

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, MgCl<sub>2</sub> concentrations [mM].

Primer pair	Forward primer, 5'→3'	Reverse primer, 5'→3'	DNA/cDNA	Amplification program/ MgCl <sub>2</sub>
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	GCAGGCGTGCGGGGG	TGGAGAAGGCAACGGTGAGGAC	476 / 403	2 / 1.5
Chi_6F / Chi_6R	CTCGCCGCCAAGTGGG	TTCTCGAACTCGCCGGTGAC	- / 89	1 / 1.8

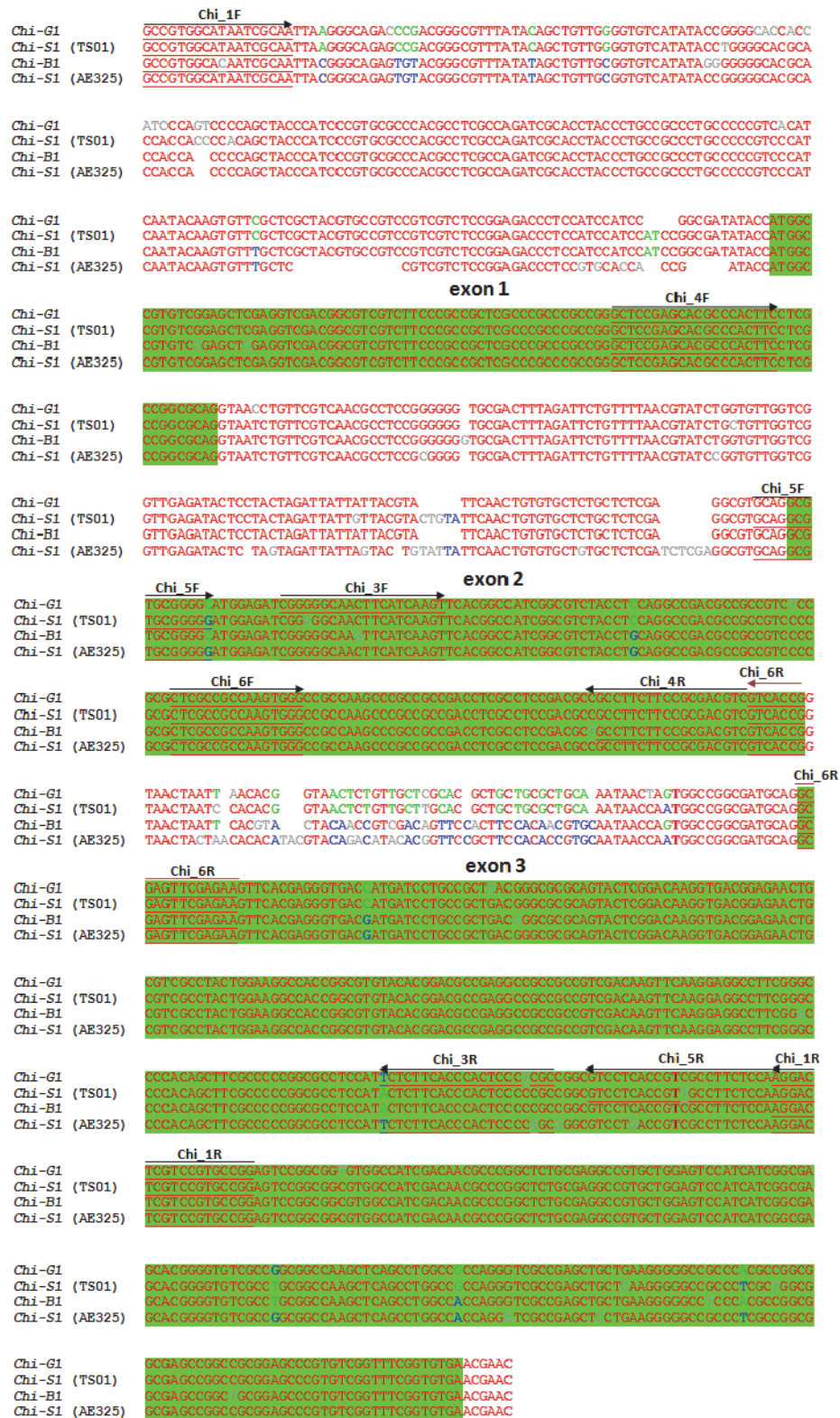


Fig. 1 Suppl. Multiple alignment of S, G, and B genome *chalcone-flavanone isomerase* (*Chi-1*) nucleotide sequences. The sites of primer pairs (Table 1 Suppl.) annealing are shown with the arrows. The length of the *Triticum timopheevii* sequence was 1 142 bp, that of the *Aegilops speltioides* 'TS01' copy 1 151 bp, and that of the *Ae. speltioides* '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.

