ORIGINAL PAPER

Characterizing HMW-GS alleles of decaploid *Agropyron elongatum* in relation to evolution and wheat breeding

Shuwei Liu · Xin Gao · Guangmin Xia

Received: 6 February 2007 / Accepted: 23 October 2007 / Published online: 9 November 2007 © The Author(s) 2007

Abstract Bread wheat quality is mainly correlated with high molecular weight glutenin subunits (HMW-GS) of endosperm. The number of HMW-GS alleles with good processing quality is limited in bread wheat cultivars, while there are plenty of HMW-GS alleles in wheat-related grasses to exploit. We report here on the cloning and characterization of HMW-GS alleles from the decaploid Agropyron elongatum. Eleven novel HMW-GS alleles were cloned from the grass. Of them, five are x-type and six y-type glutenin subunit genes. Three alleles Aex4, Aey7, and Aey9 showed high similarity with another three alleles from the diploid Lophopyrum elongatum, which provided direct evidence for the E^e genome origination of A. elongatum. It was noted that C-terminal regions of three alleles of the y-type genes Aey8, Aey9, and Aey10 showed more similarity with x-type genes than with other y-type genes. This demonstrates that there is a kind of intermediate state that appeared in the divergence between x- and y-type genes in the HMW-GS evolution. One x-type subunit, Aex4, with an additional cysteine residue, was speculated to be correlated with the good processing quality of wheat introgression lines. Aey4 was deduced to be a chimeric gene from the recombination between another two genes. How the HMW-GS genes of A. elongatum may contribute to the improvement of wheat processing quality are discussed.

Communicated by M. Morell.

S. Liu · X. Gao · G. Xia (⊠) School of Life Sciences, Shandong University, 250100 Jinan, People's Republic of China e-mail: xiagm@sdu.edu.cn

Introduction

High molecular weight glutenin subunits (HMW-GS) are conserved endosperm storage proteins in the seeds of wheat and wheat-related species (Lawrence and Shepherd 1981; Shewry et al. 1995, 2003a). They explain up to 70% of the variation in bread making performance among European wheat cultivars (Branlard and Dardevet 1985; Payne et al. 1987, 1988), despite they only accounting for up to about 12% of the total protein in the endosperm of common wheat (Halford et al. 1992).

Due to the importance of HMW-GS to the improvement of wheat processing quality, genes encoding these subunits have been cloned from wheat and wheat-related species (Forde et al. 1985; Sugiyama et al. 1985; Thompson et al. 1985; Halford et al. 1987; Anderson and Greene 1989; Anderson et al. 1989; Halford et al. 1992; Reddy and Appels 1993; De Bustos et al. 2001; Wan et al. 2002; Liu et al. 2003; Wang et al. 2004; Guo et al. 2005; Wang et al. 2006; Yan et al. 2006). It has been confirmed that the HMW-GS genes are located on the long arms of the homoeologous group 1 chromosomes of hexaploid bread wheat at loci designated as Glu-1 (Lawrence and Shepherd 1980; Payne et al. 1980; Lawrence and Shepherd 1981; Payne et al. 1982). Each locus consists of two tightly linked genes which encode two types of subunits, the greater one termed x-type and the smaller one y-type (Harberd et al. 1986). Complete amino acid sequences of these subunits include three distinct domains: two highly conserved N- and C-terminal domains and a central repetitive domain. The central repetitive domains of both x- and y-type subunits comprising of hexapeptide and nonapeptide motifs while x-type subunits also contain tripeptide motifs.

The sequences of known HMW-GS genes shed light on how the different allelic genes have evolved and diverged. The similarity in structures of different HMW glutenin subunits indicates that they probably evolved from the same ancestor (Shewry and Tatham 1990; Shewry et al. 1995). Analyses through aligning N- and C-terminal sequences of some known HMW-GS confirmed that x- and y-type subunits represent two different subclasses, which indicated that the first step in the evolutionary process of HMW subunits was the duplication of a single ancestral gene into two closely linked copies (Wan et al. 2002; Shewry et al. 2003b). These copies diverged to be distinguishable (x- and y-type) before the speciation of wheat and wheat-related species (Shewry et al. 2003b).

Decaploid Agropyron elongatum (syn. Lophopyrum *elongatum* = *Thinopyrum ponticum*, 2n = 10x = 70) has many excellent characteristics such as high content of seed protein and high resistance to stress (Xia et al. 2003). So, it is an important resource for improving cultivated wheat (Triticum aestivum L). A great deal of hybrid cultivars with good processing quality were derived from sexual hybridization between A. elongatum and T. aestivum, e.g., Xiaoyan no. 6 (1, 14 + 15, 2 + 12) (Zhou et al. 1995) and Xiaoyan no. 54 (1, 14 + 15, 2 + 12); moreover, some somatic hybrid introgression lines with good processing quality were obtained from somatic hybrids (Xia et al. 2003; Liu et al. 2006) between *T. aestivum* cv. Jinan 177 (7 + 9, 2 + 12) and A. elongatum (most of the HMW-GS of A. elongatum are not clear before this report). A series of novel HMW-GS correlated with good bread-making quality were present in these hybrid progenies (Zhao et al. 2003; Feng et al. 2004a; Liu et al. 2006). It is necessary to investigate the contribution of HMW-GS of A. elongatum to those of hybrids.

In the work reported in this paper, we cloned and sequenced the open reading frames (ORFs) encoding HMW-GS from the decaploid *A. elongatum*. These results could enable us to compare the primary structure of HMW-GS from this wheat related polyploid with the published HMW-GS from wheat and other wheat-related grasses. In addition, the result will also assist us to understand the evolutionary process of HMW-GS genes in this decaploid grass and even the process of allopolyploidization of many *Triticeae* species.

Materials and methods

Cloning and sequencing of HMW glutenin gene ORFs

Seeds of *A. elongatum* stored in our laboratory were grown for 20 days at room temperature. Genomic DNA was extracted from a single seedling by the CTAB method according to Murray and Thompson (1980). HMW subunit genes contain no introns; so, genomic DNA is suitable as a template for PCR amplification of the entire coding region. In order to amplify the complete ORFs of HMW-GS genes of *A. elongatum* via genomic PCR, we designed a pair of degenerate

primers according to published DNA sequences of HMW-GS alleles of wheat and wheat-related grasses. The sequences of the two primers were P1 (5'-ATGGCTAAGCGGC/TTA/ GGTCCTCTTTG-3') and P2 (5'-CTATCACTGGCTA/ GGCCGACAATGCG-3'), respectively. Genomic PCR was carried out using the LA Taq polymerase (TaKaRa Biotechnology) with GC buffer for GC-rich template. The parameters for the reaction were: one cycle at 95°C for 5 min, followed by 30 cycles of 94°C for 40 s, 68°C for 4 min, and a final extension step at 72°C for 7 min. PCR products were separated in 1.0% agarose gels. All of the amplicon were recovered from the agarose gels and cloned into pUCm-T vector, then transferred into Escherichia coli DH10B competent cells. By restriction enzyme digestion mapping and terminal DNA sequencing, we found a series of new inserts that are not published before. To determine the complete DNA sequences of selected inserts, a series of subclones were prepared for each insert using the nested deletion method of Sambrook et al. (1989). Sequencing was performed commercially (Invitrogen). Sequence analyses were performed with the help of MEGA (Version 3.1, Kumar et al. 2004) and programs from the NCBI and EBI networks.

Bacterial expression of cloned ORFs

For bacterial expression of the mature proteins of HMW-GS from A. elongatum, two sets of PCR primers were designed for amplifying mutant ORFs without signal peptides and introducing appropriate restriction enzyme sites for the mutant ORFs to facilitate subsequent cloning experiments. The sequence of forward primer is PF: 5'-ACCCATATGGAAGGTGAGGCCTCT-3', while the sequences of two reverse primers are PR1: 5'-CTAGAAT TCCTATCACTGGCTGGCCGA (for Aex1, Aex4, Aey7, and Aey9) and PR2: 5'-CTAGAATTCCTATCACTGGC TAGCCGA (for Aey2). Introduced restriction site is Nde I for forward primer and EcoR I for both reverse primers, respectively. Mutant ORFs of these five alleles were cloned into the expression vector pET-24a (Novagen). The constructs were transferred into E. coli DE3 competent cells (Promega) for inducing bacterial expression. Inducement of bacterial expression of these five alleles was carried out according to Sambrook et al. (1989). Expressed proteins were extracted by dissolving cells in SDS-PAGE sample buffer (Wan et al. 2002).

Results

HMW-GS genes in A. elongatum

The amplicon of *A. elongatum* genomic DNA included over ten bands in all (Fig. 1). We reclaimed and cloned all





CK

Aey9

Aey7

Aex1

Ag

Aex4

Aey2

Ag

Fig. 2 Expression of the modified ORFs of five alleles *Aex1*, *Aex4*, *Aey2*, *Aey7*, and *Aey9* in *E. coli* and SDS-PAGE analysis of expressed products. The modified ORFs were prepared by removing the signal peptide sequence from each of the sequences by mutagenesis. Protein extracts were prepared by dissolving cells directly in SDS-PAGE sample buffer. The glutenin proteins synthesized in *E. coli* directed by *Aex1*, *Aex4*, and *Aey7* under IPTG induction showed identical electrophoretic mobility to those from seeds of *A. elongatum* (shown by *arrows*). No proteins from seeds of *A. elongatum* displayed similar mobility with those directed by *Aey2* and *Aey9* in bacteria (shown by *arrows*). *CK* proteins extracted from bacteria harboring pET–Aex1 without IPTG induction for control, *Ag* proteins extracted from seeds of *A. elongatum*



Fig. 1 PCR amplification of HMW-GS coding sequences from genomic DNA of *A. elongatum*. *M* lambda DNA digested by *Eco*R I + *Hind* III, *Ag* amplicon of *A. elongatum*

the amplicon together. After restriction enzyme digestion mapping and terminal DNA sequencing, we confirmed that 15 different inserts were obtained; five for x-type subunits and ten for y-type subunits. These inserts were designated as Aex1-Aex5 (x-type) and Aey1-Aey10 (y-type) according to their type and length. The length of these sequences shows a very large range, with the largest, Aex1, containing 2,424 base pairs while the smallest, Aey10, comprised only 1,150 base pairs. The latter is one of the smallest known HMW-GS genes identified to date. The five x-type sequences were larger than most of the y-type genes except that Aex3 (2,184 bp), Aex4 (2,082 bp), and Aex5 (2,004 bp) were smaller than the largest y-type allele Aev1 (2,219 bp). Of the 15 HMW-GS alleles, Aev2, Aey6, Aey8, and Aey9 were the same as those published by Feng et al. (2004b, c) in our lab, while the other 11 were found for the first time, which included five x-type and six y-type ones.

🖄 Springer

Derived amino acid sequences of HMW-GS genes

Analysis of the amino acid sequences deduced by the DNA sequences showed that 11 HMW-GS possessed a typical primary structure shared by other published HMW-GS (Figs. 3, 4). Each subunit consists of a signal peptide of 21 amino acid residues, a conserved N-terminal region, a central repetitive domain, and a conserved C-terminal region (Figs. 3, 4). The N-terminal regions of x-type subunits possess 86 amino acid residues except that Aex4 contains only 81 amino acid residues, while that of y-type subunits include 104 amino acid residues except that Aey4, Aey8, Aey9, and Aey10 have 105 amino acid residues. N-terminal regions of these four y-type subunits contain an extra glutamine (Q) residue compared to other y-type subunits with only 104 residues (Fig. 4). This glutamine residue is also present in all the known x-type subunits. Conserved C-terminal regions of all the 11 subunits comprise 42 amino acid residues. Central repetitive region of all the 11 subunits consists of hexapeptide and nonapeptide motifs; however, the five x-type subunits also contain tripeptides (GQQ), which is the typical character of x-type HMW glutenin subunits. Difference between these subunits and those from wheat is mainly due to single residue substitution and insertion or deletion of repeat motifs in central repetitive region (Figs. 3, 4).

Fig. 3 Comparison of primary structure of five x-type subunits from A. elongatum with that of three representative x-type subunits from common wheat. The N- and C-terminal regions were boxed. The tailed arrows indicated the cysteine residues and the additional cysteine residues of 1Dx5, Aex2, and Aex4 were underlined. The glutamine (Q) residues conserved in N-terminal domain of x-type subunits but absent from most y-type subunits were shown by non-tailed arrows. The in-frame stop codon was represented by asterisk. The Genbank accession numbers of these sequences were displayed in Table 1

