

Supplementary Text

Computational identification of putative barley TGases

Coding sequences of putative barley TGase genes were searched for using Arabidopsis AtPng1p (Della Mea et al. 2004a) and maize TGZ15 and TGZ21 (Villalobos et al. 2004) protein sequences to query the public databases. One predicted barley protein has been identified (BAK01337.1, hereafter named HvPng1-like) as exhibiting a high degree of similarity to the AtPng1p reference protein sequence. The corresponding cDNA sequence was successfully verified by assembling it with eight ESTs from the NCBI UniGene Hv.16669 (Figure S1). HvPng1-like has a high level of similarity to multiple proteins from both monocots and dicots (Table S1), all of which possess a conserved core domain (pfam01841) and whose Cys-His-Asp residues form the catalytic triad, a characteristic of TGases (Figure S2). According to the TargetP1.1 Server search, none of these proteins possess a classical signal peptide capable of targeting it to any cellular compartment.

When the maize TGZ21 protein was used for the identification of barley homologs, four predicted proteins with partial homology were identified, BAJ92539.1 being the most similar. We refer to this putative protein as HvTGZ21-like, whose cDNA sequence has been confirmed by assembly with 18 other cDNAs/ESTs from the NCBI UniGene Hv.1565 (Figure S3). Interestingly, HvTGZ21-like shows a very high degree of similarity to TGZ21 at the N-terminus, but the C-termini of the two proteins are drastically different. A more detailed investigation revealed that although numerous monocot cDNA sequences are highly similar to the *TGZ15* and *TGZ21* cDNA sequences, these two maize cDNAs lack a conserved G in position 747, which results in a frame shift that extends the TGZ15/TGZ21 protein length, with respect to the other *Poaceae* sequences (Figures S4 and S5). According to Villalobos et al. (2004), the TGase catalytic triad in the TGZ15 and TGZ21 proteins is placed in this unique C-terminal region. As we did not find any proteins with similarity to this region in the databases nor did we observe the G deletion in any other maize DNA sequence (including genomic DNA; see Figure S6), we focused on the functional analysis of only the *HvPng1-like* gene.

Table S1. Plant proteins highly similar to barley HvPng1-like protein.

Organism	NCBI accession	Length	Identities	Positives
<i>H. vulgare</i> (HvPng1-like)	BAK01337	777 aa	-	-
<i>O. sativa</i>	EEC82091	802 aa	76 %	83 %
<i>B. distachyon</i>	XP_003563069	719 aa	75 %	81 %
<i>S. bicolor</i>	XP_002462888	727 aa	70 %	79 %
<i>R. communis</i>	XP_002512221	719 aa	52 %	70 %
<i>S. lycopersicum</i>	NP_001234560	725 aa	53 %	69 %
<i>V. vinifera</i>	XP_002278422	726 aa	51 %	66 %
<i>M. truncatula</i>	XP_003626428	719 aa	49 %	66 %
<i>A. thaliana</i> (AtPng1p)	NP_199768	721 aa	51 %	66 %

Figure S1. CAP3 assembly of sequences from the UniGene Hv.16669. Sequence AK370136 is a cDNA corresponding to HvPNG1-like protein, acc. no BAK01337.1. Coding sequence has been highlighted.

***** Contig 1 *****

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 8/gb=BJ485214- is in 12/gb=AK370136+
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DETAILED DISPLAY OF CONTIGS

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Figure S2. Catalytic transglutaminase core in plant proteins closely related to AtPng1

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	↓	↓	↓
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B.distachyon	TRFPRYNDPSKLLQTRKGRCGEWANCFTFYCRAFGYEARLILDFTDHWVTECFNSLYGRWMHLDPCEGVY		
HvPNG-like	TRFPRYNDPHKLIQTRKGRCGEWANCFTFYCRVFGYEARLILDFTDHWVTECFNSLYGRWIHFDPCEGVY		
M.truncatula	TRFPRYNDPKKLVETREGRCGEWANCFTLYCRAFGYESRLIQDFTDHWVTECYSQFLGRWMHLDPCEAIY		
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	*****	***:***	*****:*
	:	*****:*	***:***

Figure S3. CAP3 assembly of sequences from the UniGene Hv.1565. Sequence AK361332 is a cDNA corresponding to HvTGZ21-like protein, acc. no BAJ92539.1. Coding sequence has been highlighted.

