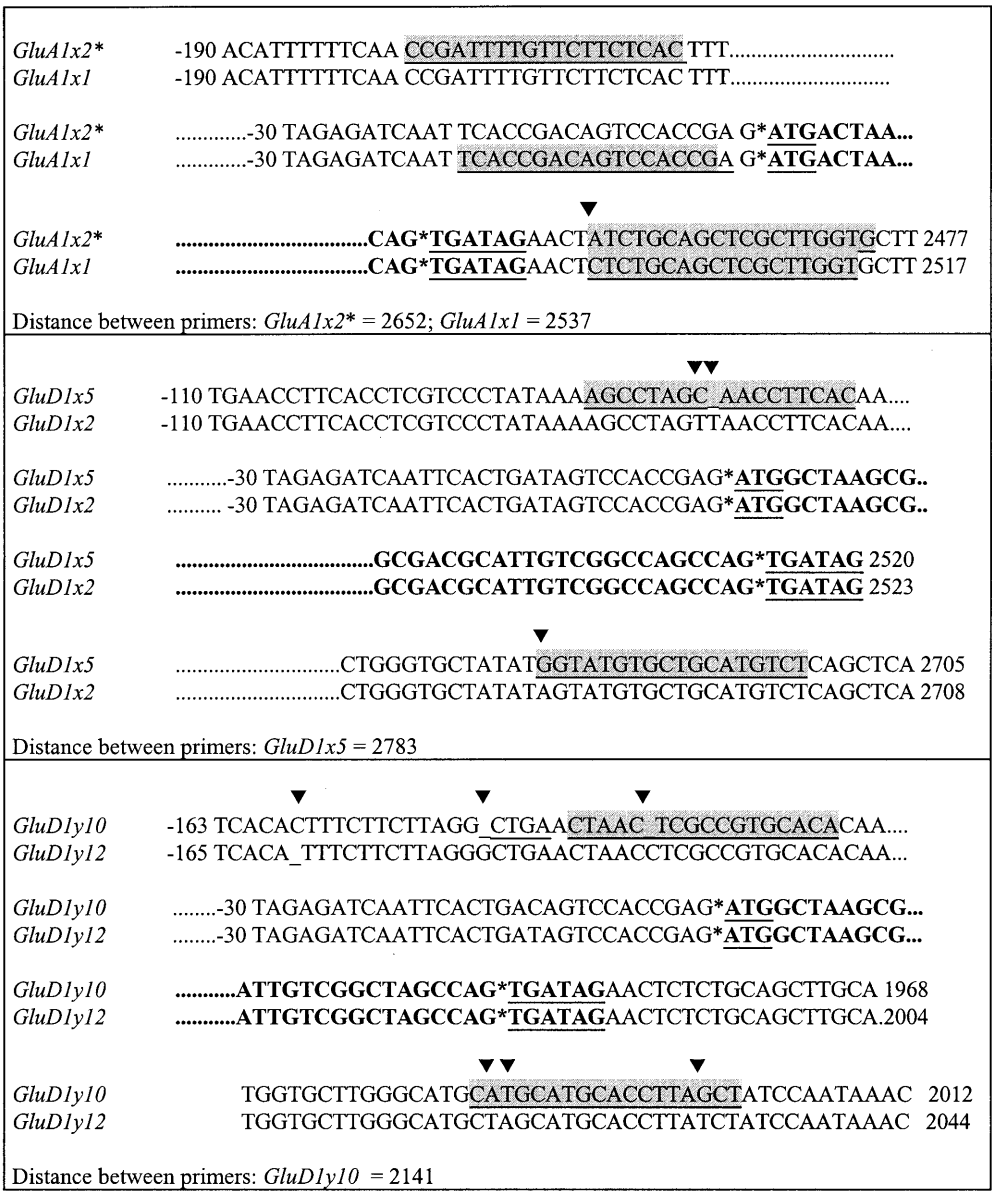


Fig. 2 Comparative details of the sequences of the *Glu-A1×2*, Glu-A1×1, Glu-D1×2, Glu-D1×5, Glu-D1y10* and *Glu-D1y12* genes. The coding regions (*black letters between asterisks*) are flanked by *ATG* (start codon) and *TGATAG* (terminal codons). The *grey boxes* indicate the selected sites used to design the allelic-specific primers (*underlined*) that are external to the coding regions (*head of arrows* indicate the differences between each pair of sequences)