| | | | TREDUCE CONTRACTOR OF TRACTON I DUUDUEC OC | CUVI COA |
|-------|--|---|---|-----------|
| Aex5 | NAKKUVLPAAVVVALVALIAAEGEASGQLQUERELREHELEAU NAKRI VI FTAVOOOJ VAI TAAFGFASGOLOGERFI FF | RQVVDQQLRDASPECRPVIVSPVARQIERQ ROVI DOOI RDASPECRPVIVSPVARQIERQ | UIVVPPKGGSFYPGELIPPQQLQQEIPWGIPALLNKYPFKVIS-QQG | SYYLCOA |
| 1By7 | MARKEVEL TAVVVVE VAL TAAFGFASGOLOCK | | PVVPSKAGSFYPSFTTPSOOLOGUTPWOTPALLINGTPAUS | SYYPGOA |
| Aex4 | MAKRSVI FAAL VVAL VAL TAAEGEASGOLOCERELOAC | QQVVNQQL RDASPECRPVAVSPVARQYEQQ | TVVPPKGGSFYPGETTPPQQLQQEIFWGIPTLLRRYYPSVTSPQQG | SYYPGOT |
| Aex1 | NAKRLVLFVAVVVALVALTVAEGEASGQLQCERELQERELKAC | QQVMDRQLRDVSPECHPVVI SPVAGQYGQQ | IVVPPKGGSFYPGETAPPOOLOOSIFWGIPALLRRYYPSVTSPOOV | SYYPGOA |
| 1Dx5 | NAKRLVLFVAVVVALVALTVÆGEASEQLQCERELQELQERELKAC | QQVMDQQLRDI SPECHPVVVSPVAGQYEQQ | IVVPPKGGSFYPGETTPPQQLQQRIFWGIPALLKRYYPSVTCPQQV | SYYPGQA |
| Aex2 | NAKRLVLFAAVVVALVALTVSEGEASGQLQCERELQERSLKAC | RQVMDQQLRDVSLECHPVVVSPVARQYEQQ | 2VVVPPKGGSFYPGETTPPQQLQQSIPWGIPALLRRYYPSVTSPQQV | SYYPGQA |
| 1Ax1 | NTKRLVLFAAVVVALVALTAAEGEASGQLQCERELQEHSLKAC | RQVVDQQLRDVSPECQPVGGGPVARQYEQQ | <u>vvvppkggsfypgettppqqlqqsilwgipallr</u> ryylsvtspqqv | SYYPGQA |
| | ↑ ↑ | · 🛉 🔺 | ↓ | |
| Aex3 | PSQWSGRGQHPGQGQQPGQGQQGQQPGQGQQGHY | PTSPQQPGQGQQLG | QWQQPVQGQPGYYPTSPQ-QLGQGQQPGQ | 3Q |
| Aex5 | SSUWSGUGUHPGUGUUPGUGUUGUUPGUGUUGYY | PISPQQPGQGQQLGP | | JU |
| 1DX1 | 2D0B1 C0C002C0C00BE0C00C00BC0C00 | | | FO |
| Aex1 | SPORPGODOOPGOGORSGOGOOGYYPTSPOOSGOWOOPFOGOPGYY | PTSPAAI GALAAPAAGAAGAAGAA | | 50 GQ |
| 1Dx5 | SPORPGOGOOPGOGOOGYYPTSPOOPGOWOOPEOGOOGYY | PTSPQQPGQLQQPAQGQQP | GOGOOGOOPGOPGOPGYPTSSOLOPGOLOOPAO | GQ |
| Aex2 | SPQRPGQGQQPGQGQQGYYPTSSQQSGQWQQPGQGQSGYY | PTSPQQSRQEQPGYYPTS | SQ-QPGRLQQPAQ | GQ |
| 1Ax1 | SSQRPGQGQQPGQGQQEYYLTSPQQSGQWQQPGQGQAGYY | PTSPQQSGQEQPGYYPTSPWQPEQLQQPTQ | CQQRQQPGQGQQLRQGQQGQQSGQGQPRYYPTSSQ-QPGQLQQLAQ | GQ |
| | | | | |
| Aex3 | -QPRQGQPGYYPTSSQQPGQGQQPGQGQQPGQGQQPGQ | GQQGYYPTSPQQPGQGQ | QPGQGQPGYYPTSLQQPGQGQQPGH | |
| Aex5 | -QPRQGQPGYYPTSSQQPGQGQQPGQGQQPGQGQQPGQ | GQQGYYPTSPQQPGQGQ | QPGQGQPGYYPTSLQQPGQGQQPGQ | |
| 1Bx7 | TSPQQSGQWQQPGQGQQPGQGQQSGQGQQPGQGQRGQCQPGQGQRPGQ | GQQGYYPISPQQPGQGQ | QSGQGQPGYYPTSLRQPGQWQQPGQ | |
| Aex4 | -UPGUGUPGYYPJSPUUPGUGUGGGGGGGGGGGGGGGGGGGGGGGGGGGGG | GUPGYYPISPQQPGQGQ | | 0000000 |
| 1Dy5 | | | | CUUDGUG |
| Aex2 | | GOOGYYPTSPOOSGOGOOSGOGOOPG | COOPGYYPTSPOOPGOGOLGYYPTSPOOSGOLOOPTOEOOPGOEOO | FAASGAG |
| 1Ax1 | OGOOPERGOOGOOSGOGOOLGOGOOGOOPGOKOOSGO | GOOGYYPI SPOOLGOGOOSGOGO | LGYYPTSP00SG0G0SGYYPTSA00PG0L00ST0E00LG0E00 | DOOSGOG |
| | | | ↓ | |
| Aex3 | GQQSGQGQQPGQGQQGQQSGQGQQGYYPT | SPQQSGQGQQAGQWQQPGQGQPGYYPTSPQ |)PGQGQQPGQGQPGYYPTSPQQPGQGQ | QG |
| Aex5 | GQRSG*GQQPGQGQQGQQSGQGQQGYYPT | SPQQSGQGQQAGQWQQRGQGQPGYYPTSPQ | QPGQGQQPGQGQPGYYPTSPHQPGQGQ | QG |
| 1Bx7 | GQQPGQGQQGQQPGQGQQSGQGQQGYYPT | SLQQPGQGQQLGQGQPGYYPTSQQ | ISEQGQQFGQGKQPGQGQQGYYPTSPQQSGQGQQ | LGQG |
| Aex4 | GQQSGQGQQGYYPTSPQQPGQBQQPGQGQPGYYPT | SPQQPGQGQQSGQGQPGYYPTSPQ |)PGQGQQPGQGQQPGQGQQPGQGQQPGQGQQ | LGQG |
| Aex1 | QQCQQPGQGQQGQQFGQCQQ-GQ-QPGYYPTSSQQSGQGQPGYYPT | SSQLPGQSQQPGQGQQGQQLGQGQ | QAQQPGQGQQPGQGQPGYYPTSPQQSGQGQLGHY | LTSPQQ- |
| 10x5 | QQGQQPGQGQQCGQQPGQGQPGYYP1SPQQSQGQPGYYP1 | SSQQPTQSQQPGQGQQQQQQQQQQQ | WAUPUPGUGUUPGUGUPGYYPTSPOOSOGOGOPGYY | LISPQUS |
| Aex2 | POCOOSCOPODOOSCOCODOCOPODOCASCOCOLICI BOCOOSCOPODOOSCOCODOCASCOCOLICITICISCOCODOSCASCO BOCOOSCOPODOOSCOCODOCASCOCOLICITICISCOCODOSCASCO BOCOOSCOCOCOCODOCASCOCOCOLICISCOCOCOCODOSCASCOCOCOCOCOCOCOCOCOCOCOCOCOCOCOCOCOC | ZDOUDGUEUUDBUI OUDEUGUGGGGURGLOGOG SDOUDGUEUUDBUI OUDEUGUGGGGGURGLOGOG | \@G@*FG&G&@&&G&G&G&FG11F13F&&3G&G&G&FG11. | PTCDUUC |
| 11181 | 14044D06164D46D060640 06160 011D1D1 66E0606111111 | di éél lépéél ivéréél délééléél délé | | . 101 990 |
| Aex3 | QTSSQQPGQEQQPGQGQQGYYPTSSQQPGQGQQ | PGQGQPGYYPTSPQKLGQGQQPGQGQ |)QSGQGQQPGQGQQSEQPGQGQQGYYPTSLQQSGQ | |
| Aex5 | QTSSQQPGQGQQGYYPTSSQQPGQGQQ | PGQGQPGYYPTSPQKLGQGQQPGQGQ | QSGQGQQPGQGQQSEQPGQGQQGYYPTSLQQSGQ | |
| 1Bx7 | QPGYYPTSPQQSGQGQQSGQGQQGYYPTSPQQSGQGQQ | PGQGQSGYFPTSRQQSGQGQQPGQGQ |)QSGQGQQGQQPGQGQQAYYPTSSQQSRQ | |
| Aex4 | QQGQQPRQGQQPGQGQQGYYPTSPQQPGQEQQSG | QWQQPGRGQPGYYPTSPQQPRQGQQPGQEQ |)QPGQGQQSQQPRQGQQGHYPTSPQQPGE | |
| Aex1 | | QQPGQGQPGYYPTSPQQSGQVQQPGQWQ | PQPGQGQPGYYPTSPLQPGQGQPGYDPTSPQQPGQ | LQQP |
| 10x5 | | WWFGWGWFGIIFISFWWSGWGWGWFGWWW | ICALCODCAAD CONTRACTOR CONTRACT | . OULUUP |
| 1 Av1 | | PGOGOPGYYPTSPOOSGOROOI ROWO | PCITEISELQEGQGQFGTTFTSELQFGQGQFGTTFTSEQQTGQ=== | |
| 111/1 | 067661190 66106766066106066066106066 | 10606101111016600606650656 | | |
| Aex3 | GQQAG | QWQQPGQGQPGYYPTSQQQPGQEQ | HPGQAQQSRQGQPVHYPTSPQQPGQGQQPA*GQQG | |
| Aex5 | GQQAG | QWQQPGQGQPGYYPTSQQQPGQEQ | HPGQAQQSRQGQPVHYPASPQQPGQGQQPA*GQQG | |
| 1Bx7 | RQQAG | QWQRPGQGQPGYYPTSPQQPGQEQ | QSGQAQQSGQWQLVYYPTSPQQPGQLQQPAQGQQPAQGQQSAQ | EQQPGQA |
| Aex4 | NQQAR | QWQQPGQGQPGYYPTSPQQPGQGQ | IQPGQAQQSGQGQPGYYPSSPQQPGQLQQPTQGQQG | |
| Aex1 | AQGQQGQQPAQGQQGQQPAQGQQGQQPGQGQQPA | QGQQGQQPAQGQQGQQPAQGQQGQQPGQGQ | QGQQPGQGQQPGQGQPGYYPTSPQQSGQGQQPGQWQQPGQGQPG | |
| 1Dx5 | | QGQQGQQPAQGQQGQQPGQGQHGQQPGQGQ | QGQQPGQGQQPGQGQPWYYPTSPQESGQGQQPGQWQQPGQGQPG | |
| Aex2 | GRUP | | UGUUPGUGUUPGUGUPGYYPTSI OOSCOCOOPCOWOUPGUGULG | |
| TAXI | | ~~~&L&&F_&F_I&F&&F&&F&&F&&F&&F&&F&&F&&F&&F&&F&&F&&F& | WWWWWWWWWWWWWWWWWWWWWWWWWWWWWWWWWWWWWW | |
| Aex3 | YYPTSP00PG0G00GYYPTSP00PG0G0 | OPGQWQQPGQGQQGYYPTSPQQPGQGQQQPG | :0W00PG0G00GYYPTSP00PG0WI 0PG0G00-GYYPTSP0 | apgagaa |
| Aex5 | YYPASLQQSGOGOOGYYTTSPOOPGOGO | RPGQGQQGYYPTSPOOLGOGOOSG | HGQQPGQWLOPGOEOO-GYYPISPO | QPG |
| 1Bx7 | 00SG0W0LVYYPTSP00PG0L00PA0G00GYYPTSP00SG0G00GY | YPTSPOOSGOGOOGYYPTSPOOSGOGOOPG | agaapragaagyypispaasgagaapgagaa-gyyptspaasgaga | OPGHEOO |
| Aex4 | YYPTSPQQTGQGQQGYYPTSPQQPGQGQ | QPGQWQQPGQGQQGYYPTSPQQSGQGQQSG | QWLQPGQGQQ-GYYPTSPQQLGQGQ | LSGHGQQ |
| Aex1 | YYLTFPLQSGQGQQWYYPTSLQQPGQGQ | QPGQWQQSGQGQQGYYPTSPQQSGQGQQPG | QGYYPTSPQ | QSGQGQQ |
| 1Dx5 | YYLTFSVAARTGQQGYYPTSLQQPGQGQ | QPGQWQQSGQGQHWYYPTSPKLSGQGQRPG | ;QGYYPTSPQ | QPPQGQQ |
| Aex2 | YYPTSPLQPGQGQQGYYRTSPQQPGQGP | QPGQWQQLGQGQQ <u>C</u> HYLTSPQQSGQGQQSG | QWLQSGQGQQ-GYYPTSLQ | QSGQGQQ |
| l¥Xl | YYPISSLQPEQGQQGYYPTSQQQPGQGP | QPGQWQQSGQGQQQPG | ₩₩LQPGQ₩LQSGYYLTSPQ | aregegg |
| 40.43 | PC001 0P | | ALCOARD AND AND AND AND AND AND AND AND AND AN | 25 |
| Aex5 | | 0 | YDSPYHVSAEYQAPSI.KVAKAQQI AAQI PANCRI EGGDAL SASQI A | 64 |
| 1Bx7 | PGQWLQPGQGQQGYYPTSSQQSGQG | HQSGQGQQGYYPTSLWQPGQGQQG | YASPYHVSAEYQAARLKVAKAQQLAAQLPANCRLEGSDALSTRO 7. | 89 |
| Aex4 | PGQWLQPGQGQQGYYPTSPQQSGQG | QQSGQGQQGYYPTSLQQPAQGQQG | YUSPYHVSAEYQAASLKVAKAQQLAAQLPAMCRLEGGNALSASQ 6 | 92 |
| Aex1 | PGQWLQPGQGQQGYYLTSPQQPGQG | QQSGQGRQG | YDSSYHVSAGHQAASLKVAKAQQLAAQLPAMCRLEGGDALSASQ 8 | 06 |
| 1Dx5 | LGQWLQPGQGQQQGYYPTSLQQTGQG | QQSGQGQQG | YYJSSYHVSVEHQAASLKVAKAQQLAAQLPANCRLEGGDALSASQ 8 | 39 |
| Aex2 | PGQWLQPGQVQQGYYPTSPQQ | SGQGQQG | YDGPYHVSAEQQAASLKVAKAQQLAAQLPAMCRLEGGDALSATQ 7 | 70 |
| 1Ax1 | PKQWLQPRQGQQGYYPTSPQQSGQGQQLGQGQQGYYPTSPQQ | SGQGQQG | YUSPYHVSAEHQAASLKVAKAQQLAAQLPANCRLEGGDALLASQ 8 | 30 |
| | | | Ŧ | |
| | | | | |

Fig. 4 Comparison of primary structure of all the ten y-type subunits from A. elongatum and that of three representative y-type subunits from common wheat. The N- and C-terminal regions were boxed. The tailed arrows indicated the cysteine residues and additional cysteine residues of Aey1, Aey3, and Aey10 were underlined. The sequences of Aey1, Aey4, and Aey5 were rectified to diminish the influence of frame shift. The revised amino acid residues were substituted by underlined X. The in-frame stop codon was represented by asterisk. The extra glutamine (Q) residues in N-terminal domain of Aey4, Aey8, Aey9, and Aey10 were shown by non-tailed arrows. The Genbank accession numbers of these sequences were displayed in Table 1