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DETAILED DISPLAY OF CONTIGS

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Figure S4. Clustal2W sequence alignment of plant proteins showing partial similarity to maize TGZ21 protein. Significant similarity only to ~220 N-terminal amino acids of the TGZ21/TGZ15 sequences is maintained. Downstream of this region, the protein sequences become more diverse and several subgroups can be easily identified, with the *Poaceae* forming a distinct subgroup. However, the TGZ21 sequence similarity to other *Poaceae* proteins rapidly discontinues after Gly249. Accession IDs of the proteins are: HvTGZ21-like (BAJ92539.1), TGZ21 (CAD32336.1), *S.bicolor* (XP_002448045.1), *B.distachyon* (XP_003579974.1), *O.sativa* (NP_001053092.1), *V.vinifera1* (XP_002277975.1), *V.vinifera2* (XP_002282805.1), *P.trichocarpa* (XP_002308428.1), *R.communis1* (XP_002528217.1), *R.communis2* (XP_002531483.1), *A.lyrata* (XP_002887107.1), *A.thaliana* (NP_176888.2), *G.max1* (XP_003551793.1), *G.max2* (XP_003531907.1). Except for barley and maize proteins, all the other sequences are from the NCBI refseq database.



Fig S4 Continued.

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A.thaliana	-----YIPQP-----VAGN-----ATGPNSV	291
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G.max2	ITAGGQPGPAPAGTG-----YDAPRPGPYGASAGPA-	308
G.max1	ITAGGQPGPAPAGTG-----YDAPIGPGYGASTGST-	309
V.vinifera2	AYAGPQSGPAASTRPG-----YDGRPGPYDAPRPGP-	325
R.communis2	TYTGAQSGSASTRSA-----YDTPRGPNYEATKGPS-	338
A.lyrata	VGAAQYPYPGVVTQPG-----YFPPR-PGYNFPRGP-	320
A.thaliana	VGAAQYPYPGVVTQPG-----YFPQR-PGYNFPRGP-	320
P.trichocarpa	GPAGEGIIPYNSNS-----VVSNVGFGGAAMSTTG-	281
R.communis1	GPAVNGTIPYDNGNN-----AAIICGIGGSVSSAG-	296
V.vinifera1	GTPSAGMIPY-----GSVAIPAS-	287
B.distachyon	AYQHGTPQAAAYQQG-----TLQGGAYQQGTPAGTYAYPTA-	319
HvTG221-like	AYQQGTPQAAAYQQGGHPQAAAYQQGGHPQAGSYQQGPPQAGAYQQGPPQAGAYAYPTA-	372
O.sativa	VYQQGTTQAGAYQQG-----STQAGAYAYPT	306
S.bicolor	AYQQG-AQAAAYQLG-----AYQHGTPQAGAYQQGN-	314
TG221	REPRLLGHISRVLRLGLYISREPRLLGHISRPRLLGHISRARMGHISRGLRLG	400
G.max2	-----YDAQRVATYDAQRLIGYDAFRGSAYDSKRGPIFDAQRT-	346
G.max1	-----YDAQRGATYDTRQLTGSNAFRGSAYDSKRGSYDMQRAP-	348
V.vinifera2	-----YDAPRPGPYDPRGPGYDAQRGPGYDAQRGPAVDGQRGP-	364
R.communis2	-----YDVSRRSGYDLRGAAYDAQRGHSYDAQRGPGYDMQRGSPSYDAQQRVP-	386
A.lyrata	-----PGSYDPTIR-----LPTGPGAPFPPG	342
A.thaliana	-----PGSYDPTIR-----LPTGPGAPFPPG	342
P.trichocarpa	-----GVAQWVGFDP-SHARG-----	297
R.communis1	-----GVAPLGGSDP-SLAEK-----	312
V.vinifera1	-----SGAAWGGAYDPNSHTQH-----	304
B.distachyon	-----FDTANAYQPHAAQASAYAGYSYGVVAGYAQ-----PSYPGTYAAPQHFV-	363
HvTG221-like	-----YDSATAYQ-----LQANAYASYSYGVVAGYAQ-----PNYPGTYAAPQHFV-	413
O.sativa	-----YDAATAYQMHAQAANAYAGYPYGVVAGYTAALPGYPSAYAAAPQPI-	353
S.bicolor	-----QAGAYTYAYDAATAYAYAGYSYGVV-----GYAQSVPNYS--YAVPPQP-	356
TG221	HISRPRLLGHISRPRLLGHISRGLSLGHISRPRLLGHISRPRMGHISRPRMGI-	456
G.max2	-----GYD-----	349
G.max1	-----AYDAPRAAGYDAQSRGVAG-PHGHPMNNMPYGSIT	384
V.vinifera2	-----VYDAPRGTNYDAPTRAAPG-PHGQVPSGNNVYGSAT	400
R.communis2	GYDVQRIPGYAPRIPGYDVQQGPHYDASRVAGYDPAARGTAVPPHGQMTANNVYGSAT	446
A.lyrata	-----PSNNTPYGSAA	353
A.thaliana	-----PSNNTPY	349
P.trichocarpa	-----	
R.communis1	-----	
V.vinifera1	-----	
B.distachyon	-----TSGTATDVICTYAVGSAGYPAGVVQPSTGAANAGQA	400
HvTG221-like	-----ASGAATDATSAYGAAGSTGYPAAPVQASSGAANAGQA	450
O.sativa	-----SSGVATDVASMYGAISSAGYPAGVVQSSSGAANAGQA	390
S.bicolor	-----SSGATTEAASMYGAAGSAGYPTAQVQPSATANAAP	393
TG221	-----SRVLRLEHTTMLMMLARLHMVTLAIQLQATRKVQC	493
G.max2	-----	
G.max1	PPARSGGGYEPA-RGVNPARR-----	404
V.vinifera2	PPARSGGGYEAPRGGNPVRR-----	421
R.communis2	PSTRAVSGYEAPRGGNPVRR-----	467
A.lyrata	-----TASARGNPSCR-----	364
A.thaliana	-----AGTHGNPSRR-----	359
P.trichocarpa	-----	
R.communis1	-----	
V.vinifera1	-----	
B.distachyon	PPAP--PAPYPATYDPTRGAQR-----	420
HvTG221-like	P--PAPYPATYDPTKAAQR-----	467
O.sativa	P--ATYPVAYDPTRAGQR-----	406
S.bicolor	PPPPPPAAPYPSTYDQTRGAQR-----	415
TG221	PTIPLHLRSQAAVQLRTPQEAASMGLVVLDDILLGKFSRAVALQMRKHLLHHRQH	553

