

Table 1 Suppl. Polymerase chain reaction (PCR) primers used for amplifying chalcone-flavanone isomerase (*Chi*), PCR product lengths, and PCR conditions. Details of amplification programs - see the main text, MgCl₂ concentrations [mM].

| Primer pair | Forward primer, 5'→3' | Reverse primer, 5'→3' | DNA/cDNA | Amplification program/ MgCl ₂ |
|-----------------|-----------------------|------------------------|--------------------|---|
| Chi_1F / Chi_1R | GCCGTGGCATAATCGCAA | CCGGCACGGACGAGTCCT | 948, 951, 956, 960 | - / 1 / 1.5 |
| Chi_3F / Chi_3R | CGGGGGCAACTTCATCAAGT | GCGAGGGAGTGGGTGAAGAGA | 427 / 354 | 1 / 1.8 |
| Chi_4F / Chi_4R | GCTCCGAGCACGCCCACTTC | GACGTCGCGGAAGAAGGCA | 325 / 188 | 1 / 1.5 |
| Chi_5F / Chi_5R | GCAGGGCGTGCGGGGG | TGGAGAAGGCAACGGTGAGGAC | 476 / 403 | 2 / 1.5 |
| Chi_6F / Chi_6R | CTCGCCGCCAAGTGGG | TTCTCGAACTCGCCGGTGAC | - / 89 | 1 / 1.8 |

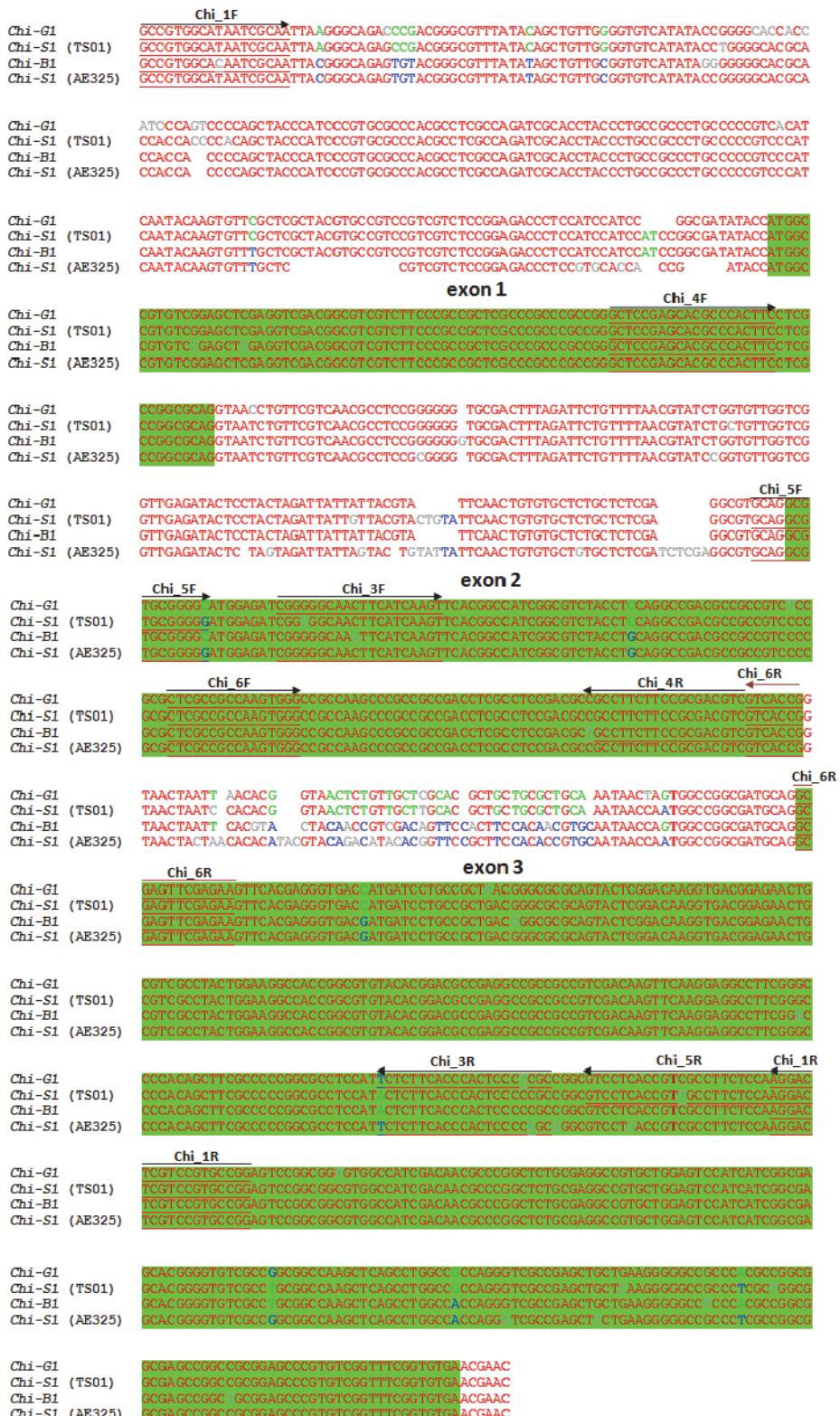


Fig. 1 Suppl. Multiple alignment of S, G, and B genome *chalcone-flavanone isomerase (Chi)-1* nucleotide sequences. The sites of primer pairs (Table 1 Suppl.) annealing are shown with the arrows. The length of the *Triticum timopheevii* sequence was 1 142 bp, that of the *Aegilops speltoides* 'TS01' copy 1 151 bp, and that of the *Ae. speltoides* 'AE325' copy 1 139 bp. The coding sequence was split into three exons, and was 696 bp in each case, as it was also for the *T. aestivum* *Chi-B1* gene (JN039038, Shoeva *et al.* 2014). The exons are shown in green.