| Aev3 | MAKRLVLFATVVIALVALTVÆDEASRKLQCECELQESSLEACE | RLVVDQQLAGRLPWSTGLQWRCC | QQLRDVSAKCRPVAVSQVARQYEQ-TAVPPKGG | SFYPRETTPLQQLQQGIFWGT] |
|---|---|---|--|--|
| Aev7 | MAKRI VI FATVVTALVALTVARGEASROLOCERELOESSI FARE | NUTRICIA AGRI PUSTGI ONRCC | OOI RDVSAKCRPVAVSOVAROVRO-TAVPPKGG | SEVESETTEL OOL OOG LEWGT |
| 1 4 | MAKELVEL HEVTELT HEVELT HEVELANDELEVELEVELEVELEVEL | UVDOOL ASEL BUSTCLOVECC | OOI PDI SAKCEPVAI SOVAPOVCO-TAVPPKCCI | FYURFTTRI OOL OOG FCCT |
| 1 AY | WANNE ALLAND AND AND ALLAND AND ALLAND AND ALLAND AND AND AND AND AND AND AND AND AND | CTANDOOL VOLT HOLOWICC | COLDDUG NODDULUGOUTOOUTOO | PIREITFLOOLOOUT WAT |
| Aeyı | MARKLYLFATYYTTLYALTAAEGEASRQLRCERELQESSLEAGT | WAAADAATAA WAAAAAAAAAAAAAAAAAAAAAAAAAAAA | QULUPANCIPAN SQAAMOIDG-IAALLUG | SF IFGEI IFLQQLQQVILWGI |
| 1By8 | MAKRLVLFATVVITLVALTAAEGEASRQLQCERELQESSLEACH | QVVDQQLAGRLPWSTGLQ#RCC | QQLRDVSAKCRLVAVSQVVRQYEQ-TVVPPKGG; | SFYPGETTPLQQLQQVIFWGT |
| Aey2 | MAKRLVLFAAVVIALVALTTÆGEASRQLQCERELQESSLEACH | RQVVDQQLAGRLPWSTGLQMRCC | QQLRDVSAKCRSVAVSQVARQYEQ-TVVPPKGG: | SFYPGETTPLQQLQQGIFWGT |
| 1Dy10 | MAKRLVLFAAVVIALVALTTÆGEASRQLQCERELQESSLEACH | RQVVDQQLAGRLPWSTGLQMRCC | QQLRDVSAKCRSVAVSQVARQYEQ-TVVPPKGG: | SFYPGETTPLQQLQQGIFWGT |
| Aey4 | MAKRLVLFATVVVALMALTAAEGEASRQLQCERELQESSLEACH | QVVDQQ*AGQLPWSTGLQMRCC | QQLRDVSAKCRLIAVSQVARQYEQQTAVPPKGG: | SFYPGETTPLQQLQQGIFWGT |
| Aev5 | MAKRLVLFATVVIALVALTTAEGEASRQLQCERELQESSLEACH | OVVDOOLAGRLRWSTGLOMRCC | OOLRDVSAKCRPVAVSQVARQYEQ-TVVPPKGG | SFYPGETTPLOOLOOGIFWGT |
| Aev6 | MAKRI VI FATVVI ALVALTTARGEASROLOCERDI QESSI BACH | ROVVDOOLAGRI PWSTRI OMRCC | OOL RDVSAKCRPVAVSQVARQYEQ-TVVPPKGG | SEVEGETTEL OOL OOGTEWGT |
| Acre ⁰ | NAKEI W FAARMAI VALTTAFCFASEOLOCEPELOFSCI FACE | OWNOOI ACOL DESTCI ONDCC | OOI PDVCTKCPDI AVCOVAPOVEOOTANDDKCC | FYPCDTTPTOOL OOPTEWCP |
| ACYO | MARINE VELYAR VALVALI I ABOBASINQEQUENEEQESSEEACI | OTADOOT YOU DIRECT OF DESILECT | QUIND & STRONT IN CONTRACT DOWN ON THE PROP | TP IF OD I IF TOOLOOD IF WOR |
| Аеуу | MAKKLVLFAVVVVALVALTTAEGEASKQLQCEKELQESSLEACI | QVVDQQLAGQLPWSIGLQMRCC | UQLRDVSAKCRPIAVSQVARQYEQQIIMPPKGG | SFYPGDIIPIQQLQQRIFWGR |
| Aey10 | MAKRLVLFATVVVALMALTAAEGEASRQLQCERELQESSLEACH | REVVDQQLAGQLFWSTGLQMRCC | QQLRDVSAKCRLIAVSQVARQYEQQTAVPPKGG: | SFYPGETTPPQQLQQRIFWGR |
| | <u>↑</u> ↑ ↑ | ŤŤ | ↑ ∧ | |
| Aey3 | SSQTVQGYYPSVTSPQQGSYYEGQASPQQPGQGQRPGQGQQ | -PGKWQELGQGHQGYYPT | SLQQPGQGQQTGQGQQGYYPTSL(| QQPGQGQQIGLWQQGYYPTSP |
| Aev7 | SSQIVQGYYPGVTSPQQGSYYRGQASPQQPGQGQRPG | KWOELGOGOOGYYPT | OGOOGYYPTSLO | OPGOGOOIGOWOOGYYPTSP |
| 1Av | SSQTVQGYYPSVI SPOQGSYYPGQASPQQPGKWQELGQGQQWYY | PTSLOOPGOGOOGYYRT | OROOGYYRTSL | OPGOGOQIGOWQQGYYPTSP |
| Aov1 | SOTVECVVPSVSSPOOGPVVPGOASPOOPCOCOOPC | | | OPCOCOTCOCOCYPTSP |
| 10.0 | COTTOCTUCETUCETCOCEPTUCECA CROOPERCOOPE | KNOELGOOGGUDTELLIGS | 202002020202010202001202002020202020202 | |
| 1Dy8 | DSQ1VQ511PSVSSPQQ6P11PGQASPQQPQ0Q0Q0PG | NWQELGQGQQQGTTPISLHQS | COCOUNT LESSED COCOUNT COCOUNT LESSED | 2QFGQGQQIGQGQQGIIPISP |
| Aey2 | SSQTVQGYYPSVTSPRQGSYYPGQASPQQPGRGQQPG | KWQEPGQGQQWYYPI | SLQQPGQWQQIGKGKQGYYPASLQ | YOLOOCOOTCOCOOCAALISL |
| 1Dy10 | SSQTVQGYYPGVTSPRQGSYYPGQASPQQPGQGQQPG | KWQEPGQGQQWYYPT | SLQQPGQGQQIGKGQQGYYPTSL | QQPGQGQQGYYPTSL |
| Aey4 | SSQTIQGYYPSVTSPRQGSYYPGQASPQQPRQGQQPG | KWQEPGQGQQGYYPT | SLQQPGQGQQIGKGQQGYYPTSL | QQPGQGQQGYYPTSP |
| Aev5 | SSQTICGYYPSVTSPRQGSYYPGQASPQQPRQGQQPG | KWQEPGQGQQGYYPT | SLOOPGOGOOIGKGOOGYYPTSLO | OOPEQGOOGYYPTSP |
| Aevô | SSOTVOGYYPSYTSPROGSYYPGOASPOOPGOGOOPG | KWQEPGQGRQGYYPT | SI GOPGOGOOTGKGOOGYYPTSI (| OPGOGOOTGOGOOGYYPTSP |
| Acres 0 | | CVVPT | FI 001 C | |
| L O | | 01111 | L TOL S | HOTHISL |
| Аеуу | DSQ1VQ511PSV1SPQQ6S11P6QASPQQP6Q6QQP6Q | GUQFGQGQQGYYPI | SLHQLG | HGTTPISP |
| Aey10 | SSQTVQGYYPSVTSPQQGSYYPGQASPQQPGQGQ*PGQ | WQE | PGQGQ | QGYYPTSL |
| | | | | |
| Aev3 | OHPGOGOOPGOGOOIGOGOOSGOGOOIGOGOSROGOOPGOGOG | GYYPTSPOOLGOGOOPGOWOOP | ROGOOGYYPTSLOOPGOGOO | GHVP |
| Aeu7 | | GYYPTSSOOI COCOOPCOMOOP | GOGOOGYYPTSI DOPCOGOO | |
| 1.0y1 | | 20111110000000000000000000000000000000 | 00000077000000000000000000000000000000 | |
| IAY | wnrowowwrowywaliowowywerewali | aTOARAAN RAAANA COORDAN | oqoqqoiiiiiiislqqbGqGqqPGQWQQP | GQGQQGYYP |
| Aey1 | QHPGQRQQPGQGQQIGQGQQLGQGRQIGQGQQSGQGQ | JGYYPTSPQQLGQGQQPGQWQQS | GQGQQGYYPTSQQQPGQGQQGQYPASQQQPGQG | QQGQYPASQQQPGQGQQGQYP |
| 1By8 | QHPGQRQQPGQGQQIGQGQQLGQGRQIGQGQQSGQGQ | GYYPTSPQQLGQGQQPGQWQQS | GQGQQGYYPTSQQQPGQGQQGQYPASQQQPGQG | QQGQYPASQQQPGQGQQGQYP |
| Aev2 | OHTGOROOPVOGOOIGOGOOPFOGOOPCOWO | GYYPTSPOOLGOGOOPGOWOOS | GOGQOGHYPTSLROPGOGOOGHYI ASOCOPAOG | DOGHYPASOCOPGOGOOGHYP |
| 10.010 | | CAAbarden Gunden of Country And Co | COCOOCHYPTSI OOPCOCOCHYI & COCOPCOC | JOGHYP& SOOOPCOCOCUVE |
| 1.0y10 | OTECODO DO | CONTRACTOR CONCOURSES | COCOOCINETSI OODAOGOOGIEE 120002 | ACCOUNT NOT ACCOUNT ACCOUNT NOT ACCOUNT NOT ACCOUNT NOT ACCOUNT NOT ACCOUNT AC |
| Aey4 | enrowneeroeue | 2011F13FWWLGWGWPGWWQQS | owowwonirialsouweredeedeentheredeedeedeedeedeedeedeedeedeedeedeedeed | GQGQQGHYP |
| Aey5 | QHPGQKQQPGQGQQPEQGQQPGQWQG | JGYYPTSPQQLGQGQQPGQWQQS | GQGQQGHYPTSLQQPGQGQQGHYLASQQQP | GQGQQGHYP |
| Aey6 | QHPGQRQQPGQGQQIGQGQQPEQGQQPGQWQ | QGYYPTSPQQLGQGQQPGQWQQS | GQGQQGHYPTSLQQPGQGQQGHYLASQQQP | GQGQQGHYP |
| Aev8 | OOSROROOPGOGOOIGOGOOPEOGEO | GYYPTSPOOPGO*OOPGOWOOP | GOGOOGYYPTSLOOPGOGOOT | GOEQQS |
| Aerr9 | 00SR0R00PG0G00PF0R00 | GYCPTSPOP | GOGOOGYYPTSI OOPGOGOOT | |
| Accel 0 | | | | 200203 |
| Aey10 | QQLQAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA | ۸ | | |
| | | 1 | | |
| Aey3 | ASLQPPGQGQQGHYPASQHQPGQGQQG | HYPASLQQS | GQGQQGHHPASMQQPGQGQQIGRPGQRQQPGQG | QETGQGQQPEQEQQPGQGQ |
| Aey7 | ASLQQPGQGQQGHYPASQHQPGQGQQG | HYPASLQQS | GQGQQGHHPASLQQPGQGQQTGQPVQRQQPGQG | QETGQGQHPEQEQQPGQGQ |
| 1Av | TSLOOPVOGOOGHYPASOHOPGOGOOG | HQPASLQ-S | GOGOOGHHPASLOOPGOGKOTGOREOROOPGOG | DOTGOGOOPEOEOOPGOGO |
| 1 av1 | ASOODPACCOCCUPA SOODPCCCCOCCUVI A SOODPCCCCOCPU | PASI OOPGOGOOGHVTASI OOP | COCOOCHYPASI OOVCOCOOTCOI COROOPCOC | ROTROGOOI FOCOOPCOGOOT |
| 1D.O | VOODDYGGGGGGGII VOGGGG GGGGGGUI TVOGGGI GGGGGGGI | IT ASLOOD OGGOOOD WTA CLOOD | 2020001MD 1 CL 00M00001 201 20D00D2D0 | COTROCOL ECCORD COOR |
| IDys | ASQUQFAQQQQQUIFASQQQFQQQQQHILASQQQFQQQQH | (PASLQQPGQGQQGHI IASLQQP | CACAMONICAL CONTRACTOR CONTRACTOR | |
| Aey2 | ASQQQPGQQQQHYPASQQEPGQGQQGQIPASQQQPGQQQQ | GHHPASLQQP | GGAGHALIZEAAEAGAATAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA | JUIGUGUUPEUEUUPGUGU |
| 1Dy10 | ASQQQPGQGQQGHYPASQQEPGQGQQGQIPASQQQPGQGQQ | GHYPASLQQP | GQGQQGHYPTSLQQLGQGQQTGQPGQKQQPGQG | QQTGQGQQPEQEQQPGQGQ |
| Aey4 | ASQQQPGQGQQGHYPASQQEPGQGQQGQIPASQQQPGQGQQ | GHYPTSLQQP | GQGQQGHYPASLQQLGQGQQIGQPGQXQQPGQG | QQTGQGQQPEQEQQPGQGQ |
| Aev5 | ASOCOPEOGOCHYPASOCEPEOGOCOTPASOCOPEOGOC | GHYPTSI OOP | COCOCHYPASI OOL COCOCTCOPCOXOOPCOC | OUTGOGOOPFOFOOPGOGO |
| Acres | TSOODPCOCOCUVEASOOFPCOCOCOCIT ASOOOPCOCOCO | | | OTCOCOOPEOEOPCOCO |
| VCAC V | LONOUDIMANOLANDLADOUDAGAGAGITUGAGGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG | | | 56106060 D6D661.0606 |
| Aeys | GGWGGPVKGHGGYYPISPGQGGGGP | GQWQQ- | GITPISPQQPGQGQQIGQGQQGTPISP | 4620909 |
| Aey9 | GQWQQLGKGHQGYYPTSPQQPGQGQQP | GQWQQP | GQGQQGYYPTSPQQPGQGQQIGQGQQGYLPTSS | QQSGQGQ |
| Aey10 | GQWQQPGQGHQGYYPTSPQQPGQGQQP | GQWQQP | GQGQQGH <u>C</u> PTSP | 20 |
| | | | 4 | |
| 1000 | | PCOCOOPEO | | |
| A 7 | QUITIIDEQ OCUMPTOLO | 2 DECODEC | MOODGOGOOGUWDAGLOODGOGO | DCOTOODCOCODE |
| Aeyr | | 2FR&G&&FE&======= | | PGQIQQPGQGQQPD |
| 1Ay | QGYYPTYLQQ | 2PGQGQQPEQ | WQQPGQGQQGHYPASLQQSGQGQQGHY | PASLQQLGQGQ*PG |
| Aey1 | RQGQQLEQGQQPGQGQQTRQGQQPEQGQQPGQGQQGYYPTSPQ | QSGQGQQPGQSQQPGRGQQGYYS | TSLQQPGQGQQGHYPASLQQPGQGH | PGQRQQPGQGQQPK |
| 1By8 | RQGQQLEQGQQPGQGQQGYYPTSPQ | SGQGQQPGQSQQPGQGQQGYYS | SSLQQPGQGLQGHYPASLQQPGQGH | PGQRQQPGQGQQPE |
| Aev2 | | PGQGQQQGQCOOCVVP | TSLOOPGOGOOGHYPASI OOPGOGO | PGOROOPCOCOHPR |
| 10-10 | QUITIDLQ | | TOLOGOCOCOCUVE ACLOGOCOCO | |
| 10310 | | 21 JANARAANA | LONG CONTRACTOR CONTRACTOR | rownwwrowownPE |
| Aey4 | QGYYPTSPQ0 | ଽୢୄ୵୰୶ଡ଼ଢ଼ଢ଼ଢ଼ଢ଼ଢ଼ଢ଼୷୷୷୷୶ୡୡୡୡୡୡୡୡ | I SLEWFGUGUUUHIPASLUUPGUGU | PGQKQQPGQGQHPE |
| Aey5 | QGYYPTSPQ0 | 25GQGQQQGQGQQGYYP | TSLQQPGQGQQGHYPASLQQPGQGQ | PGQRQQPGQGQHPE |
| Аеуб | QGYYPTSP00 | <u>}</u> | PGQGQQGHYPASLQQPGQGQ | PGQRQQPGQGQHPE |
| Aev8 | QGYYPTSI 00 | PGQGQQTGQGQQS | GOWQOPAKGHOGYYPTSPOOPGOGO | |
| Aerro | | | | |
| A1 0 | LOUGH AND A LOUGH AND AND A LOUGH AND A LOUGH AND AND AND A LOUGH AND | | CONTINUES CONTINUES AND COULD CALLER CONTINUES | |
| veA10 | Q011115EQ | | GQWQQPGKGHQGYYPTSPQQPGQGQ | |
| | PRQAQ | PGQGQQTGQ | GQWQQPGKGHQGYYPTSPQQPGQGQ SQQPGQEQ | |
| | PRQAQ | PGQGQQTGQ | GQWQQPGKGHQGYYPTSPQQPGQGQ SQQPGQBQ | |
| Aey3 | QEQQPGQGQ-GYYSTSSQQPGQGQHPGQGQQGYYPTSPQHPGQ | QPCQGQQTGQ | GGWQQPGKGHQGYYPTSPQQPGQGQ SQQPGQBQ QPGQGQQIGQVQQLGQGQQGYYPTSLQQPGQGQ | QLGQGQQSGQGRQPGQGQ*SG |
| Aey3 Aey7 | QUITIOLQ PRQAQ QEQQFGQGQ-GYYSTSSQQPGQGQHPGQGQQGYYPTSPQHFGQ QEQQFGQGQQCYYPTSSQQFGQGQHFGQGQQGYYPTSPQHFQQF | | GQWQQPGKGHQGYYPISPQQPQGQ | 2LGQGQQSGQGRQPGQGQ*SG QSGQGQQLGQGHQPGQGQQSG |
| Aey3 Aey7 1Ay | QEQPEQGQQ-GYYTSSQQPGQQHPGQQQQGYYTSQAPPGQ QEQQPGQGQQGYYTSSQQPGQQHPGQQQQGYYTSQQPGQ QEQQPGQGQQGYYTSSQQPGQQHPGQCQQGYYTSSQQPGQ QEQQPGQCQGYYTSSQQPGQCQHPGQCQGGYYTSSQ | JPGQCQQTCQ JQQPGQCQQGHCPTSPQQPGQAQ JQQPGQCQQGHCPTSPQQPGQAQ QPGQCQQGHFPTSGQAQ | GGWQQPCKCHQCYPTSPQPCQQQ | 2LGQGQQSGQCRQPGQGQ*SG 2SGQGQQLGQCHQPGQGQSG 2SGQGQQLGQCHQPGQGQOSG |
| Aey3 Aey7 1Ay Aey1 | | JPGQGQQTCQQPGQAQGQGHCPTSPQQPGQAQ JQQPGQGQQGHCPTSPQQPGQAQ QPGQGQQGHFPTSPQAQ JQDPGQGQXGHCPTSPQDTCOAO | GawqQPGKGHQYYYTSPQQPGQQQ | 2LGQGQQSGQCHQFGQGQSG 2SGQGQQLGQCHQFGQGQQSG 2SGQGQQSGGCHQFGQGQSG 2SGQGQQSGGCHRTGCGCQPSG |
| Aey7 Aey7 1Ay Aey1 1By-9 | GENT FISCA GENERATING CONTRACT CONTRACT CONTRACT GENERATING CONTRACT CONTRACT CONTRACT CONTRACT GENERATING CONTRACT CONTRACT CONTRACT GENERATING CONTRACT CONTRACT CONTRACT CONTRACT GENERATING CONTRACT CONTRACT CONTRACT GENERATING CONTRACT GENERATING CONTRACT | | Gawaareeksinay yy hispaarodoga | 2LGQGQQSGQGRQPGQGQ+SG 2SGQGQQLGQGHQPGQGQSG 2SGQGQQLGQCHQPGGQQSG 2SGQGQQSGQCHRLGQQRSG 2SGQGQCQCGHRLGQQQSG |
| Aey7 1Ay Aey1 1By8 | | | GawaqPericenzey YYTSPaqPeage SQRFCQEQ QPCGCQQICQVQLLCQCQQCYYTSLQPCCQC QPCGCQQICQVQLLCQCQQCYYTSLQPCCQC QPCGCQQICQAQLCQCQQCYYTSLQQPCCQC QPCGCQQICQAQCEQCQCYYTSLQQSCQCQ QPCGCQQICQVQEFCQCQCCYTFISLQQSCQCQ QPCGCQQICQVQEFCQCQCCYTFISLQQSCQCQ | 2LGQGQQSGQCRQPGQGQ+SG 2SGQGQLGQCHQPGQGQSG 2SGQGQLGQCHQPGQGQSG 2SGQGQQSGQCHQLGGQQSG 2SGQGQQSGCGHQLGGQQSG |