Figure S5. Multiple sequence alignment of coding sequences of TGZ15, TGZ21 and Poaceae proteins. Significant sequence similarity across the whole coding region is maintained, but lack of conserved guanosine in position 747 of the TGZ15 and TGZ21 cDNA (marked with black solid arrow) results in a frame shift. It preserves the Gly249 amino acid (encoded by GGA instead of GGG) but changes all the consecutive amino acids and extends the protein length with respect to other Poaceae sequences (see Fig.S4 for comparison).

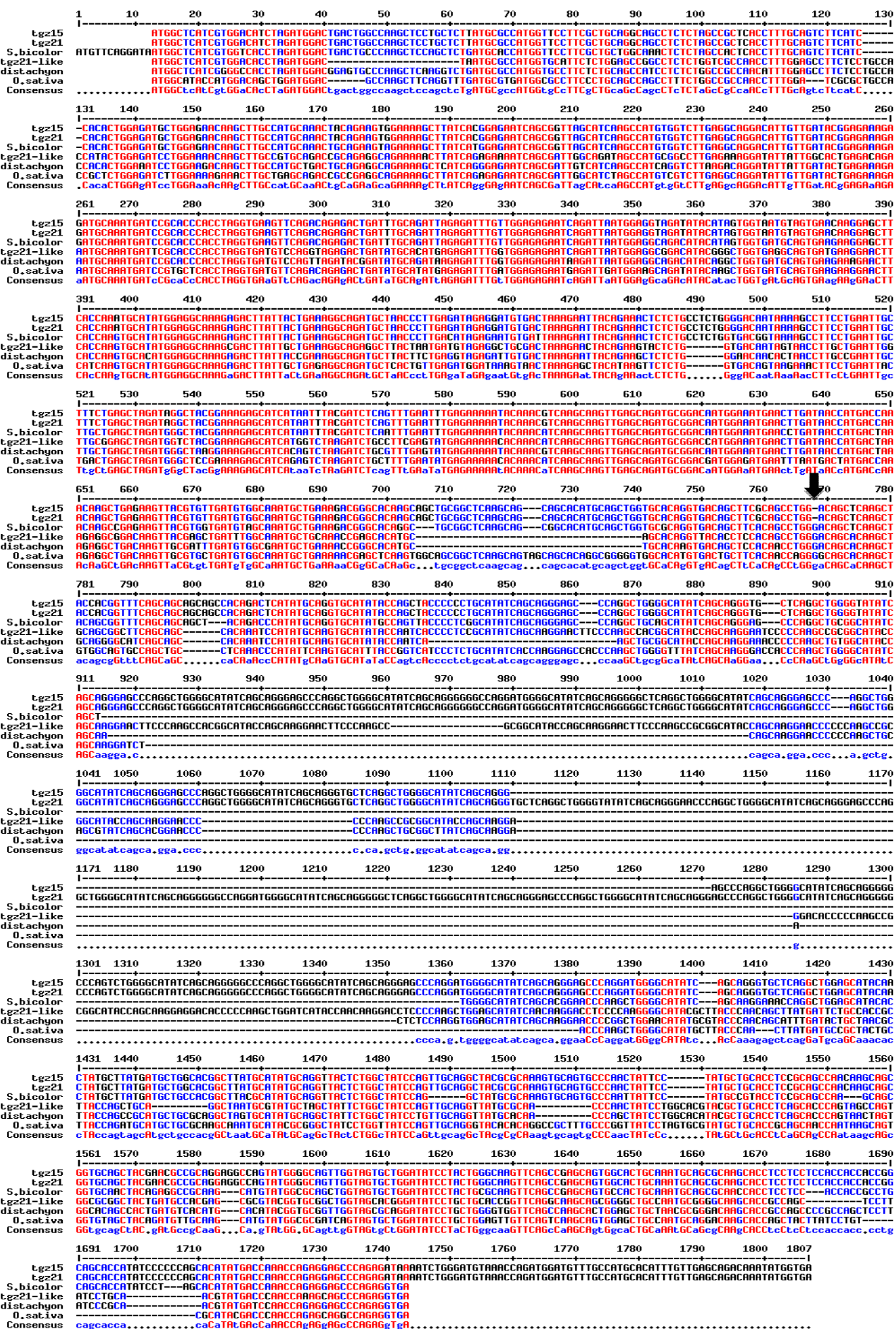


Figure S6. Alignment of TGZ15 and TGZ21 115-nt fragments (704:818) with maize DNA sequences. Sequences were retrieved by blastn search with the query cDNA fragment in the MaizeGDB database. AJ421525 and AJ488103 are TGZ15 and TGZ21 database records, respectively. chr2 is genomic DNA of maize chromosome 2.

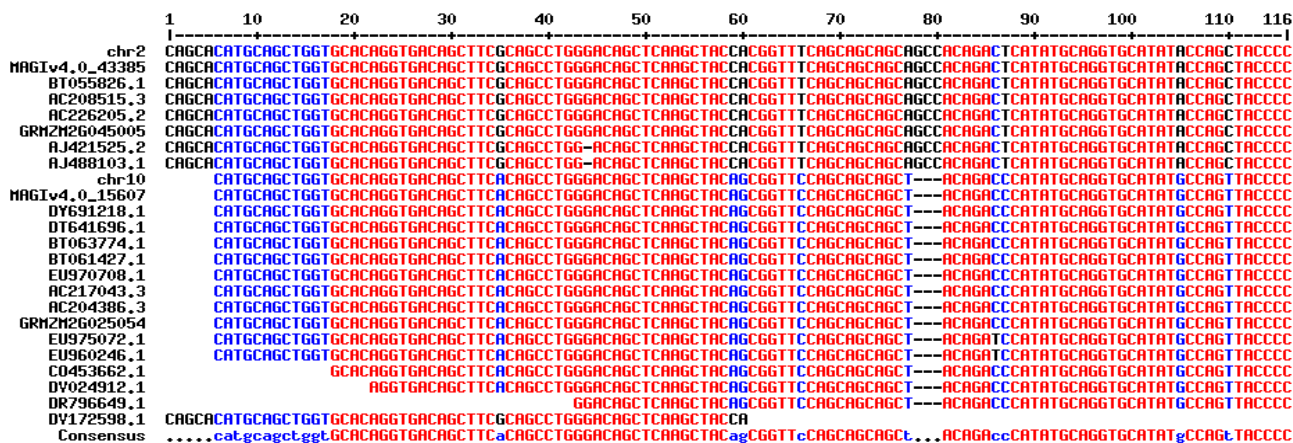
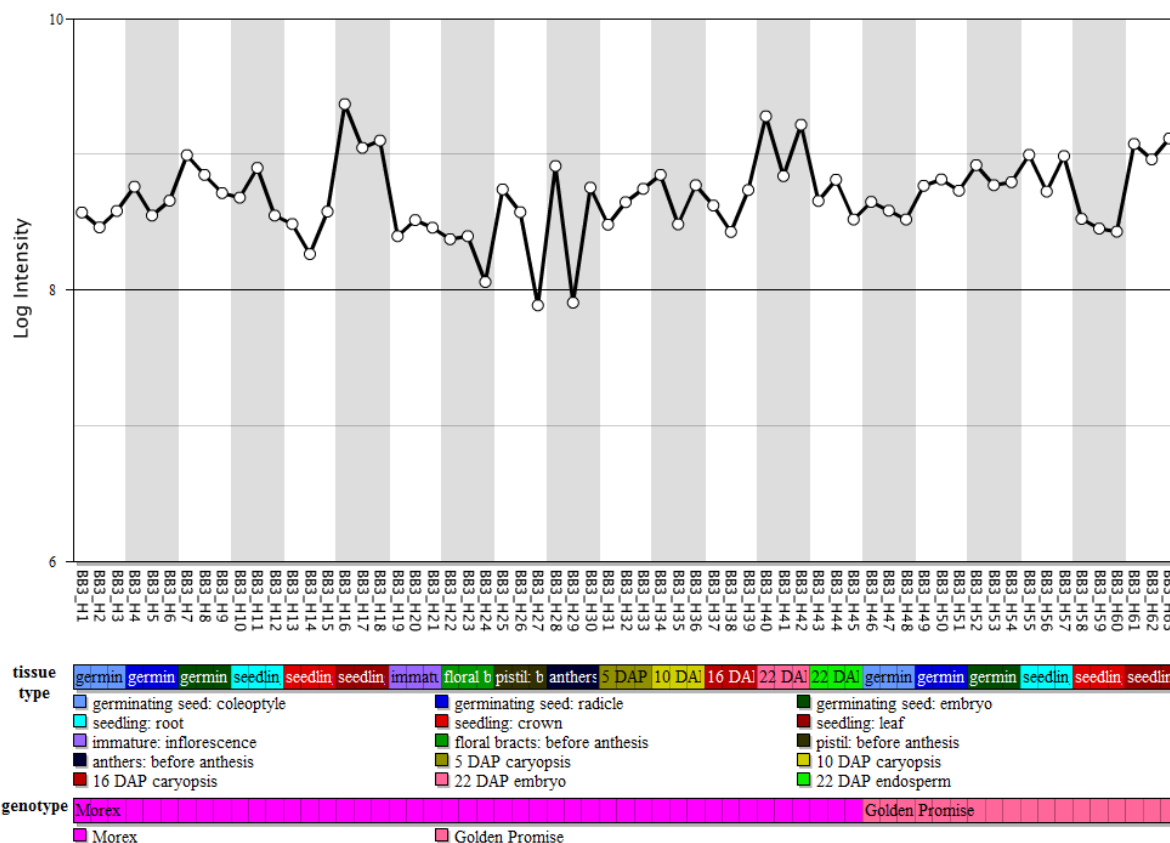