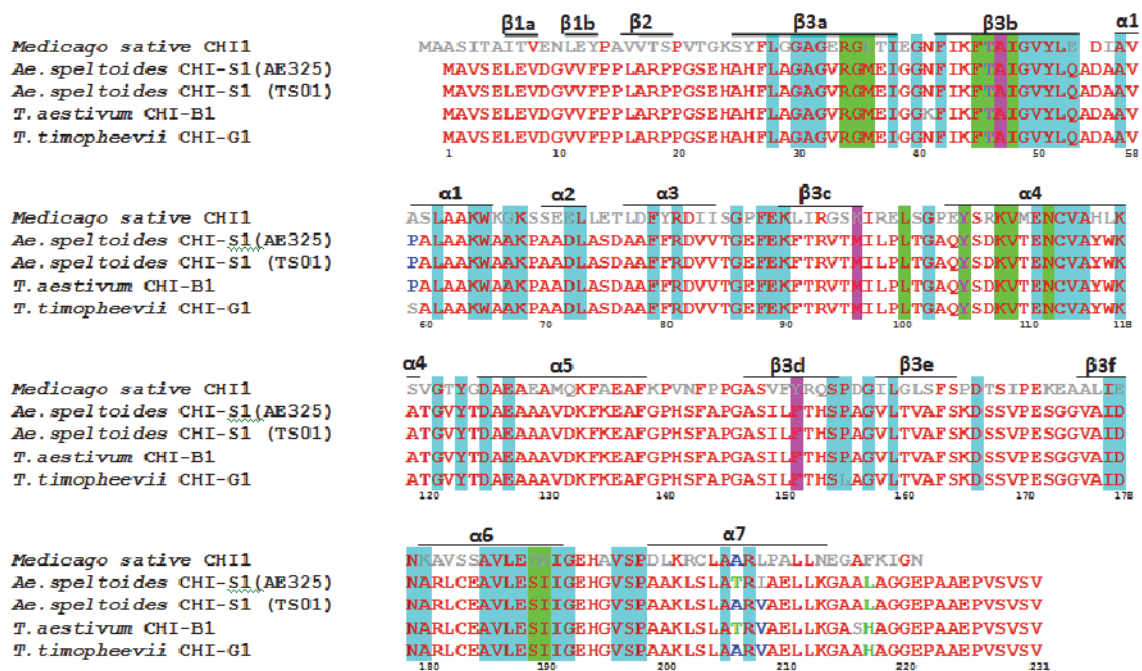


Fig. 2 Suppl. Multiple alignment of S, G, and B genome chalcone-flavanone isomerase (CHI) deduced polypeptide sequences. Residues associated with a substrate binding cleft are shown in *green*, those with an active site hydrogen bond network in *pink*; other conserved residues are shown in *blue*; residues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

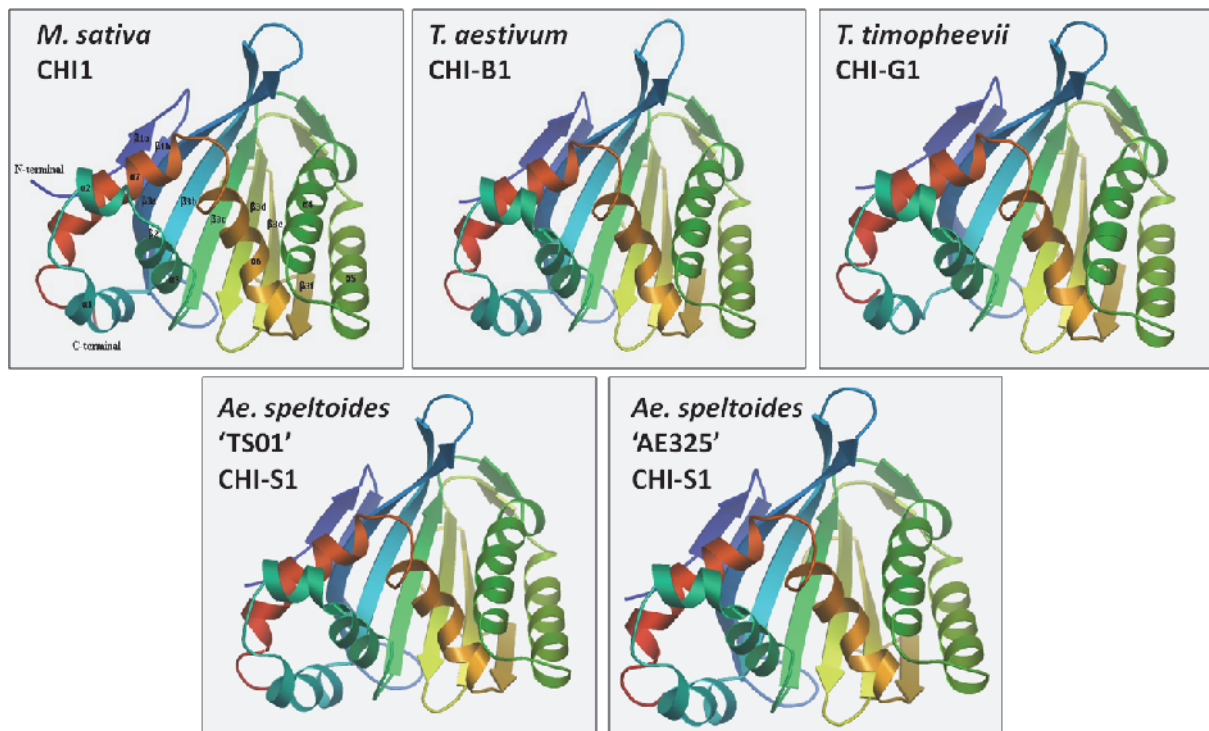


Fig. 3 Suppl. Three dimensional structures of *Medicago sativa* chalcone-flavanone isomerase (CHI) (Jez *et al.* 2000) and its homologs in *Triticum timopheevii* and *Aegilops speltoides* determined using the *SWISS-MODEL* program.