| Aey7 1Ay Aey1 1By8 Aey2 | QEDAPFGQQ QEDAPFGQQ PRQAQ QEQQPGQQ QEQQPGQQQCYYPTSSQQPGQQHPGQQQQCYYPTSPQHPGQ QEQQPGQQQCYYPTSSQQPGQCYYPTSPQ QEQQPGQQQQCYYPTSSQQPGQCKQLGQCQCYYPTSSQQPGQ QEQQPGQQQCYYPTSPQPGQCKQLGQCQCYYPTSPQPGQQQQCYYPTSPQQPGQQQCYYPTSPQQPGQQC | | GawadyekGHagYYYTSP4QF6Q6q | 2LGGGQQSGQCRQPGQGQ+SG 2SGGGQQLGQCHQPGQGQQSG 2SGGGQQCQLGQCHQPGQGQQSG 2SGGGQSGCQCHRLGQGQRSG 2SGGQQSCGCHQLGQGQQSG 2SGGGQQSGGCHQPGQGQQSG |
| Aey3 Aey7 1Ay Aey1 1By8 Aey2 1Dy10 | QUITITISEQ QEQQPGQCQ-GYYTSSQQPGQCQHPOQCQCQYPTSPQHPOQ QEQQPGQCQGYYTSSQQPGQQHPOQCQCQYPTSPQHPOQ QEQQPGQCQGYYTSSQQPGQQQHPOQCQQCYYTSPQ QTQQPGQCQCYTTSSQQPCQCQLOCQCCQYYTSPQ QGQQPGQCQQCYYTSSQQPCQCQLOCQCQCYYTTSPQPCQ QGQQPGQCQQQYYTTSSQQPCQCQLOCQCQCYYTTSPQPCQ QGQQPGQCQQQYYTTSSQQPCQCQLOCQCQCYYTTSPQPCQ QGQQPGQCQQQYTTSPQQPCQ QGQQPGQCQQQYTTSPQQPCQ QGQQPGQCQQQYTTSPQQPCQ QGQQPGQCQQQYTTSPQQPCQ QGQQPGQCQQQYTTSPQQPCQ QGQQPGQCQQQYTTSPQQPCQ QGQQQCQQQQQYTTSPQQPCQ QGQQPGQCQQQYTTSPQQPCQ QGQQPGQCQQQQYTTSPQQPCQ QGQQPGQCQQQYTTSPQQPCQ QGQQQQQQQQQT QGQQQQQQT QGQQQQQQT QGQQQQQT QGQQQQQQT QGQQQQQQT QGQQQQQT QGQQQQQT QGQQQQQQT QGQQQQQT QGQQQQQQT QGQQQQQQQT QGQQQQQQT QGQQQQQT QGQQQQQQT QGQQQQT QGQQQQQQT QGQQQQQT | | Gawaarekschartyryrsaarekscoor | 21.646442564264264264256 255666421.642642664256 255666421.642642664256 2556664256426411.64664256 255666425642641264664256 255666425564126466425642564256 25566642556412666425642564256 |
| Aey3 Aey7 1Ay Aey1 1By8 Aey2 1Dy10 Aey4 | QEQREGGQ GYTTSSQQPGQQHPGQGQQGYYPTSPQHPGQ QEQQPGQQQ GYTTSSQQPGQQHPGQQQQGYYPTSPQHPGQ QEQQPGQQQGYYPTSSQQPGQQGQGYYPTSPQ QEQQPGQQQQGYYPTSPQPGQQQGQGQGYYPTSPQPFQQ QGQQPGQQQQGYYPTSPQPFQQQQLGQCQQGYYPTSPQPFQQ QGQQPGQQQQGYYPTSPQQPGQQQLGQCQQGYYPTSPQPFQQ QEQQPGQQQQGYYPTSPQQPFQQQQLGQCQQGYYPTSPQPFQQ QGQQPGQQQQGYYPTSPQQPFQQQQLGQCQQGYYPTSPQQPGQ QEQQPGQQQQYYPTSPQQPFQQQQLGQCQQGYYPTSPQPFQQ QGQQPGQQQQYYPTSPQQPFQQQQLGQCQQGYYPTSSQQPFQQ | | GAWQUPKGEH2YYYTSPQQFGQQG | 2LCGCQQSCQCRQPCQCQ+SC 2SCQCQQLCQCHQPCQCQQSC 2SCQCQQLCQCHQPCQCCQSC 2SCQCQQSCQCHQCQQSC 2SCQCQQSCQCHQCQQCQSC 2SCQCQQSCQCHQPCQCQQSC 2SCQCQQSCQCHQPCQCQQSC 2SCQCQQSCQCHQPCQCQQSC 2SCQCQQSCQCHQPCQCQQSC |
| Aey3 Aey7 1Ay Aey1 1By8 Aey2 1Dy10 Aey4 Aey5 | QEDITIDE QEQQPGQGQ-GYYTSSQQPGQGQHPGQGQGQYPTSPQHPGQ QEQQPGQGQGYYTSSQQPGQGQHPGQGQGQYYTSPQHPGQ QEQQPGQGQGYYTSSQQPGQGQHPGQGQGQYYTSPQ QGQQPGQGQQGYYTSSQQPGQGQLGQGQGYYTSPQPGQ QGQQPGQGQQGYYTSSQQPGQGQLGQGQGYYTSPQPGQ QGQQPGQGQQGYYTSSQQPGQGQLGQGGQGYYTSSQQPGQ QGQQPGQGQQGYYTSSQQPGQGQLGQGGQGYYTSSQQPGQ QGQQPGQGQQGYYTSSQQPGQGQLGQGGQGYYTSSQQPGQ QGQQPGQGQQGYYTSSQQPGQGQLGQGGQGYYTSSQQPGQ QGQQPGQGQQGYYTSSQQPGQGQLGQGGQGYYTSSQQPGQ QGQQGQGQQGYYTSSQQPGQGQLGQGQGYYTSSQQPGQ QGQQPGQQQQGYYTSSQQPGQ QGQQPGQQQQYYTSSQQPGQ QGQQPGQQQQYYTSSQQPGQ QGQQPGQQQQYYTSSQQPGQ QGQQPGQQQQYYTSSQQPGQ QGQQPGQQQQYYTSSQQPGQ QGQQPGQQQYYTSSQQPGQ QGQQPGQQQYYTSSQQPGQ QGQQPGQQQYYTSSQQPGQ QGQQPGQQQYYTSSQQPGQ QGQQPGQQQYYTSSQQPGQ QGQQPGQQQYYTSSQQPGQQQPG QGQQPGQQQYTSSQQPGQQQQPG QGQQPGQQQYTSSQQPGQQQPGQQQPG QGQQPGQQQYTSSQQPGQQQQPS QGQQPGQQQYTSSQQPGQQQQQYTSSQQPGQQQQQYTSSQQPGQQQQQQQPS QGQQPGQQQYTSSQQPGQQQQYTSSQQPGQQQQYTSSQQPGQQQQQYTSSQQPGQQQQQYTSSQQPGQQQQYTSSQQPGQQQQQQQQYTSSQQPGQQQQQQYTSSQQPGQQQQQYTSSQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQ | | GawaqPerkGHaQYYYTSPQQPGQQQ | 2LEQEQEQEGEQEQEQEQESQ 2SEQECQLEQEHQEQEQESG 2SEQECQLEQEHQEQEQESG 2SEQECQEQEQEGEHLEQEQEQESG 2SEQECQESCQEHLEQEQEQESG 2SEQECQESCQEHDPEQECQESG 2SEQECQESCQEHDPEQECQESG 2SEQEHDPEQECQESG |
| Aey3 Aey7 1Ay Aey1 1By8 Aey2 1Dy10 Aey4 Aey5 Aey6 | QEOTITISEQPCQQUPCQCQCGYPTSPQPCQQQQ QEQQPCQQQ-GYYSTSSQQPCQQUPCQCQQCYYPTSPQPCQQ QEQQPCQQQQYYPTSSQQPCQCQUPCQCQQCYYPTSPQPCQQ QCQQPCQCQQCYYPTSSQQPCQCQLQCQCQCYYPTSPQPCQQQQQCYYPTSPQPCQCQQCQYYPTSPQPCQCQLQCQCQYYPTSPQPCQQQLQCQCQYYPTSPQPCQQQQLQCQCQYYPTSSQQPCQQQQCYYPTSSQQPCQQQQCYYPTSSQQPCQQQQCYYPTSSQQPCQQQQCYYPTSSQQPCQQQLQCQCQYYPTSSQQPCQQQLQCQCQYYPTSSQQPCQQQLQCQCQYYPTSSQQPCQQQLQCQCQCQUPCYPTSSQQPCQQQDQQQCQYYPTSSQQPCQQQLQCQCQQCQYYPTSSQQPCQQDQCQQCQQCQYYPTSSQQPCQQQDQQQCQYYPTSSQQPCQQQDQCQQCQQCQYPTSSQQPCQQQDQQQYPTSSQQPCQQQDQQQQYPTSSQQPCQQQDQQQQYPTSSQQPCQQQDQQQYPTSSQQPCQQQDQQQQYPTSSQQPCQQQDQQQYPTSSQQPCQQDQQQQYPTSSQQPCQQQDQQTYPTSSQQPCQQDQDQTYPTSSQQPCQQDQDQTQDDDDDDDDDDDDDDDDDDDDDDDDDDDD | | GAWQUPGKGHQYYYTSPQQPGQQQ | 2LCGCQ2SCQCRQPGQCQ+SC 2SCQCQ2LCQCRUPCQCQQ2SC 2SCQCCQ2LCQCRUPCQCQQ2SC 2SCQCQ2SCQCRUPCQCQQ2SC 2SCQCQ2SCQCRUPCQCQQ2SC 2SCQCQ2SCQCRUPCQCQQ2SC 2SCQCRUPCQCQQ2SC 2SCQCRUPCQCQQ2SC 2SCQCRUPCQCQQ2SC |
| Aey3 Aey7 1Ay Aey1 1By8 Aey2 1Dy10 Aey4 Aey5 Aey6 Aey6 | QEQPFGQQQGYYPTSPQPFQQQQLGQCQGYYPTSQQPGQ QEQQPGQQQGYYPTSSQQPGQQHPQQQQQGYYPTSPQPFQ QEQQPGQQQGYYPTSSQQPGQQHPQQQQGYYPTSPQ QCQQPGQQQQGYYPTSPQPCQQLQQQGQGYYPTSPQPCQ QGQQPGQQQQGYYPTSPQQPCQQQLGQCQQGYYPTSQQPCQ QGQQPGQQQQGYYPTSPQQPCQQQLGQCQQGYYPTSSQQPCQ QEQQPGQQQQGYYPTSPQQPCQQQLGQCQGCYYPTSSQQPCQ QEQQPGQQQQGYYPTSPQQPCQQQLGQCQGCYYPTSSQQPCQ QEQQPGQQQQGYYPTSPQQPCQQQLGQCQGCYYPTSSQQPCQ QEQQPGQQQQYYPTSPQQPCQQQLGQCQGCYYPTSSQQPCQ QEQQPGQQQQYYPTSPQQPCQQQLGQCQGCYYPTSQQPCQ | | GawagPeakGHagYYYTSPQDPG2GQG4 | 2LEQGQQSGQGRQFQQGQ+SG 2SCQCQLCQCHDPCQCQQSG 2SCQCQLCQCHDPCQCQQSG 2SCQCQQLCQCHDPCQCQQSG 2SCQCQSQCQHDLCQCQRSG 2SCQCQSCQCHQFCQCQQSG 2SCQCQSQCGHDPCQCQQSG 2———————————————————————————————————— |
| Aey3 Aey7 1Ay Aey1 1By8 Aey2 1Dy10 Aey4 Aey5 Aey6 Aey8 | Generation G | | Ganagheraddian Carlong | 2L.GGGQQSCGCRAPGQGQ+SG 2SGGCQQL.GQCRAPGQGQQSG 2SGGCQQQL.GQCRAPGQGQQSG 2SGGCQQSCQCRAPGQGQQSG 2SGGCQSCGCRAPGCQGQSG 2SGGCQSCGCRAPGCQQQSG 2————SGQCRAPGCQQQSG 2————SGQCRAPGCQQQSG 2———————————————————————————————————— |
| Aey3 Aey7 1Ay Aey1 1By8 Aey2 1Dy10 Aey4 Aey5 Aey6 Aey8 Aey9 | QETITI TSC. PRQAC QEQQPCQCQ-GYYTSSQQPCQCQHPCQCQQQYPTSPQHPCQ QEQQPCQCQQCYYTSSQQPCQCQHPCQCQQCYYTSPQPCQ QCQQPCQCQQCYYTSSQQPCQCKQLCQCQCYYTTSPQPCQ QCQQPCQCQQCYYTSPQPCQCKQLCQCQCYYTTSPQPCQ QCQQPCQCQQCYYTSPQPCQCKQLCQCQCYYTTSPQPCQ QCQQPCQCQQCYYTSPQPCQCQQLCQCQCQYTTSPQPCQ QCQQPCQCQQCYYTSPQPCQCQQLCQCQCQYTTSSQQPCQ QEQQPCQCQQCYYTSPQPCQCQQLCQCQCYYTTSQQPCQ QEQQPCQCQQCYYTSPQPCQCQQLCQCQCQYTTSSQQPCQ QEQQPCQCQQCYYTSPQPCQCQQLCQCQCQYTTSQQPCQ QEQQPCQQCQCYYTSPQPCQCQQLCQCQCQYTTSSQQPCQ QEQQPCQQCQCYYTSPQPCQCQQLCQCQCQQCYYTTSQQPCQ QEQQPCQQCQCYYTSPQPCQCQQLCQCQCQQCYYTTSQQPCQ QEQQPCQQCQCYYTSPQPCQCQQLCQQCQQCYYTTSQQPCQ QEQQPCQQCQCYYTSPQPCQCQQLCQQCQQCYYTTSQQPCQ QEQQPCQQCQCYYTSPQPCQCQQLCQQCQQCYYTTSQQPCQ QEQQPCQQCQQCYYTSPQPCQCQQLCQQCQQCYYTSPQPCQ QEQQPCQQCQQCYYTSPQPCQCQQLCQQCQQCYYTSPQPCQ QEQQPCQQQCQQCYYTSPQPCQCQQLCQQQQCQQQCYYTSPSQPCQU QEQQPCQQQCQQQCQQQCYYTSPQPCQCQQUCQQCQQQQUCQQQCQQQQUCQQQCYYTSPQPCQUCQQUCQQQCQQQUCQQCQQUTSPSQPCQUCQQUTSPSQPCQUCQUCQUCQUCQQQUTSPSQPCQUCQUCQUCQUCQUCQUCQUCQUCQUCQUCQUCQUCQUC | | GWAQPEGGAQCSCRGQCYPTISAQPCGQQ | 2LEQGQQSGQGRQFQQGQ+SG 2SCQCQLEQCHDPQQCQSS 2SCQCQLEQCHDPQQCQSS 2SCQCQLCQCHDPQQCQSS 2SCQCQSQCGHDLQQCQSS 2SCQCQSCQCHDPQQCQQSS 2SCQCQSCQCHDPQQCQQSS 2SCQCHDPQCQCQSS 2SCQCHDPCQCQQSS 2SCQCQPQCQQQSS |
| Aey3 Aey7 1Ay Aey1 1By8 Aey2 1Dy10 Aey4 Aey5 Aey5 Aey6 Aey8 Aey9 Aey10 | QEONTIFICAC QEQQPGQQQ-CYYTSSQQPGQQUPCQGQQGYYTSPQPFQQ QEQQPGQQQQCYYTSSQQPGQGQUPCQGQQGYYTSPQPFQQ QEQQPGQQQQCYYTSSQQPGQGQUPCQGQQGYYTSPQPFQQ QGQQPGQQQQCYYTSSQQPGQGQUPCYTSPQPFQQ QGQQPGQQQQYYTSSQQPGQQQQCYYTSPQPFQQ QEQQPGQQQQYYTSSQQPGQQQQCYYTSSQQPGQQQYYTSSQQPFQQ QEQQPGQQQQYYTSSQQPGQQQQQQCYYTSSQQPFQQ QEQQPGQQQQYYTSSQQPGQQQLGQGQQYYTSSQQPFQQ QEQQPGQQQQYYTSSQQPGQQQLGQQGQQYYTSSQQPFQQ QEQQPGQQQQYYTSSQQPGQQQLGQGQQQYYTSSQQPGQ QCQQPQQQQQQQQQYYTSSQQPGQQQQCYYTSSQQPGQ QCQQPQQQQQQQQQYYTSSQQPGQQQQQYYTSSQQPGQ QCQQQQQQQQQQQQQYYTSSQQPGQQQYYTSSQQPGQQ QCQQQQQQQQQQQQYYTSSQQPGQQQQYYTSSQQPGQ QCQQQQQQQQQQQQYYTSSQQPGQQQYYTSSQQPGQ QCQQQQQQQQQYYTSSQQPGQQQQQQQQYYTSSQQPGQQ QCQQQQQQQQQQYYTSSQQPGQQQQQQQQYYTSSQQPGQ QCQQQQQQQQQQYYTSSQQPGQQQQQQQQQQQQQQQQQQQQQYYTSSQQPGQQ QCQQQQQQQQQQQQQQQQQQQQQQQQQQYTSSQQDQTQUTSSQQPGQQ QCQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQ | PORCOCTO CC PORCOCTO CC SQRPQCQCQCHPTSPQPQQAC OPAGQCQCHPTSPQPQQAC OPAGQCQCGCHPTSPQTGQAC CQAPCQCQCHPTSPQTGQAC SQRPQCQCQCHCPTSPQTGQAC CQAPCQCQCHCPTSPQTGQAC SQRPQCQCQCHCPTSPQTGQAC CQAPCGQCQCHCPTSPQTGQAC SQRPQCQCQCHCPTSPQTGQAC CQAPCGQCQCHCPTSPQTGQAC SQRPQCQCQCHCPTSPQTGQAC CQAPCGQCQCHCPTSPQQTGQAC SQQPCGQCQCHCPTSPQQTGQAC CQAPCTGQACTGAC SQQPCGQCQCHCPTSPQQTGQAC CQAPCTGQACTGAC SQQPCGQCQCHCPTSPQQTGQAC CQAPCTGACAC SQQPCGQCQCHCPTSPQQTGQAC CQAPCTGACAC SQQPCGQCQCHCPTSPQQTGQAC CQAPCGQCQCHCPTSPQQTGQAC SQQPCGQCQCHCPTSPQQTGQAC CQAPCTGACAC SQQPCGQCQCHCPTSPQTGQAC CQAPCGQCQCHCPTSPQTGQAC SQQPCGQCQCHCPTSPQTGQAC CQAPCTGACAC SQQPCCGQCHCPTSPQTGQAC CQAPCTGACAC SQQPCCGQCHCPTSPQTGACAC CQAPCGQCQHCPTSPQTGACAC SQQPCCGQCHCPTSPQTGACAC CQAPCGQCHCPTSPQTGACAC SQQPCCGQCHCPTSPQTGACAC CQAPCGQCHCPTSPQTGACAC SQQPCCGQCHCPTSPQTGACAC CQAPCGQCHCPTSPQTGACAC SQQPCCGQCHCPTSPQTGACAC CQAPCGQCHCPTSPQTGACAC | GawadyekGhayyyrishqurodda | 2LGGGQQSCqCRAPGQGQ+SG 2SGGGQLGQCRAPGQGQQSG 2SGGGQQLGQCRAPGGGQQSG 2SGGGQQSCqLGQCRAPGGGQQSG 2SGGGQQSCGCRAPGGQQSG 2SGGGQQSCGCRAPGGQQSG 2SGQCRAPGGQQSG 2SGQCRAPCGQQSG 2 |
| Aey3 Aey7 1Ay Aey1 1By8 Aey2 1Dy10 Aey4 Aey5 Aey5 Aey6 Aey8 Aey9 Aey10 | QETITISEQ PRQAQ QEQQPGQQQ QEQQPGQQQ QEQQPGQQQQ QEQQPGQQQQ QEQQPGQQQQQY QEQQPGQQQQQY QEQQPGQQQQQY QEQQPGQQQQQY QEQQPGQQQQY QEQQPQQQQQY QEQQPQQQQQY QEQQPQQQQQY QEQQPQQQQQY QEQQPQQQQQY QEQQPQQQQQY QEQQPQQQQQY QEQQPQQQQQY QEQQPQQQQQY QEQQPQQQQQQY QEQQPQQQQQQQQQQQQQQQQQQY QEQ | QQQPQQQQQCHCPTSPQQPQQQ QQQPQQQQQCHCPTSPQQPQQQ QQQPQQQQQCHCPTSPQQPQQQ QQQPQQQQQCHCPTSPQQTQQQ QQQPQQQQQCHCPTSPQQTQQQ QQQPQQQQQCHCPTSPQQTQQQ QQQPQQQQQCHCPTSPQQTQQQ QQQPQQQQQCHCPTSPQQTQQQ QQQPQQQQQCHCPTSPQQTQQQ QQQPQQQQQCHCPTSPQQTQQQ QQQP GQQ GQQQ GQQ GQQ GQQQQ GQQQQQQ GQQQ GQQQQQQ | GawagPerkGHagYYYTSPQ4PGaGa | 2LGGGQQSGGGRQPGQGQ+SG 2SGGGQLGGGHQPGQGQSG 2SGGGQQSGGGHCGQGSG 2SGGGQQSGGGHCGQGQSG 2SGGGQQSGGGHDCQGQGQSG 2SGGGQQSGGGHQPGQGQQSG 2———SGGGHQPGGGQQSG 2———SGGGHQPGGGQSG 2———SGGGHQPGGGQSG 2———SGGGHQPGGQQSG 2———SGGGQQPGGQQSG 2———SGGGQQPGGGQQSG -———SGGGQQPGGGQQSG -———SGGGQQPGGGQQSG |
| Aey3 Aey7 1Ay Aey1 1By8 Aey2 1Dy10 Aey4 Aey5 Aey5 Aey9 Aey10 Aey3 | QEODITISEQPECTION QEQQPGQQQ-GYYTSSQQPGQQHPGQGQQGYYPTSPQPEQQ QEQQPGQQQQYYPTSSQQPGQGQHPGQGQQGYYPTSPQPEQQ QGQQPGQQQQGYYPTSPQPEQQGQLGQGQQGYYPTSPQPEQQ QGQQPGQQQQGYYPTSPQPEQQQLGQGQQGYYPTSPQPEQQ QEQQPGQQQQGYYPTSPQPEQQQLGQGQQGYYPTSPQPEQQ QEQQPGQQQQGYYPTSPQPEQQQLGQGQQGYYPTSPQPEQQ QEQQPGQQQQGYYPTSPQPEQQQLGQGQQGYYPTSPQPEQQ QEQQPGQQQQGYYPTSPQPEQQQLGQGQQGYYPTSPQPEQQ QEQQPGQQQQGYYPTSPQPEQQQLGQGQQGYYPTSPQPEQQ QEQQPGQQQQGYYPTSPQPEQQQLGQGQGYYPTSPQPEQQ QEQQPGQQQQGYYPTSPQPEQQQLGQGQGYYPTSPQPEQQ | POECQCQTCQ POECQCQTCQ >>>>>>>>>>>>>>>>>>>>>>>>>>>> | GawaqPerkGH2YYYISPQQPGQQ | 2LCQGQQSCQCR0PCQCQ+SC 2SCQGQQLCQCH0PCQCQQSC 2SCQGQQLCQCH0PCQCQQSC 2SCQGQQSCQCH0LCQCQCQSC 2SCQCQQSCQCH0LCQCQCQSC 2SCQCQQSCQCH0PCQCQQSC 2=SCQCH0PCQCQQSC 2=SCQCH0PCQCQQSC 2=SCQCH0PCQCQQSC 2=SCQCH0PCQCQQSC 2=SCQCH0PCQCQQSC 2=CQCH0PCQCQQSC |