of about 2783 bp and 2141 bp in agarose gels, corresponding to the *x5* and *y10* primer sets. As for the alleles of *Glu-A1*, the results of AS-PCR fitted well with those of SDS-PAGE, with the sole restriction of the inability to distinguish heterozygous plants with only one set of primers, respectively (Figs. 2 and 3c–d).

The next step was to verify the identity of the amplified products. Three approaches were used to determine whether the single bands separated in the agarose gels corresponded to the amplification of the expected HMW genes:

(1) Each set of allele-specific primers amplified a single product with the expected fragment size. No non-specific products were observed in any case.

(2) In all cases the amplification product was present only in the cultivars, tester lines, parentals and segregant plants possessing the corresponding HMW glutenin, as demonstrated by SDS-PAGE.

(3) Each single band was sliced from the agarose gels, extracted, purified and directly sequenced in both directions using the designed primers. In all cases, the comparison of a sequence of about 400 bases from both ends of each fragment confirmed their identity to the HMW subunits analysed.

The efficiency of allele-specific screening developed from RFLP or other molecular markers tightly linked to genes of agronomic value has been discussed by Rafalsky and Tingey (1993) and demonstrated by Williams et al. (1990). The advantages of using PCR-based systems as valid alternatives to standard techniques for selecting genotypes with particular glutenin genes was previously reported by D'Ovidio and Anderson (1994). These authors designed specific primers to study the gene *Glu-D1-x5* (without any allelic distinction) and the subunits of the gene *Glu-A1*. Later,

