

Phylogenetic Study of *Aegilops* Species and their Relation with *Triticum* and *Secale* Analysed by Glutenin Genes

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Abstract: This work reports the molecular characterization of HMW glutenin subunits belonging to the diploid species *Aegilops comosa*, *Ae. uniaristata* and *Ae. speltoides*. The sequences of the genes are used to clarify the phylogenetic relationships between these species and others of the tribe *Triticeae*. Alignment of the sequences obtained with the corresponding orthologous genes previously published depicted a phylogenetic tree in concordance with the classification of the genus in six sections. The analysis of relationships between members of *Triticeae* group performed with orthologous glutenin genes separated the species of the genus *Secale* from the rest, whereas the species of *Triticum* and *Aegilops* appeared in a common group. The glutenin genes of *Aegilops comosa* and *Aegilops uniaristata* from *Comopyrum* section were grouped together. The HMW glutenin subunits of *Ae. speltoides* were associated with the glutenin *Glu-B1y9* of wheat, indicating a relation between S and B genomes. Our results do not support the separation of *Aegilops* and *Triticum* into two distinct genera.

Keywords: high molecular weight glutenin; orthologous genes; *Aegilops*

The genus *Aegilops* includes approximately 25 species belonging to six sections. Presumably this genus includes the diploid species that contributed to the allopolyploid origin of the genus *Triticum*. It was for this reason that BOWDEN (1959) recognised these genera as one, although they have traditionally been maintained as separate taxa (KIMBER & SEARS 1983). This controversy has inspired many taxonomic studies, but no consensus has been reached.

The high molecular weight (HMW) glutenin genes of wheat have been intensively analysed because of their relationship with flour quality (PAYNE 1987). These proteins are coded by two strongly linked genes (named *x* and *y*). Given the great importance of these proteins, much work has been undertaken on HMW glutenins in genera related to wheat. Despite the relationships between wheat and *Aegilops*, molecular analyses of the HMW glutenin subunits have been performed in

only a few diploid species of the genus *Aegilops* (LIU *et al.* 2003).

This work reports the identification of new HMW glutenin genes in three diploid species of the genus *Aegilops* and its use to clarify the phylogenetic relationships between *Aegilops* and species from the *Triticeae* group.

MATERIALS AND METHODS

The plant material examined included three diploid species: *Aegilops comosa* Sibth. et Sm. ($2n = 14$; genome M), *Ae. uniaristata* Vis. ($2n = 14$; genome Un) and *Ae. speltoides* Tausch ($2n = 14$; genome S). The isolation and characterisation of the genes was performed as previously reported by DE BUSTOS *et al.* (2001). Nucleotide sequences were aligned using the Clustal W 1.5 program. Phylip programme was used to construct phylogenetic trees. The robustness of the trees was assessed