| Aey3 Aey7 1Ay Aey1 1By8 Aey2 1Dy10 Aey4 Aey5 Aey5 Aey6 Aey9 Aey9 Aey10 Aey3 Aey7 | QEQQPGQQQ-GYYSTSSQQPGQQHPGQQQQGYYFTSPQHPGQ QEQQPGQQQ-GYYSTSSQQPGQQHPGQQQQGYYFTSPQHPGQ QEQQPGQQQQGYYFTSSQQPGQQHPGQQQQGYYFTSPQPFQQ QGQQFQQQQQGYYFTSPQPFQQQQLGQCQQGYYFTSPQPFQQ QGQQFQQQQQGYYFTSPQPFQQQQLGQCQQGYYFTSPQPFQQ QEQQPGQQQQGYYFTSPQPFQQQQLGQCQQGYYFTSPQPFQQ QEQQPGQQQQGYYFTSPQPFQQQQLGQCQQGYYFTSPQPFQQ QEQQFQQQQGYYFTSPQPFQQQQLGQCQQGYYFTSPQPFQQ QEQQFQQQQGYYFTSPQPFQQQQLGQCQQGYYFTSPQPFQQ QEQQFQQQQGYYFTSPQPFQQQQLGQCQQGYYFTSPQPFQQ QFQQMQQPGQCQQGYYFTSPQPFQQQQLGQCQQYYFTSPQPFQQ QFQQMQQPGQCQQGYYFTSPQPFQQ QFQQMQQPGQCQQGYYFTSQPFQ QFQQMQPGQCQQGYYFTSQPFQ QFQQMQPGQCQQGYYFTSQPFQ QFQQMQPGQCQQGYYFTSQPFQ QFQQMQPGQCQQGYYFTSQPFQ QFQQMQPGQCQQGYYFTSQPFQ QFQQMQPGQCQQGYYFTSQPFQ QFQQMQPGQCQQGYYFTSQPFQ QEQQYGEQQGYYFTSQPFQ QFQQMQPGQCQQGYYFTSQPFQ QEQQYGEQQGYYFTSQPFQ QFQMQPGQCQQQYFTSQPFTSQPFQ QFQMQPGQCQQQYFTSQQPFQ QFQMQPGQCQQQYFTSQQPFQ QFQMQPGQCQQQYFTSQQPFQ QFQMQAQPGQCQQQYFTSQQPFQ QFQMQAQPGQCQQQYFTSQQPFTSQQPFQ QFQMQAQPGQCQQQYFTSQQPFQ | LEPEGQQCTQQ | GawagPerGGaptery YYTSPQ4PCdQ3q | 2LCGCQQSCQCRQPCQCQ+SC 2SCQCQQLCQCHQPCQCQQSC 2SCQCQQLCQCHQPCQCQQSC 2SCQCQQLCQCHQPCQCQQSC 2SCQCQQSCQCHQPCQCQQSC 2SCQCQQSCQCHQPCQCQQSC 2SCQCQQSCQCHQPCQCQQSC 2=SCQCHQPCQCQQSC 2=SCQCHQPCQCQQSC 2=SCQCHQPCQCQQSC 2=SCQCHQPCQCQQSC 2=SCQCQQPCQCQQSC 2= |
| Aey3 Aey7 1Ay Aey1 1By8 Aey2 1Dy10 Aey4 Aey5 Aey5 Aey5 Aey5 Aey9 Aey9 Aey10 Aey3 Aey7 | CONTINUE | PO20000100 400 PO20000100 | GawagPesGebagYYF15Pqdpr6d3da | 2L.GQGQQSCQCRQPCQGQ+SG 2SGQGQQLGQCHQPCQCQQSG 2SGQGQQCQLGQCHQPCQCQQSG 2SGQGQQSCQCHULGQGQGSG 2SGGGQSCQCHULGQGQSG 2SGGGQSCQCHUPCQCQQSG 2=SGQCHQPCQCQQSG 2=SGQCHQPCQCQQSG 2=SGQCHQPCQCQQSG 2=SGQCHQPCQCQQSG 2=SGQCHQPCQCQSG 2=SGQCHQPCQCQSG 2=SGQCHQPCQQQSG |
| Aey3 Aey7 1Ay Aey1 1By8 Aey1 1By8 Aey2 1Dy10 Aey4 Aey5 Aey5 Aey5 Aey9 Aey10 Aey3 Aey7 1Ay | QEOTITISEQ QEQQPGQQQ-GYYSTSSQQPGQQHPGQQQQYPTSPQHPGQ QEQQPGQQQGYYTSSQQPGQQHPGQQQQGYYTSPQHPGQ QEQQPGQQQQGYYTSSQQPGQQHPGQQQQGYYTSPQPGQ QGQQFGQQQQGYYTSSQQPGQQGQGGQGYYTSSQQPGQ QGQQFQQQQQGYYTSSQQPGQQQQGQGYYTSSQQPGQ QGQQFQQQQQGYYTSSQQPGQQQQGQGGYYTSSQQPGQ QGQQFQQQQQGYYTSSQQPGQQQQQGQGQYYTSSQQPGQ QGQQFQQQQQYYTSSQQPGQQQQQGQGYYTSSQQPGQ QQQGYQQQQQYYTSSQQPGQQQQQQGQGYYTSSQQPGQ QQQQGQQQGYYTSSQQPGQQQQQQQYYTSSQQPGQ QCQQGQQQYYTSSQQPGQQQQQQQQQYYTSSQQPGQ QCQQQQQQQQYYTSSQQPGQQQQQYYTSSQQPGQ QCQQQQQQQQQQQQYYTSSQQPGQQQYYTSSQQPGQ QCQQQQQQQQQQQQQQYYTSSQQPGQQQYYTSSQQPGQ QCQQQYQSQQQQQYYTSSQQPGQQQYYTSSQQPGQ QCQQQQQQQQQQQQQQQQQQYYTSSQQPGQ QCQQYSYTSYDQQAASSKVXAAHPXQLPTICQMBCRD QCQQYSYTSYHYSSQQAASSKVXAAHPYAQLPTICQMBCRD QCQQYSYHSYHYSSQQAASSKVAAAHPYAQLPTICQMBCRD QCQQYSYNSYHYSSQQAASSKVAAAHFYAQLPTICQMBCRD QCQQYSYNSYHYSSQQAASSKVAAHFYAQLPTICQMBCRD QCQQYSYNSYHYSSQQAASSKVAAHFYAQLPTICQMBCRD QCQQYSYNSYHYSSQQAASSKVAAHFYAQLPTICQMBCRD QCQQYSYNSYHYSSQQAASSKVAAHFYAQLPTICQMBCRD QCQQYSYNSYHYSSQQAASSKVAAHFYAQLPTICQMBCD | Interpretation Interpretation | GawadyekGrayeyYYYISPQ4PG4304 ———————————————————————————————————— | 2LCGCQQSCQCRAPGQCQ+SG 2SCQCCQLCQCRAPGQCQQSC 2SCQCCQLCQCRAPGCQCQQSC 2SCQCCQQSCQCRAPCQCQQSC 2SCQCQQSCQCRAPCQCQQSC 2SCQCQQSCQCRAPCQCQQSC 2=SCQCRAPCQCQQSC 2=SCQCRAPCQCQQSC 2=SCQCRAPCQCQQSC 2=SCQCQQPQCQQSC 2=GQCRAPGQCQQSC |
| Aey3 Aey7 1Ay Aey1 1By8 Aey1 1By8 Aey2 1Dy10 Aey4 Aey5 Aey6 Aey6 Aey8 Aey9 Aey10 Aey3 Aey7 1Ay Aey1 | Construction | POPOGQUTQ | GawagPerkGHagYYYTSPQ4Pcdq3q | 2L.GGGQ2SGGCR0PGQG4+SG 2SGGGQ2L.GGCH0PGQGQ2SG 2SGGGQ2GCLGGCH0PGQGQ2SG 2SGGGQ2SGCH0LGQGGQSG 2SGGGQ2SGCGH0LGQGQSG 2SGGGQ2SGCGH0PGQGQ2SG 2SGQCH0PGQGQ2SG 2SGQCH0PGQGQ2SG 2SGQCH0PGQGQ2SG 2GQGH0PGQGQ2SG |
| Aey3 Aey7 1Ay Aey1 1By8 Aey2 1Dy10 Aey2 1Dy10 Aey4 Aey5 Aey6 Aey8 Aey9 Aey7 1Ay Aey7 1Ay Aey1 1By8 | CONTINUE | QQPQQQQQQHCPTSPQQPQQQQ QQPQQQQQQHCPTSPQQPQQQ —QPQQQQQQQHCPTSPQQTQQQ QQPQQQQQQQCHCPTSPQQTQQQ QQPQQQQQQQCHCPTSPQQTQQQ QQPQQQQQQQCHCPTSPQQTQQQ QQPQQQQQQCHCPTSPQQTQQQ QQPQQQQQQCHCPTSPQQTQQQ QQPQQQQQQCHCPTSPQQTQQQ QQP GQP | GawadpekGHagYYYTSPQ4PG4364 ———————————————————————————————————— | 2L.GGGQQSCQCRAPGQGQ+SG 2SGQGQQLGQCRAPGQGQQSG 2SGGGQQSCQLGQCRAPGQGQQSG 2SGGGQQSCQCRAPLOQGQQQSG 2SGGGQQSCQCRAPGCQQQSG 2SGGGQQSCQCRAPGCQQQSG 2SGQCRAPGCQQQSG 2SGQCRAPGCQQQSG 2SGQCRAPGCQQSG 2 |
| Aey3 Aey7 1Ay Aey1 1By8 Aey1 1By8 Aey2 1Dy10 Aey4 Aey5 Aey5 Aey5 Aey5 Aey5 Aey9 Aey10 Aey3 Aey7 1Ay Aey1 1By8 Aey7 1Ay | Generation G | LEADEQUE OF, CL | GawagPedGaBapYIYISPQ4PG2G30 | 2LGGGQQSGGGRQFQGQ4*SG 2SGGGQLGGGHBPGQGQ4SG 2SGGGQQSGQGHBLGQGQRSG 2SGGGQQSGGGHBLGQGQRSG 2SGGGQQSGGCHBLGQGQRSG 2SGGGQQSGGCHBPGQGQQSG 2=SGGCHBPGQGQQSG 2=SGGCHBPGQGQQSG 2=SGGCHBPGQGQQSG 2=SGGCHBPGQGQQSG 2=SGGCHBPGQGQQSG 2= |
| Aey7 Aey7 IAy Aey1 IBy8 Aey2 IDy10 Aey4 Aey5 Aey6 Aey8 Aey9 Aey10 Aey3 Aey7 IAy Aey1 IBy8 Aey1 IBy8 Aey1 IBy8 | Construction C | Interpret of the second seco | GawadpekGHagYYYTSPQ4PG4Q44 | 2LGGGQQSCQCRAPGQGQ+SG 2SGQGQQLGQCRAPGQGQQSG 2SGQGQQLGQCRAPGQGQQSG 2SGQGQQSGQCRAPLGQGQQSG 2SGQGQQSGQCRAPLGQGQQSG 2SGGQQSGQCRAPGCQQSG 2SGQCRAPGQGQQSG 2SGQCRAPGCQQSG 2SGQCRAPGQGQQSG 2SGQCRAPGQGQQSG 2 |
| Aey3 Aey7 1Ay Aey1 1By8 Aey2 1Dy10 Aey4 Aey5 Aey6 Aey6 Aey7 Aey10 Aey3 Aey7 1Ay Aey1 1By8 Aey2 1Dy10 | Construction | QQPGQQQQGHCPTSPQQPQQQQ QQPQQQQQGHCPTSPQQPQQQQ QQPQQQQQGHCPTSPQQTQQQ QQPQQQQQGHCPTSPQQTQQQ QQPQQQQQGHCPTSPQQTQQQ QQPQQQQQGHCPTSPQQTQQQ QQPQQQQQGHCPTSPQQTQQQ QQPQQQQQGHCPTSPQQTQQQ QQPQQQQQGHCPTSPQQTQQQ QQPQQQQQGHCPTSPQQTQQQ QQP GQP | GWAQEPSGERRYYYTSPQQPGQQQ | 2LCGCQQSCQCRQPCQCQ+SC 2SCQCQQLCQCHQPCQCQQSC 2SCQCQQLCQCHQPCQCQQSC 2SCQCQQSCQCHQLCQCHQCQQSC 2SCQCQQSCQCHQPCQCQQSC 2SCQCQQSCQCHQPCQCQQSC 2SCQCQQSCQCHQPCQCQQSC 2SCQCHQPCQCQQSC 2SCQCHQPCQCQQSC 2SCQCHQPCQCQQSC 2SCQCHQPCQCQQSC 2SCQCQQPCQCQQSC 2 |
| Aey3 Aey7 IAy Aey1 IBy8 Aey2 IDy10 Aey4 Aey5 Aey6 Aey8 Aey9 Aey10 Aey3 Aey7 IAy Aey7 IAy Aey1 IBy8 Aey2 IDy10 Aey4 | Construction C | JCACCONCIDENTIAL JCACCONCIDENTIAL JCQPCQCCQCQCHPTSPQQPQQQC JCQPCQCQCQCCHPTSPQQPQQQQCQCGCHPTSPQQTQQQQQQCQCGCGCGCGCGCGCGQQQQQCGCGCGCGCG | GAWQUPEGGAQLGQVQLGQCQCYTTSLQQPGQCQ | 2LGGGQQSGGRAPGQGQ+SG 2SGGGQQLGGGRAPGQGQQSG 2SGGGQQLGGCRAPGGGQQSG 2SGGGQQSGGRAPLGGGQQSG 2SGGGQQSGGRAPLGGGQQSG 2SGGGQQSGGRAPGQGQQSG 2SGGGRAPGQGQQSG 2SGGGRAPGQGQQSG 2SGGGRAPGQGQQSG 2 |
| Aey3 Aey7 IAy Aey1 IBy8 Aey2 IDy10 Aey4 Aey5 Aey6 Aey8 Aey9 Aey10 Aey3 Aey7 IAy Aey1 IBy8 Aey2 IDy10 Aey4 Aey1 IBy8 Aey2 IDy10 Aey4 Aey5 Aey7 | Construction Con | Interpret of the second sec | GawagPerGGaptery TYTSPQ4PCdQ3q | 2LCQGQQSCQCRQPGQQQ+SG 2SGQGQQLGQCHQPGQGQQSG 2SGQGQQCQLGQCHQPGQGQQSG 2SGQGQQSGQCHQLGQCGQSG 2SGQGQQSCQCHQPGQGQQSG 2=SGQCHQPGQGQQSG 2=SGQCHQPGQCQQSG 2=SGQCHQPGQCQQSG 2=SGQCHQPGQCQQSG 2= |
| Aey3 Aey7 IAy Aey1 IBy8 Aey2 IDy10 Aey4 Aey5 Aey6 Aey6 Aey8 Aey9 Aey10 Aey3 Aey7 IAy Aey1 IBy8 Aey2 IDy10 Aey2 IDy10 Aey2 Aey5 Aey6 Aey7 Aey7 Aey7 Aey7 Aey7 Aey7 Aey7 Aey7 | Construction C | Veccourto Veccourto Veccourto SeqPeccourto SeqPeccourto Percourto SeqPeccourto | GAWQUPENGERLAYYYTISPQQPGQQQ | 2LGQGQQSGQGRQPGQGQ+SG 2SGQGQQLGQGRQPGQGQQSG 2SGQGQQLGQGRHQPGQGQQSG 2SGQGQQSGQHQPGQGQQSG 2SGGGQQSGGHQPGQGQQSG 2SGQGHQPGQGQQSG 2SGQGHQPGQGQQSG 2 |
| Aey3 Aey7 1Ay Aey1 1By8 Aey2 1Dy10 Aey2 1Dy10 Aey2 Aey2 Aey6 Aey8 Aey9 Aey10 Aey3 Aey7 1Ay Aey7 1Ay Aey1 1By8 Aey2 1Dy10 Aey4 Aey5 Aey5 Aey6 Aey7 | Construction C | Lickeyede Lickeyede SQQPQGQQQGHPTSPQQPQQQ SQQPQQQQQGHPTSPqQPQQQ —QPQQQQQQGHPTSPQQTQQA GQQPQQQQQGHPTSPQQTQQA GQQPQQQQQGQHPTSPQQTQQA GQQPQQQQQGHPTSPQQTQQA GQQPQQQQQGHPTSPQQTQQA GQQPQQQQQGHPTSPQQTQQA GQQPQQQQQGHPTSPQQTQQA GQQPQQQQQGHPTSPQQTQQA | GAWQUPEGGAQLGQVQFYTFSLQQPGQGQ | 2L.GGGQQSGQGRAPGQGQ+SG 2SGQGQQLGQGRAPGQGQQSG 2SGGGQQSGQLGQGRAPGQGQQSG 2SGGGQQSGCGRAPGQGQQSG 2SGGQQSGCGRAPGQGQQSG 2SGGQQSGCRAPGQGQQSG 2SGQGRAPGQGQQSG 2SGQGRAPGQGQQSG 2SGQGRAPGQGQQSG 2GQGRAPGQGQQSG |
| Aey3 Aey7 1Ay Aey1 1By8 Aey2 1Dy10 Aey4 Aey5 Aey6 Aey7 Aey7 Aey10 Aey3 Aey7 1Ay Aey7 1Ay Aey7 1Ay Aey7 1Dy10 Aey4 Aey5 Aey5 Aey5 Aey2 1Dy10 Aey4 Aey7 Aey7 Aey7 Aey7 Aey7 Aey7 Aey7 Aey7 | Construction C | Jeneral Color Jeneral Color JogPGQCQQQGHPTSPQQPQQQQGHPTSP GQAPQQQQQGHPTSPQQPQQQQGHPTSPQQTQQAQ JOQPGQQQQGHPTSPQQTQQAQ GQAPQQQQQHPTSPQQTQQAQ JQQPGQQQGHPTSPQQTQQAQ GQAPQQQQQHPTSPQQTQQAQ JQQPGQQQGHPTSPQQTQQAQ GQAPQQQQQHPTSPQQTQQAQ GQAPGQQQGHPTSPQQTQQAQ GQAPQQQQCHPTSPQQTQQAQ JQQP GHPTSPQTQAQAQ GQAPGQQQGHPTSPQQTQQAQ GQAPQQQQQHPTSPQQTQQAQ GQAPGQQQGHPTSPQQTQQAQ FOUDQQ JQQ GHPTSPQTQAQAQQQQQQ GQAPGQQQGHPTSPQQTQQAQ FOUDQQ JQAPGQQQGHPTSPQQTQQAQ FOUDQQ JQAPGQQQCHPTSPQQTQQAQ FOUDQQ JLSASQ 594 JLSASQ 659 JLSASQ 658 JLSASQ 652 JLSASQ 621 JLSASQ 621 JLSASQ 612 | GWAQPEGKGH2YYYTSPQQPGQQ SQQPGQBQ SQQPGQBQ QPGQGQIGQWQQLGQCQQGYYPTSLQQPGQGQ QPGQGQIGQWQPGQGQQGYYPTSLQQPGQBQ QPGGQQIGQWQPGQGQQGYYTTSLQQPGQQ QLGQQQIGQWQQPGQGQQGYYTTSLQQPGQQ QPGGQQIGQWQPGQGQQGYYTTSLQQPGQQ PPGGQQIGQWQPGQGQQGYYTTSLQQPGQQ PPGGQQIGQWQPGQGQQGYYTTSLQQPGQQ QIGQGQPGQGQQGSQQQGYYTTSLQQPGQQ QIGQGQQPGQGQQSGQQGYYTTSPQP QSGQQQQP | 2L.GGCQQSCQCRQPCQQQ+SC 2SCQCQQLCQCHQPCQCQQSC 2SCQCQQLCQCHQPCQCQQSC 2SCQCQQSCQCHQLCQCCQSC 2SCQCQQSCQCHQLCQCCQSC 2SCQCQQSCQCHQPCQCQQSC 2=SCQCHQPCQCQQSC 2=SCQCHQPCQCQQSC 2=SCQCHQPCQCQQSC 2=CQCHQPCQCQQSC 2=CQCHQPCQCQQSC |
| Aey3 Aey7 1Ay Aey1 1By8 Aey2 1Dy10 Aey2 1Dy10 Aey4 Aey5 Aey6 Aey7 Aey7 Aey7 Aey7 1By8 Aey7 1By8 Aey7 1By8 Aey7 1By8 Aey1 1By8 Aey2 1Dy10 Aey4 Aey5 Aey5 Aey7 | Construction C | QQPQQQQQQHPTSPQQPQQQQ QQPQQQQQQHPTS QQQPQQQQQQHPTS QQQPQQQQQQHPTSPQQQQ QQPQQQQQQQHPTSPQqQQ QQPQQQQQQQHPTSPQqQQQ QQPQQQQQQQHPTSPQqQQQ QQPQQQQQQQHPTSPQqQQQ QQPQQQQQQHPTSPQqQQQ QQPQQQQQQHPTSPQqQQQ QQP GHPTSPQQTQQQ QQP GHPTSPQQTQQQ QQP GHPTSPQQTQQQ QQP GHPTSPQQTQQQ QQP GHPTSPQQTQQQ QQP GHPTSPQQTQQQ QQP GHPTSPQQTQQQQ QQP GHT QQP GHT QQP GHT QQP GHT QQP GHT QQP GHT LSASQ G1 LSASQ G58 LSASQ G21 LSASQ G12 LSASQ G12 LSASQ G14 LSASQ G14 | GAWQUPEKGH2YYYTSPQQPGQQQ | 2LGGGQQSCQGRAPGQGQ+SG 2SGQGQQLGQGRAPGGQQQSG 2SGQGQQLGQGRAPGGQQQSG 2SGQGQQSGQLGQGRAPGGQQQSG 2SGQGQQSGQRAPGGQQQSG 2SGGQQSGQCRAPGGQQQSG 2SGQGRAPGQGQQSG 2SGQGRAPGQGQQSG 2SGQGRAPGQGQQSG 2SGQGQPGQGQQSG 2 |
| Aey3 Aey7 1Ay Aey1 1By8 Aey2 1Dy10 Aey4 Aey5 Aey5 Aey6 Aey6 Aey7 Aey7 Aey7 1Ay Aey1 1By8 Aey7 1Dy10 Aey4 Aey5 Aey5 Aey5 Aey5 Aey5 Aey5 Aey5 Aey7 Aey10 Aey7 Aey10 Aey7 Aey10 Aey7 Aey10 Aey7 Aey10 Aey7 Aey7 Aey7 Aey5 Aey5 Aey5 Aey5 Aey5 Aey5 Aey5 Aey5 | Construction | QQPQQQQQQCHCPTSPQQPQQQQ QQPQQQQQQCHCPTSPQQPQQQQ QQPQQQQQQCHCPTSPQQPQQQQ QQPQQQQQQCHCPTSPQQTQQQ QQPQQQQQQCHCPTSPQQTQQQ QQPQQQQQCHCPTSPQQTQQQ QQPQQQQQCHCPTSPQQTQQQ QQPQQQQQCHCPTSPQQTQQQ QQQPQQQQQCHCPTSPQQTQQQ QQQPQQQQQCHCPTSPQQTQQQ QQQPQQQQQCHCPTSPQQTQQQ QQQPQQQQQCHCPTSPQQTQQQ QQQPQQQQQCHCPTSPQQTQQQ QQQPQQQQCHCPTSPQQTQQQ QQQPQQQQCHCPTSPQQTQQQ QQQPQQQQCHCPTSPQQTQQA QQQPQQQQCQCHCPTSPQQTQQA QQQPQQQQCQCHCPTSPQQTQQA QQQPQQQQCQCHCPTSPQQTQQA QQQPQQQQCQCHCPTSPQQTQQA QQQPQQQQCQCHCPTSPQQTQQA QQQPQQQQCQCHCPTSPQQTQQA QQQPQQQQCQCHCPTSPQQTQA QQQPQQQQCQCHCPTSPQQTQA QQQPQQQQCQCHCPTSPQQTQA QQQPQQQQCQCHCPTSPQQTQA QQQPQQQQQCQCHCPTSPQTQA QQQPQQQQQCQCHCPTSPQTQA QQQPQQQQQCQCHCPTSPQTQA QQQPQQQQQCQCHCPTSPQTQA QQQPQQQQQCQCHCPTSPQTQA QQQPQQQQQQTQQQQTQQQQQQQQQQQQQQQQQQQQQQ | GWAQPEGKGH2YYYTSPQQPGQGQ | 2L.GGCQQSGQCRQPGQGQ+SG 2SGQGQQLGQCHQPGQGQQSG 2SGQGQQSGQLGQCHQPGQGQQSG 2SGQGQQSGCGHQPGQGQQSG 2SGGGQQSGCGHDLGQGQGQSG 2SGGGQQSGCGHDPGQGQQSG 2SGQCHQPGQGQQSG 2SGQGHQPGGQQSG 2SGQGHQPGGQQSG 2 |