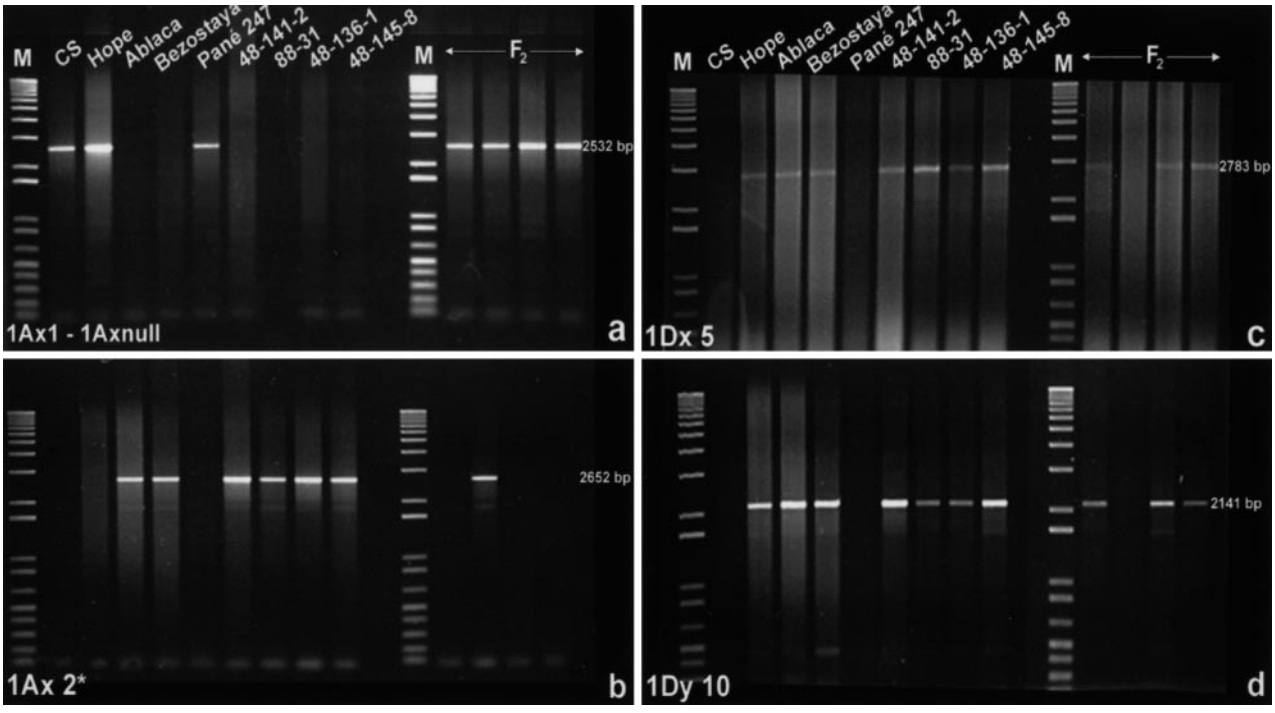


Fig. 3a–d Separation of amplification products on agarose gels (1.8%) using the designed primers to distinguish the alleles of *Glu-A1* and *Glu-D1*. From left to right are included the molecular-weight marker (*M*) and the same wheats, parental lines and F_2 segregants as included in the Fig. 1

Varghese et al. (1996) and Lafiandra et al. (1997) used new primers that amplified, in the N-terminal region, amplified the different *Glu-1* genes previously designed by D'Ovidio et al. (1995). All these studies agree on the importance of this type of marker in the diagnosis and selection of genes for bread flour quality.

The present results confirm the usefulness of the method in selecting recombinant plants with desired glutenin alleles. This method uses only small amounts of DNA from leaves and avoids damage to embryos by using only small pieces of the endosperm. This enables the gathering of direct information on the genetic composition of selected plants at any moment in their life cycle.

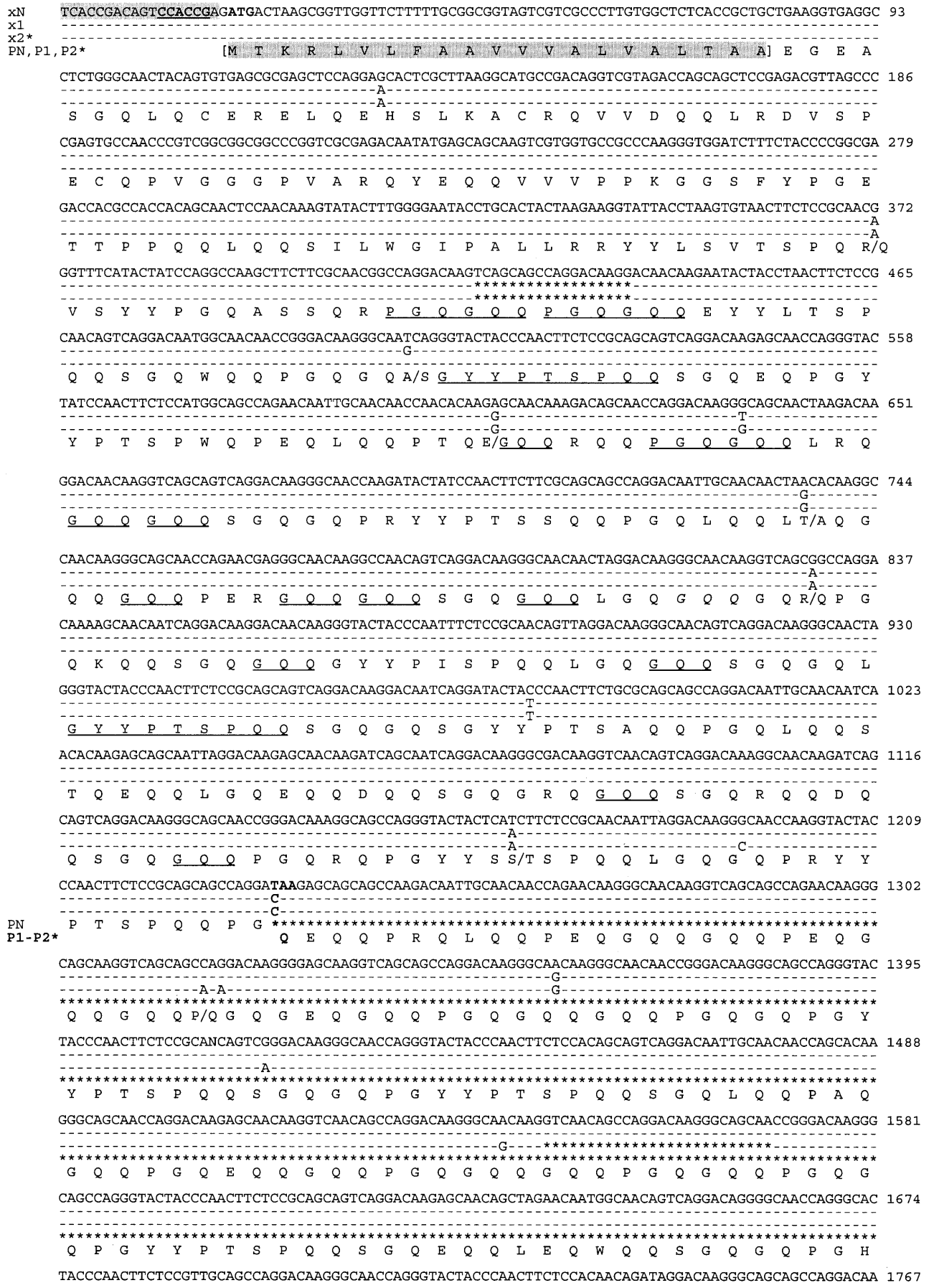
Isolation, cloning and sequencing of *Glu-A1 x-null*