Medicago sativa CHI1
Ae. speltoides CHI-S1 (AE325)
Ae. speltoides CHI-S1 (TS01)
T. aestivum CHI-B1
T. timopheevii CHI-G1

Medicago sativa CHI1
Ae. speltoides CHI-S1 (AE325)
Ae. speltoides CHI-S1 (TS01)
T. aestivum CHI-B1
T. timopheevii CHI-G1

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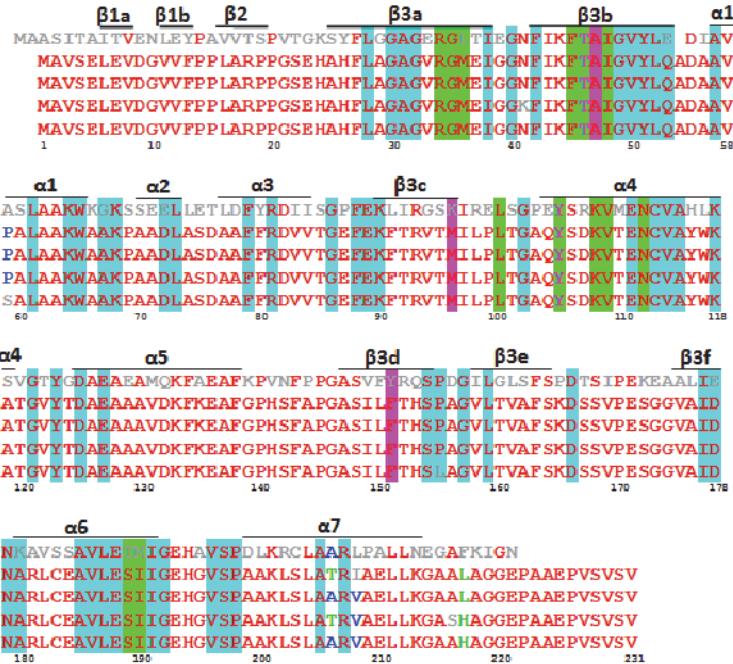


Fig. 2 Suppl. Multiple alignment of S, G, and B genome chalcone-flavanone isomerase (CHI) deduced polypeptide sequences. Residues associated with a substrate binding cleft are shown in green; those with an active site hydrogen bond network in pink; other conserved residues are shown in blue; residues from α -helices and β -strands are indicated above the sequences (after Jez *et al.* 2000).

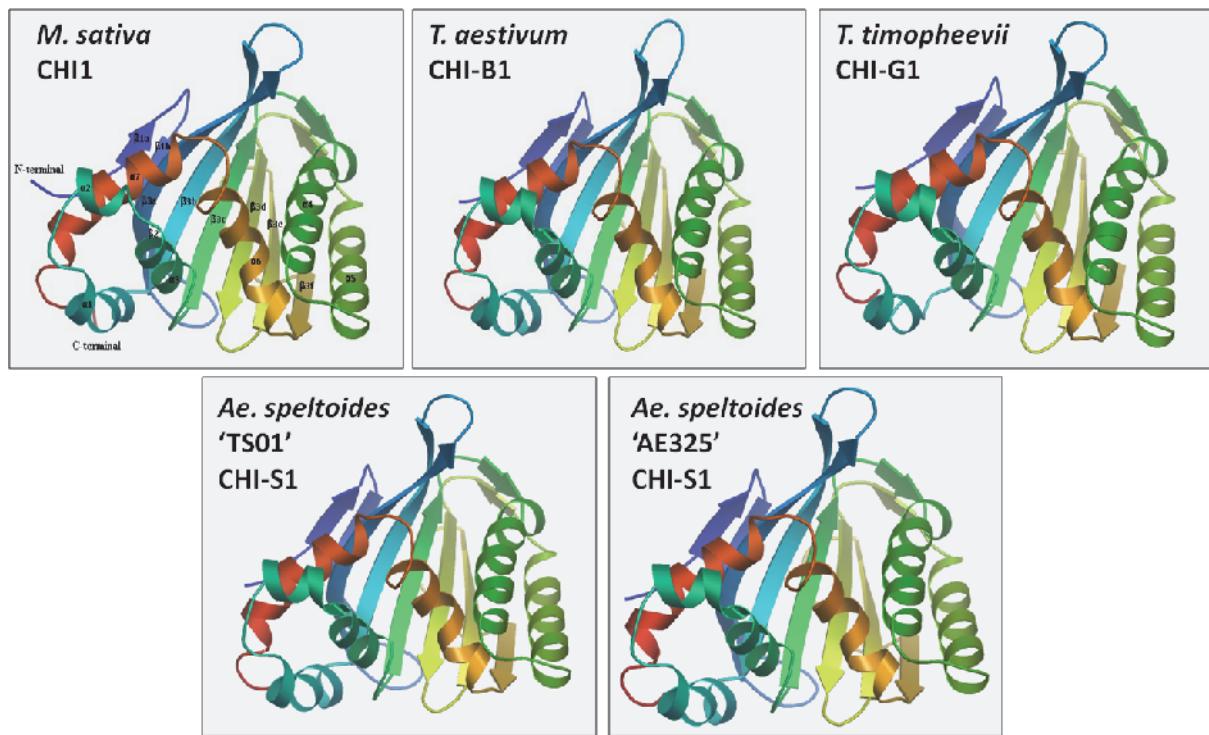


Fig. 3 Suppl. Three dimensional structures of *Medicago sativa* chalcone-flavanone isomerase (CHI) (Jez *et al.* 2000) and its homologs in *Triticum timopheevii* and *Aegilops speltoides* determined using the SWISS-MODEL program.