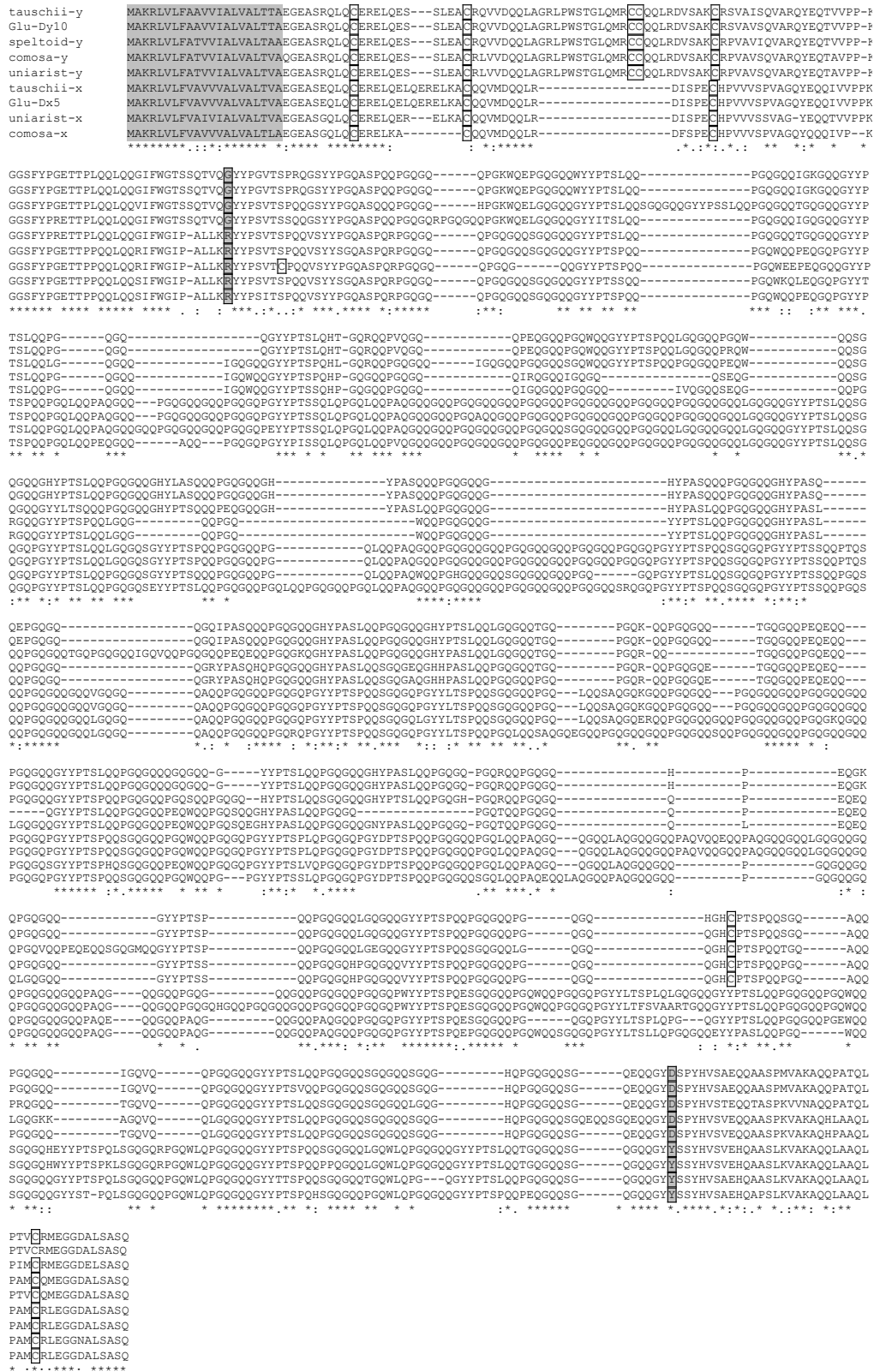


Figure 1. Sequence alignment of deduced glutenin proteins of *Aegilops* species. The grey box in the N terminal region indicates the sequence of the signal peptide. The small grey boxes indicate the first and last residues of the central repetitive region. The cysteine residues are indicated by a white box. Identical residues are marked with an asterisk, whereas ‘/’ and ‘\’ indicate that one or two samples showed different amino acids from the rest

by the bootstrap method with 1000 random replications.

RESULTS AND DISCUSSION

Molecular characterization

The glutenin genes were characterised using primers based on sequences published for related species. The primers designed amplified two products in *Ae. uniaristata* and *Ae. comosa*, but only one in *Ae. speltoides*. The amino acid composition of these proteins is quite similar to that of the wheat glutenins (Figure 1), especially with respect to the number and position of cysteine residues, to which dough quality has been related (PAYNE 1987).

Species relationships

The protein sequences obtained were aligned with glutenins of *Aegilops* previously reported. Bootstrap analysis of the *y*-type genes provided better separation of the groups than *x*-type (Figure 2). The *y*-type sequences were arranged in two clearly differentiated clusters. The first included the sequences of the species of the section *Comopyrum*, close to *Ae. caudata* (section *Cylindropyrum*) pointing out relationships between these two sections (as mentioned by WANG *et al.* 2000).

