Detection and characterization of a glutenin subunit with unusual high Mr at the Glu-A1 locus in hexaploid wheat

Abstract A hexaploid wheat landrace collected from the Baluchistan province of Pakistan was found to possess a novel high-molecular-weight glutenin subunit (HMW-GS). The subunit has a very slow electrophoretic mobility as revealed by SDS-PAGE, and its molecular weight is comparable to that of the highest molecular weight glutenin subunit (“2.2” encoded in the D-genome) reported so far in hexaploid wheat varieties and landraces of Japanese origin. Evidence obtained from (PCR) gene amplification studies using the primers specific for Glu-1 loci proved that the gene coding for this novel subunit belongs to the Glu-A1 locus located on the long arm of chromosome 1A. Digestion of the amplified gene (PCR product) with restriction enzymes indicated that the novel gene differs from prevailing Glu-A1 alleles (null, 1 and 2*) by an extra DNA fragment of approximately 600 base pairs. The results also indicated that the novel subunit is most probably a derivative of subunit 2* that has very likely incorporated the 600-bp fragment following a process of unequal crossing over. The present findings were further substantiated by reserved phase high performance liquid chromatography (RP-HPLC) analysis.

Key words Wheat · HMW glutenin genes · Glu-A1 · PCR · RP-HPLC

Introduction

A series of research efforts during the past 15 years (Holt et al. 1981; Lawrence and Shepherd 1981; Payne and Corfield 1979; Payne et al. 1980, 1981; 1982, 1985) have clearly demonstrated the extent of allelic variation for high-molecular-weight glutenin subunits (HMW-GS) in wheat (T. aestivum) as revealed by SDS-PAGE. A typical hexaploid genotype usually contains from three to five HMW-GS. Allelic variation of these subunits is controlled by genes found at three Glu-1 loci (Glu-A1 Glu-B1 and Glu-D1) located on the long arm of the homoeologous group 1 chromosomes which correspond to the A, B, and D genomes of hexaploid wheat, respectively. The Glu-B1 and Glu-D1 loci usually code for two subunits (1Dx and 1Dy) and one or two subunits (1Bx or 1Bx and 1By), respectively. Only one subunit (1Ax) or no subunit at all is encoded at the Glu-A1 locus.

Early electrophoretic data showed less polymorphism at the Glu-A1 locus that at the Glu-B1 and Glu-D1 loci, and three alleles designated as a, b and c were reported (Payne and Lawrence 1983). Alleles a and b code for glutenin subunits termed 1 and 2*, respectively, while allele c corresponds to a silent gene (Thompson et al. 1983) which does not code for a protein. Waines and Payne (1987) reported four new allelic variants at the Glu-A1 locus (d, e, f, g) in hexaploid wheat that showed faster mobility on SDS-PAGE than the normal alleles. Allelic variants at the Glu-A1 locus with a faster mobility on SDS-PAGE than the normal alleles were also found by Cross and Guo (1993) in a diverse wheat germ plasm collection. In this report, we describe a new variant at the Glu-A1 locus that was found in hexaploid wheat germ plasm collection from Pakistan.

Materials and methods

Electrophoretic and chromatographic analyses

The hexaploid wheat used in this study is a landrace belonging to the Baluchistan province of Pakistan. The seeds for this landrace (PK-15684) were obtained from Plant Genetic Resources Institute, National Agriculture Research Center Islamabad, Pakistan, as a part of the wheat germ plasm collection from Pakistan. The seed for reference standards was provided by the Department of Agrobiology and
Agrochemistry, University of Tuscia, Italy, where the present investigation was carried out.

Total protein extract from the brush-half of the single kernels was fractionated by SDS-PAGE on 10% gels (C = 1.28%) according to the procedure described by Payne et al. (1980). SDS-PAGE urea gels (C = 2.67%) were prepared by incorporating 4M urea into the main gel. RP-HPLC analyses of HMW-GS were conducted following the methods described by Lafiandra et al. (1995). Fractions corresponding to peaks were collected, freeze-dried and identified by SDS-PAGE.

DNA extraction and polymerase chain reaction

The embryo-half of the seeds saved from above analyses were grown in pots to raise the individual plants. Leaves and/or boots from these plants were used to extract genomic DNA following the procedure of Dellaporta et al. (1983). Polymerase chain reaction (PCR) analysis was carried out in a total volume of 50 µl using 150 ng of template DNA. The composition of the reaction mixture was 1 x Taq PCR buffer (Life Technologies™), 0.3 mM of each dNTP, 0.3 µM of each of the primers (125 ng of 20-mer oligonucleotide) and 2.5 units of Taq DNA polymerase (Life Technologies™). Primers used for PCR analyses were as reported by D'Ovidio et al. (1995). For all reactions, a Perkin Elmer Cetus Thermocycler (Model 480) was programmed to obtain amplification conditions of 30 cycles at 94 °C for 1 min, 60 °C for 2 min. and 72 ° C for 2 min, followed by a final incubation step at 72 °C for 7 min. The amplified products were digested with restriction enzymes (HindIII and HaeIII). Agarose gel (1%) electrophoresis in 1 x TBE buffer used to separate the amplified DNA fragments as well as the digestion products of the amplified DNA.

Results

SDS-PAGE

A survey of the hexaploid wheat germ plasm collected from Pakistan was recently conducted with respect to high-molecular-weight glutenin subunit (HMW-GS) composition. Figure 1 (upper) presents the SDS-PAGE separations of HMW-GS in some of the landraces from Pakistan (lanes 4, 5, 6 and 7) as compared to some bread wheat cultivars (lanes 1, 8, 9 and 10) and germ plasm lines (lanes 2 and 3). As indicated by the arrowhead in lane 4 of Fig. 1 (upper), a landrace (PK-15684) that originated from the Baluchistan province of Pakistan was found to possess a very large HMW glutenin subunit. The mobility of this subunit was comparable to that of the highest-molecular-weight Glu-D1-encoded subunit, designated as 2.2, which was first reported by Payne et al. (1983) in Japanese cultivars, and later by Margiotta et al. (1993) in a germ plasm line (MG-7249) of Japanese origin (Fig. 1, upper, lowest moving band in lane 3). The only subunit pair which could be readily identified in the landrace PK-15684 was 7 + 8 at the Glu-B1 locus. In addition, the landrace possessed two other subunits, a subunit (second slowest band in lane 4) with a mobility slower than that of the 1Dx subunit 2* (lane 1) and a subunit with a mobility intermediate of that of subunits 9 and 10 corresponding to genes 1By and 1Dy, respectively (lane 8 and, fastest moving in lane 4). These two subunits were assigned to the Glu-D1 locus because we had already encountered Glu-D1-encoded subunits of identical mobilities in other germ plasm lines of the same origin along with the Glu-B1 subunit 2* (Fig. 1 upper, lane 5) or “null” (lane 7).

It has been reported that SDS gels containing 4 M urea can be used to further discriminate and substantiate the results obtained in SDS gels without urea (Lafiandra et al. 1993). In particular, the mobility of the HMW glutenin subunits 2* and 1, encoded at the Glu-A1 locus, is faster than that of the Glu-D1-encoded subunits 2 and 5 when this type of gel is used. To further strengthen our belief that the novel subunit 2.1* possessed by PK-15684 is encoded at the Glu-A1 locus, we separated the HMW-GS in SDS-PAGE including 4 M urea (Fig. 1, lower). It is obvious from Fig. 1 (lower) that novel subunit 2.1* (indicated by the arrowhead in lane 4) migrates faster than the 1Dx subunit 2.2 (slowest moving...