Figure S7. Expression profile of *HvPng1-like* during barley development. A. Comparison of 15 different barley tissue types representing barley developmental stages in two barley genotypes, Morex and Golden Promise. All data were generated in one experiment involving total 63 GeneChip hybridizations (Druka et al. 2006). B. General expression profile during barley development generated by meta-analysis of 1795 GeneChip hybridizations from multiple experiments.

A.



B.

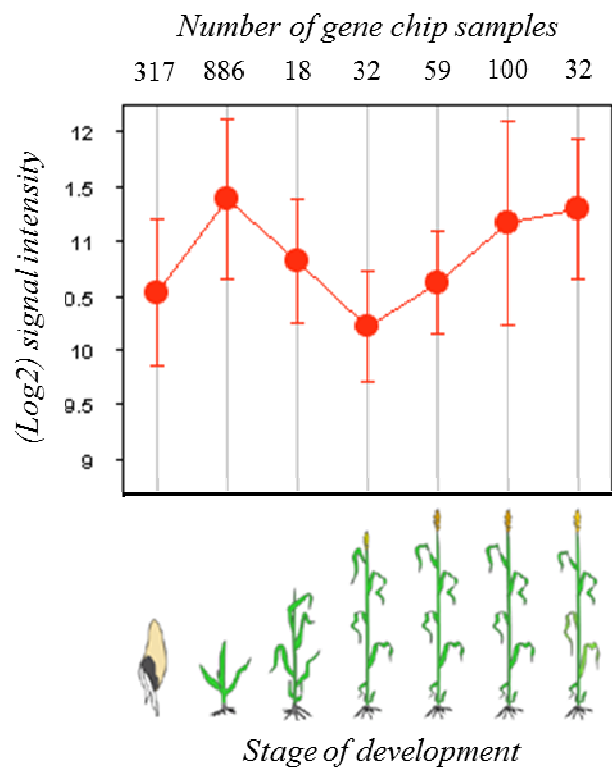


Figure S8. Visual representation of variation for HvPng1-like transcript levels during barley leaves senescence, measured relative to day 0. Data collected from 4 biological replicates, each replicate being a pool of about 30 plants. Whisker-box plots were generated with REST 2009 software.