The use of allele-specific primers that amplify the complete coding-region of the genes may also help sequence and characterise new alleles. The single PCR product obtained using the DNA template from 'Pané-247' and the *Glu-A1* primer set correspond to the coding region of the *xNull* allele, so far un-sequenced. With this idea in mind, the PCR product that was cloned into the Bluesript SK+/– phagemid vector was purified. The polylinker of the vector was cut with *EcoR* V endonuclease to insert the PCR product. The transformation product gave rise to only a few recombinant colonies, probably as a consequence of the difficulty of inserting the large PCR fragment (2537 bp) into the plasmid vector. One of them, designated UAHP16.1, was selected for sequencing. Due to the large size of the sequence it was necessary to obtain and subclone a nested set of deletions of the insert. The total sequence of the *xNull* allele was obtained by combining the overlapping terminal regions of each subclone and the partial sequences of the original long insert of UAHP16.1.

Comparison of the alleles *Glu-A1 x-null, x-1* and *x-2**

The total sequence of the insert of UAHP16.1 was obtained and the amino-acid sequence of the allele *xNull* deduced. The nucleotide sequence of this allele has been compared with the previously reported sequences of the alleles *x1* and *x2** (aligned from the top to the 3rd line in Fig. 4). The main features of the comparative study of the three sequences are the following:

Fig. 4 Comparison of the sequences of the coding regions of the alleles of *Glu-A1*: *xNull* (this paper), *x1* and *x2** (Anderson and Greene 1989; Halford et al. 1992). The *grey boxes* indicate the sites of the primers used to amplify the *x1* and *xNull* alleles. The signal peptide of the three alleles is indicated in *grey in brackets*. Differences in bases and amino acids are also shown, as is the CCACCG sequence near the start codon. This and the stop codons are *underlined*. The *dashes and small stars* in the nucleotide sequences indicate identical and deleted regions respectively. The amino-acid sequences for the mature proteins encoded by these three alleles are shown under the gene sequences. The *underlined* amino-acids correspond to repetitive peptides. The *large stars* signal the sites of the stop codons found in the *Glu-A1 xNull* sequence. The nucleotide sequence of the allele *Glu-A1 xNull* is deposited under EMBL GenBank and DDBJ accession number AF145590 ▶