Table 1 A summary of properties of the primary structure of HMW-GS from A. elongatum in comparison with some HMW-GS of common wheat

| Subunit | | Signal peptide | N-terminal region | | Repetitive region | | C-terminal region | | Total | |
|-------------------|------------------|----------------|-------------------|----------------|-------------------|----------------|-------------------|-----|-------|-----|
| Name | Accession number | Size | Size | Cys | Size | Cys | Size | Cys | Size | Cys |
| 1Ax1 | X61009 | 21 | 86 | 3 | 681 | 0 | 42 | 1 | 830 | 4 |
| 1Bx7 | X13927 | 21 | 81 | 3 | 645 | 0 | 42 | 1 | 789 | 4 |
| 1Dx5 | X12928 | 21 | 89 | 3 | 687 | 1 ^b | 42 | 1 | 839 | 5 |
| Aex1 | DQ478575 | 21 | 86 | 3 | 657 | 0 | 42 | 1 | 806 | 4 |
| Aex2 | DQ478576 | 21 | 86 | 3 | 623 | 1 ^b | 42 | 1 | 772 | 5 |
| Aex3 | DQ478574 | 21 | 86 | 3 | 577 | 0 | 42 | 1 | 726 | 4 |
| Aex4 | DQ534448 | 21 | 81 | 3 | 548 | 1 ^b | 42 | 1 | 692 | 5 |
| Aex5 | EF190195 | 21 | 86 | 3 | 517 | 0 | 42 | 1 | 666 | 4 |
| 1Ay | X03042 | 21 | 104 | 5 | 420 | 0 | 42 | 1 | 587 | 6 |
| 1By8 | AY245797 | 21 | 104 | 5 | 553 | 1 | 42 | 1 | 720 | 7 |
| 1Dy10 | X12929 | 21 | 104 | 5 | 481 | 1 | 42 | 1 | 648 | 7 |
| Aey1 | AY899822 | 21 | 104 | 5 | 571 | 2 ^b | 42 | 1 | 738 | 8 |
| Aey2 ^a | AY263343 | 21 | 104 | 5 | 491 | 1 | 42 | 1 | 658 | 7 |
| Aey3 | EF190196 | 21 | 104 | 6 ^c | 472 | 1 | 42 | 1 | 639 | 8 |
| Aey4 | EF190197 | 21 | 105 | 5 | 454 | 1 | 42 | 1 | 622 | 7 |
| Aey5 | EF190198 | 21 | 104 | 5 | 454 | 1 | 42 | 1 | 621 | 7 |
| Aey6 ^a | AY263344 | 21 | 104 | 5 | 445 | 1 | 42 | 1 | 612 | 7 |
| Aey7 | DQ078273 | 21 | 104 | 4^d | 427 | 1 | 42 | 1 | 594 | 6 |
| Aey8 ^a | AY319518 | 21 | 105 | 5 | 335 | 0 | 42 | 1 | 503 | 6 |
| Aey9 ^a | AY264065 | 21 | 105 | 5 | 328 | 1 | 42 | 1 | 496 | 7 |
| Aey10 | DQ078274 | 21 | 105 | 5 | 215 | 1 | 42 | 1 | 383 | 7 |