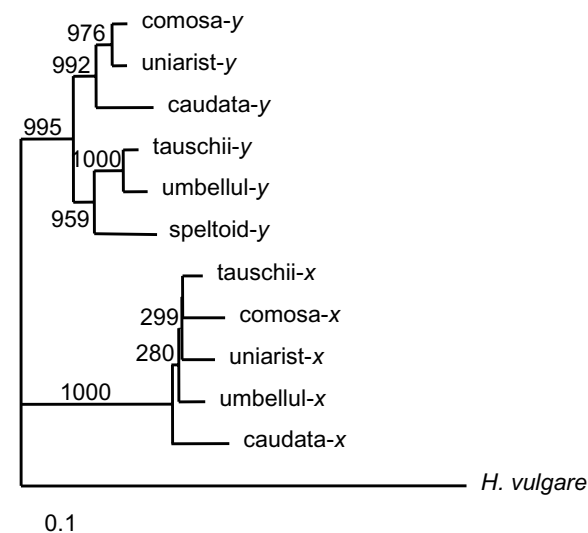


Figure 2. Phylogenetic tree obtained from the deduced amino acid sequences of the glutenins from the diploid *Aegilops* species representing the six genomes of the genus. *Hordeum vulgare* was used as the outgroup. Bootstrap values are indicated at the nodes

The other group includes the sequences belonging to *Ae. tauschii*, and *Ae. umbellulata* (sections *Vertebrata* and *Polyeides*, respectively). Relationships between the members of these sections have been also reported (BANDOPADHAYAY *et al.* 2004). The *x*-type sequence of *Ae. speltoides* appeared to be less related to the remaining orthologous sequences.

Thirty-nine HMW glutenin sequences belonging to the species of the genera *Aegilops*, *Triticum* and *Secale* were used to build a phylogenetic tree concerning the evolution of the HMW glutenin gene subunits in the Tribe *Triticeae* (Figure 3). The *x*- and *y*-type sequences of the genus *Secale* fell into subgroups within a separate clade, whereas the orthologous sequences of *Triticum* and *Aegilops* appeared intermixed in common groups. ZOHARY and FELDMAN (1962) reported the polyploids of *Triticum* to fall into three natural groups shar-

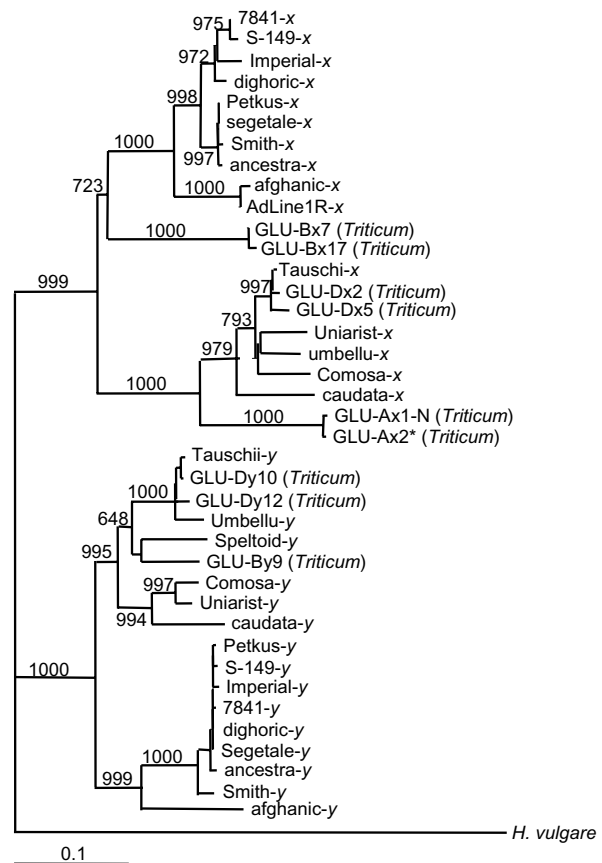


Figure 3. Phylogenetic relationships between species of the Tribe *Triticeae* belonging to the genera *Aegilops*, *Triticum* and *Secale*, determined by aligning the amino acid sequences of the *x* and *y* glutenin subunits. *Hordeum vulgare* was used as outgroup. Bootstrap values are indicated at the nodes

ing either the U genome of *Ae. umbellulata*, the D genome of *Ae. Tauschii* and the A genome of *T. monococcum*. As expected, the *x*-type and *y*-type HMW glutenins of *Ae. tauschii* are both closely related to the glutenins of the D genome of *Triticum*. However, the glutenins of *Ae. umbellulata* did not fall into the group of glutenins belonging to the B genome. Interestingly, the *y*-type glutenin of *Ae. speltoides* was associated with the glutenin subunit encoded by the allele *Glu-B1y9* of wheat. This finding agrees with the works of DAUD and GUSTAFSON (1996). The present results, which compare the sequences of the HMW glutenin subunits, do not support the separation of *Aegilops* and *Triticum* into two distinct genera.

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