xN
x1
x2*
PN,P1,P2*
P1-P2*
PN

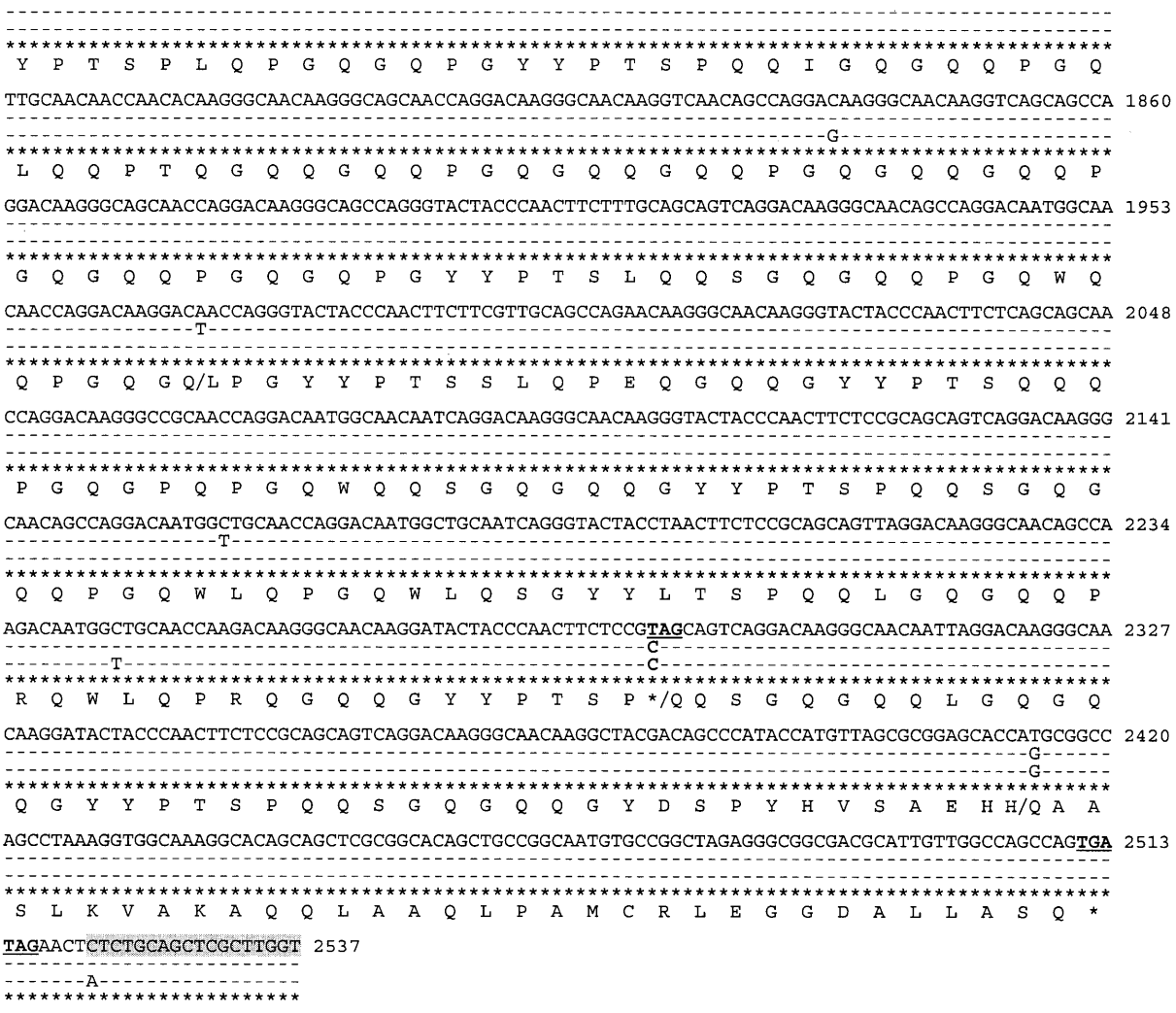


Fig. 4 (continued)

(1) The three sequences share a 21-residue signal peptide in the N-terminal amino acid sequence of the encoded protein (63 bp from the initiator codon). The three alleles share the hexanucleotide CCACC, followed by the trinucleotide GAC, just before the translation start codon ATG. The allele *xNull* lacks introns and shows the same general structure as *x2** and *x1*, previously demonstrated by Anderson and Greene (1989).