^a Aey2, Aey6, Aey8, and Aey9 were cloned by Feng et al. (2004b)

^b 1Dx5, Aex2, Aex4, and Aey1 contain extra cysteine residues in central repetitive domain

^c Aey3 contains an additional cysteine in N-terminal domain

^d The N-terminal domain of Aey7 has one cysteine residue less than other y-type subunits

All the conserved cysteine residues that present in published HMW-GS of wheat and wheat-related grasses were observed in the deduced amino acid sequences of the 11 alleles except that the conserved cysteine (TGC) mutated to arginine (CGC) at position 43 of the N-terminal region of *Aey7* (Fig. 4). Additional cysteine residues were observed at position 659, 261, 581, and 33 of the deduced amino acid sequences of *Aex2*, *Aex4*, *Aey1*, and *Aey3*, respectively. The cysteine residues situated on the bottom of repetitive regions of most y-type subunits were absent from *Aey10*, while an extra cysteine residue appeared at its position 257. The detailed properties of the 11 subunits and some representative subunits of wheat are summarized in Table 1.

Out of the 11 novel alleles, only three (*Aex1*, *Aex4*, and *Aey7*) have intact open reading frames; five (*Aex2*, *Aex3*, *Aex5*, *Aey3*, and *Aey10*) contain in-frame stop codon; the remainder three (*Aey1*, *Aey4*, and *Aey5*) show frame shift mutation resulting from single nucleotide insertion or deletion. The analysis of amino acid sequences derived from *Aey1*, *Aey4*, and *Aey5* is based on the rectified sequences. It is interesting that except N-terminal region, repetitive

region and C-terminal region of *Aey4* and *Aey5* were nearly identical, with only three SNPs. The N-terminal region of *Aey4* is the same as *Aey10* with only a SNP, and both of them contain an additional glutamine residue than most y-type subunits (Fig. 4).

Evolutionary relationship

To investigate evolutionary relationships among the subunits characterized in this study and the published HMW glutenin subunits cloned from *Lophopyrum elongatum* (E^e) and *Pseudoroegneria stipifolia* (St), phylogenetic trees were drawn from the alignment of these alleles based on both full length sequences and the two conserved terminal sequences (Fig. 5). Alignment according to full length sequences indicated that the phylogenetic tree was divided into two halves, comprising the alleles of y-type genes at the top and x-type genes at the bottom. The ten y-type alleles of *A. elongatum* have been further divided into five clades. Of them, *Aey2*, *Aey4*, *Aey5*, and *Aey6* showed close relationship, while *Aey3* with *Aey7* and *Aey8* with *Aey9* Fig. 5 Phylogenetic analysis of HMW-GS from *A. elongatum* and some other wheat-related grass. **a** Neighbor-Joining tree of full length sequences of *Glu-1* genes from *A. elongatum* and some other wheat-related grass. **b** Neighbor-Joining tree of N-terminal regions of HMW-GS from *A. elongatum*. **c** Neighbor-Joining tree of C-terminal regions of HMW-GS from *A. elongatum*. This work was done under the help of MEGA program (Version 3.1)



clustered together, respectively; moreover, Aey1 and Aey10 were located in two independent branches. The five x-type sequences were subdivided into three clades. Aex1 with Aex2 and Aex3 with Aex5 stayed together, respectively, while Aex4 was far from the other four sequences. It was noted from the phylogenetic tree that the published y-type HMW-GS alleles E1.5 and Glu-1E-y from the diploid L. elongatum were more similar to Aey9 of A. elongatum; another y-type allele E1.8 was closer with Aey7 of A. elongatum, while two x-type alleles E2.1 and E1.9 from the diploid L. elongatum showed higher similarity to Aex4 than any other alleles of A. elongatum. Sequence alignment also indicated that the sequences of Aex4, Aey7, and Aey9 displayed homology to those of E2.1, E1.8 (Wang et al. 2006) and E1.5 (Wang et al. 2004) from the diploid L. elongatum (E^{e}) , respectively (data not shown). HMW-GS sequences from the diploid P. stipifolia were very smaller in length than most alleles from A. elongatum and all of them did not exhibit homology to those from A. elongatum (Fig. 5a).

As observed for the tree based on full length sequences, phylogenetic trees based on the N- and C-terminal region sequences can be divided into two halves. The relationship of all 15 sequences from *A. elongatum* reflected by trees of terminal sequences was similar to those in the full length sequences. However, there was one obvious difference in the position of *Aey4*: the N-terminal region of *Aey4* showed a higher degree of relatedness to that of *Aey10* (Fig. 5b), while the full length sequence and C terminal region of this gene were closer to *Aey5* (Fig. 5a, c). Another difference was that the C-terminal sequences of *Aey8*, *Aey9*, and *Aey10* showed more similarity with x-type genes than with the other seven y-type genes (Fig. 5c).