(2) The *xNull* allele differs from both *x1* and *x2** in the extension of the presumptive mature protein with only 384 residues instead of the 809 (x1) or 794 (x2*) of the active alleles according to Halford et al. (1992). This is due to the presence of a stop codon (TAA) 1215 bp downstream from the start codon. The stop codon is a consequence of the transition of T by C at the first base of a glutamine codon (CAA) in *x1* and *x2**.

(3) A further stop codon (TAG) 2280 bp downstream from the starting codon is also found in the *xNull* allele.

(4) The sequence of the *xNull* allele resembles the other *Glu-A1x* alleles in that it has a clear domain structure. This result confirms the organisation of glutenin sequences deduced by treatments with restriction enzymes and PCR (Tahir et al. 1996; Lafiandra et al. 1997). All three of these alleles share an N-terminal non-repetitive domain of 86 residues, previously reported in *x2** and *x1* by Halford et al. (1992). This region is followed by a truncated repetitive domain of only 319 residues in *xNull*, instead of more than 700 residues as in the other alleles. These have a second non-repetitive domain at the C-terminal amino-acid sequence which is absent in x-Null.

(5) The repetitive domain of *xNull* consists of tandem and interspersed repeats based on the tripeptides YYP (4) and GQQ (14), the hexapeptide PGQGQQ (3) and the nonapeptide CYYPTSPQQ (2).

(6) The open reading frame of the *xNull* and *x1* alleles have the same size in bp. Both are larger than *x2** which shows two small deletions (420–437 bp and 1544–1559 bp downstream from the starting codon).

(7) The comparison of the common coding region of the presumptive protein of *xNull* with the active products of *x1* and *x2** reveals minor differences in amino-acid composition. The N-terminal region of the mature protein of the three alleles shows 378 residues in common and only six differences. The *xNull* allele shows Arg instead of Glu at residues 97 and 250, Ser instead of Ala at 142, Glx instead of Gly at 176, Tre instead of Ala at 219, Arg instead of Gln at 210, and Ser instead of Tre at 363.

It is important to clarify the possible effect on grain quality of the reduced size of the presumptive mature protein encoded by *xNull*. The importance of the size and type of glutenin polymers on baking flour quality has been studied extensively (Huebner and Wall 1976; Dachkevitch and Autran 1989; Singh et al. 1990; D'Ovidio et al. 1995). In an earlier approach (Thompson et al. 1983), no differences were distinguished in the mRNA complementary to the cDNA coding for the HMW glutenins from *Glu-A1* in aneuploids, Nulli1ATetra1D plants or euploids of 'Chinese Spring'. These authors proposed that the sequences coding for *Glu-A1 xNull* residing on the long arm of 1 A probably represent an incomplete or inactive HMW glutenin gene. The present finding of a stop codon (TAA) 1215-bp downstream from the translation start codon supports the hypothesis of an incomplete protein. However, the presence of a stop codon along the coding region could by itself only be an indication of a non-functional gene. A similar stop codon was identified in the coding region of a *Glu-1 Ay* allele (Forde et al. 1985), but subsequent analysis of this gene found the promoter to be inactive, despite the rest of the gene appearing to be intact (Halford et al. 1989). Silencing mechanisms in a *Glu-1* allele of wheat due to the insertion of transposon-like elements has also been observed (Harberd 1987). Moreover, Halford et al. (1992) reported that the poor quality associated with the presence of the inactive *Glu-A1* allele could also be explained by a lesser total proportion of HMW subunit proteins due to differences in gene expression. Functional analysis at the transcriptional or translational level would help clarify the incidence of silencing mechanisms as additional causes to explain the negative effect of the allele *Glu-A1 xNull* on grain quality.

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