Discussion

Previous research indicates that each genome of wheat and its wild-related grasses, all contain a locus consisting of two tightly linked HMW-GS genes. Hence, we deduce that there may be ten pairs of tightly linked HMW-GS alleles in cross-pollinated decaploid *A. elongatum*. We have cloned 15 HMW-GS alleles from a seedling of *A. elongatum* in all, including ten y-type and five x-type alleles. The reason why we have not obtained the other x-type alleles may be that the degenerate primer pairs we used did not match those sequences very well and/or that the x-type alleles were less polymorphic or heterozygous than the y-type ones.

Five HMW-GS alleles from *A. elongatum* were successfully expressed in *E. coli* and three of the proteins directed by *Aex1*, *Aex4*, and *Aey7* have the same mobility with those from the seeds; thus, they were the coding genes for the three subunits. However, we have not found proteins from the seeds that showed similar migration with those directed by *Aey2* and *Aey9* in bacteria, which may be due to silencing of the two alleles in the seeds. The reason for this is not clear, but it has been reported that inactivation of promoter leads to the silencing of *IAy* of bread wheat (Halford et al. 1989).

Decaploid A. elongatum is an allopolyploid, but there is still controversy about the composition of its genome. Based on the results of cytogenetics, biochemistry, RAPD, and ISH, Zhang et al. (1996) speculated that the genome composition of decaploid A. elongatum was StStStStE^eE^eE^bE^bE^xE^x, and the St genome might come from Pseudoroegneria while the E genome derived from the diploid Thinopyrum elongatum (L. elongatum, $E^{e}E^{e}$) and *Thinopyrum Bessarabicum* (E^bE^b). Sequence alignment of HMW-GS genes from the decaploid A. elongatum with that of the diploid L. elongatum and P. stipifolia indicated that Aex4, Aey7, and Aey9 from A. elongatum showed very high similarity with three alleles from the diploid L. elongatum (Fig. 5), which confirmed that the diploid *L. elongatum* was the donor of the E^e genome of A. elongatum. Because all the 15 alleles from A. elongatum showed no high similarity with those of P. stipifolia, we concluded that the diploid P. stipifolia might not be the ancestor of the decaploid A. elongatum, while the St genome of A. elongatum might come from other species of the Pseudoroegneria genera.

The C-terminal region of three y-type alleles *Aey8*, *Aey9*, and *Aey10* showed higher similarity with the five x-type alleles than other y-type alleles (Fig. 5). Therefore, the structure of these three y-type subunits was not as typical as that of other y-type ones. Their structures lied between x- and y-type but inclined to y-type subunits. The extra glutamine residues in N-terminal of *Aey8*, *Aey9*, and *Aey10* also presented in x-type subunits. Thus, we speculated that this glutamine residue might be deleted after the divergence of x- and y-type subunits that did not contain this residue. Furthermore, we concluded that there was a kind of intermediate state in the divergence between x- and y-type subunits.

The strange structure of *Aey4* indicated that it might be a chimeric gene originating from recombination between *Aey5* and *Aey10*. Chimeric HMW-GS gene has also been

reported in *Aegilops searsii* (Sun et al. 2006). Through which way were these chimeric genes created was not known, but Wang et al. (2002) and Arguello et al. (2006) have referred that some new chimeric genes observed in *Drosophila* originated through retroposition and illegitimate recombination. Therefore, the mechanism of origination of chimeric HMW-GS gene might be the same as that found in *Drosophila*.

The greater part of HMW-GS genes from A. elongatum possess in-frame stop codon or frame shift mutation which result in their inability to express normal proteins in seeds. The reason for the appearance of so many mutations in HMW-GS genes may be due to the special structure of these subunits and their biological function. The amino acid sequences of HMW-GS include many glutamine (Gln) residues whose codons, CAA or CAG, can easily convert to stop codon TAA or TAG, respectively, in the process of evolution. The in-frame stop codons in these genes that we cloned are all due to this conversion. Such circs have also been reported in silent 1Ay and 1Ax from bread wheat (Forde et al. 1985; De Bustos et al. 2000) and 1Dx from Aegilops cylindrica (Wan et al. 2002). They were also present in gliadin and LMW subunit pseudogenes (Rafalski 1986; Anderson and Greene 1997). As seed storage proteins, the biological function of HMW glutenin subunits is to provide carbon, nitrogen, and energy sources for seed germination and seedling growth. The mutation or silence of such genes is not lethal for the plant, so the selection pressure on these genes is much lower than on other functional genes in evolution; this may be another reason for these genes to accumulate more mutation.

Four of the 15 subunits Aex2, Aex4, Aey1, and Aey3 contain extra cysteine residues in their amino acid sequences, even though only one subunit, Aex4, has intact ORFs. It has been shown that in all the known HMW-GS of wheat, only the 1Dx5 subunit possesses an additional cysteine residue in its structure and this cysteine exerts a positive influence on dough properties (Lafiandra et al. 1993; Gupta and MacRitchie 1994). Because of the presence of relatively more cysteine residues in glutenin subunits of A. elongatum than those of wheat, we predicted that this grass could provide HMW-GS genes for wheat quality improvement, e.g., the extra cysteine residue may confer on Aex4 potential value in improving the processing properties of wheat. Moreover, asymmetric somatic hybrid lines between common wheat and A. elongatum have been shown to possess a series of novel HMW-GS with good processing quality (Xia et al. 2003; Feng et al. 2004a; Liu et al. 2006). It has been proved that the presence of some novel HMW-GS in the hybrid lines was correlated with HMW-GS sequences from the donor A. elongatum. For example, Aex4 and Aey1 were found to be introgressed into some high quality hybrid lines from A. elongatum, while another novel hybrid allele *H1Dy12* may be the outcome of recombination between *Aey2* and another HMW-GS allele of Jinan177 (Liu et al. 2007). Therefore, HMW-GS genes of *A. elongatum* will contribute to the improvement of wheat processing quality and they deserve further investigation.

Acknowledgments This work was supported by Projects of National 863 High Technology Research and Development (Project no. 2006AA100102), National Basic Research Program of China (Project no. 2006CB100100), and Key and Doctor Position of ministry of education.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

- Anderson OD, Greene FC (1989) The characterization and comparative analysis of high-molecular-weight glutenin genes from genomes A and B of a hexaploid wheat. Theor Appl Genet 77:689–700
- Anderson OD, Greene FC (1997) The α -gliadin gene family. II. DNA and protein sequence variation, subfamily structure, and origins of pseudogenes. Theor Appl Genet 95:59–65
- Anderson OD, Greene FC, Yip RE, Halford NG, Shewry PR, Malpica-Romero J-M (1989) Nucleotide sequences of the two highmolecular-weight glutenin genes from the D-genome of a hexaploid bread wheat, *Triticum aestivum* L. cv Cheyenne. Nucleic Acids Res 17:461–462
- Arguello JR, Chen Y, Yang S, Wang W, Long M (2006) Origination of an X-linked testes chimeric gene by illegitimate recombination in *Drosophila*. PLoS Genet 2(5):e77
- Branlard G, Dardevet M (1985) Diversity of grain protein and bread wheat quality. II. Correlation between high molecular weight subunits of glutenin and flour quality characteristics. J Cereal Sci 3:345–354
- 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, Jouve N (2001) Characterisation of two gene subunits on the 1R chromosome of rye as orthologs of each of the *Glu-1* genes of hexaploid wheat. Theor Appl Genet 103:733–742
- Feng DS, Xia GM, Zhao SY, Chen FG (2004a) Two quality-associated HMW glutenin subunits in a somatic hybrid line between *Triti*cum aestivum and Agropyron elongatum. Theor Appl Genet 110:136–144
- Feng DS, Chen FG, Zhao SY, Xia GM (2004b) High-molecularweight glutenin subunit genes in decaploid Agropyron elongatum. Acta Bot Sin 46:489–496
- Feng DS, Chen FG, Zhao SY, Xia GM (2004c) Study on a novel HMW glutenin subunit coding region from *Agropyron elongatum* (in Chinese with English Abstract). Acta Bot Boreal Occident Sin 24:237–242
- Forde J, Malpica J-M, Halford NG, Shewry PR, Anderson OD, Greene FC, Miflin BJ (1985) The nucleotide sequence of a HMW glutenin subunit gene located on chromosome 1A of wheat (*Triticum aestivum* L.). Nucleic Acids Res 13:6817–6832
- Guo ZF, Yan ZH, Wang JR, Wei YM, Zheng YL (2005) Characterization of HMW prolamines and their coding sequences from *Crithopsis delileana*. Hereditas 142:56–64

- Gupta RB, MacRitchie F (1994) Allelic variation at glutenin subunit and gliadin loci, *Glu-1*, *Glu-3* and *Gli-1*, of common wheats. II. Biochemical basis of the allelic effects on dough properties. J Cereal Sci 19:19–29
- Halford NG, Forde J, Anderson OD, Greene FC, Shewry PR (1987) The nucleotide and deduced amino acid sequences of an HMW glutenin subunit gene from chromosome 1B of bread wheat (*Triticum aestivum* L.) and comparison with those of genes from chromosomes 1A and 1D. Theor Appl Genet 75:117-126
- Halford NG, Forde J, Shewry PR, Kreis M (1989) Functional analysis of the upstream regions of a silent and an expressed member of a family of wheat seed protein genes in transgenic tobacco. Plant Sci 62:207–216
- 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 restrictionfragment variation in the gene family encoding high molecular weight (HMW) glutenin subunits of wheat. Biochem Genet 24:579–596
- Kumar S, Tamura K, Nei M (2004) MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 5:150–163
- Lafiandra D, D'Ovidio R, Porceddu E, Margiotta B, Colaprico G (1993) New data supporting high Mr glutenin subunit 5 as the determinant of quality differences among the pairs 5 + 10 vs. 2 + 12. J Cereal Sci 18:197–205
- Lawrence GJ, Shepherd KW (1980) Variation in glutenin protein subunits of wheat. Aust J Biol Sci 33:221–233
- Lawrence GJ, Shepherd KW (1981) Chromosomal location of genes controlling seed proteins in species related to wheat. Theor Appl Genet 59:25–31
- Liu Z, Yan Z, Wang Y, Liu K, Zheng Y, Wang D (2003) Analysis of HMW glutenin subunits and their coding sequences in two diploid *Aegilops* species. Theor Appl Genet 106:1368–1378
- Liu H, Shi L, Zhao JS, Xia GM (2006) Genetic characteristic of high molecular weight glutenin subunits in somatic hybrid wheat lines—potential application to wheat breeding. J Agric Food Chem 54:5007–5013
- Liu S, Zhao S, Chen F, Xia G (2007) Generation of novel high quality HMW-GS genes in two introgression lines of *Triticum aestivum*/ *Agropyron elongatum*. BMC Evol Biol 7:76
- Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4325
- Payne PI, Law CN, Mudd EE (1980) Control by homoeologous group 1 chromosomes of the high-molecular-weight subunits of glutenin, a major protein of wheat endosperm. Theor Appl Genet 58:113–120
- Payne PI, Holt LM, Worland AJ, Law CN (1982) Structural and genetical studies on the high-molecular-weight subunits of wheat glutenin. Part 3. Telocentric mapping of the subunit genes on the long arms of the homoeologous group 1 chromosomes. Theor Appl Genet 63:129–138
- Payne PI, Nightingale MA, Krattiger AF, Holt LM (1987) The relationship between HMW glutenin subunit composition and the breadmaking quality of British grown wheat varieties. J Sci Food Agric 40:51–65
- Payne PI, Holt LM, Krattiger AF, Carrillo JM (1988) Relationship between seed quality characteristics and HMW glutenin subunit composition determined using wheats grown in Spain. J Cereal Sci 7:229–235
- Rafalski JA (1986) Structure of wheat gamma-gliadin genes. Gene 43:221–229

- Reddy P, Appels R (1993) Analysis of a genomic DNA segment carrying the wheat high-molecular-weight (HMW) glutenin Bx17 subunit and its use as an RFLP marker. Theor Appl Genet 85:616–624
- Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, New York
- Shewry PR, Tatham AS (1990) The prolamin storage proteins of cereal seeds: structure and evolution. Biochem J 267:1–12
- Shewry PR, Napier JA, Tatham AS (1995) Seed storage proteins: structure and biosynthesis. Plant Cell 7:945–956
- Shewry PR, Halford NG, Tatham AS, Popineau Y, Lafiandra D, Belton PS (2003a) The high molecular weight subunits of wheat glutenin and their role in determining wheat processing properties. Adv Food Nutr Res 45:219–302
- Shewry PR, Halford NG, Lafiandra D (2003b) Genetics of wheat gluten proteins. Adv Genet 49:111–184
- Sugiyama T, Rafalski A, Peterson D, Söll D (1985) A wheat HMW glutenin subunit gene reveals a highly repeated structure. Nucleic Acids Res 13:8729–8737
- Sun X, Hu SL, Liu X, Qian WQ, Hao ST, Zhang AM, Wang DW (2006) Characterization of the HMW glutenin subunits from *Aegilops searsii* and identification of a novel variant HMW glutenin subunit. Theor Appl Genet 113:631–641
- Thompson RD, Bartels D, Harberd NP (1985) Nucleotide sequence of a gene from chromosome 1D of wheat encoding a HMW-glutenin subunit. Nucleic Acids Res 13:6833–6846
- Wang W, Brunet FG, Nevo E, Long M (2002) Origin of Sphinx, a young chimeric RNA gene in *Drosophila melanogaster*. Proc Natl Acad Sci USA 99:4448–4453

- Wang JR, Yan ZH, Wei YM, Zheng YL (2004) A novel highmolecular-weight glutenin subunit gene *Ee1.5* from *Elytrigia elongata* (Host) Nevski. J Cereal Sci 40:289–294
- Wang JR, Yan ZH, Wei YM, Zheng YL (2006) Characterization of high molecular weight glutenin subunit genes from *Elytrigia elongata*. Plant Breed 125:89–95
- Wan Y, Wang D, Shewry PR, Halford NG (2002) Isolation and characterization of five novel high molecular weight subunit of glutenin genes from *Triticum timopheevi* and *Aegilops cylindrica*. Theor Appl Genet 104:828–839
- Xia GM, Xiang FN, Zhou AF, Wang H, Chen HM (2003) Asymmetric somatic hybridization between wheat (*Triticum aestivum* L.) and *Agropyron elongatum* (Host) Nevishi. Theor Appl Genet 107:299–305
- Yan ZH, Wei YM, Wang JR, Liu DC, Dai SF, Zheng YL (2006) Characterization of two HMW glutenin subunit genes from *Taenitherum* Nevski. Genetica 127:267–276
- Zhang XY, Dong YS, Wang RRC (1996) Characterization of genomes and chromosomes in partial amphiploids of the hybrid *Triticum aestivum* × *Thinopyrum ponticum* by in situ hybridization, isozyme analysis and RAPD. Genome 39:1062–1071
- Zhao TJ, Quan TY, Xia GM, Chen HM (2003) Glutenin and SDS sedimentation analysis of the F₅ somatic hybrids between *Triticum aestivum* L. and *Agropyron elongatum* (in Chinese with English Abstract). J Shandong Univ (Nat Sci) 38(3):112–116
- Zhou HP, Li B, Li ZS (1995) The study of breeding blue-grain gene translocation of wheat (in Chinese with English Abstract). Acta Bot Boreal Occident Sin 15:125–128