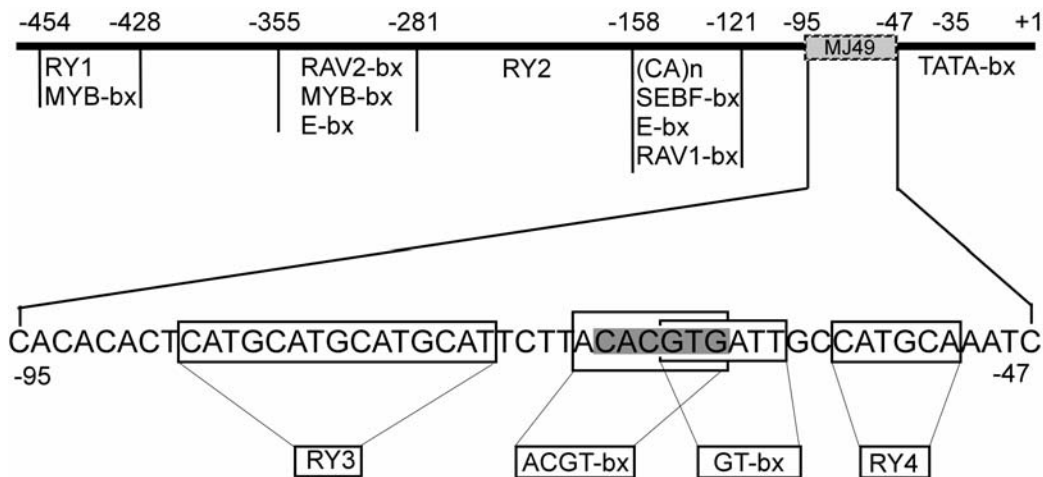


Supplemental Data Gao et al., 2009 Repression of Seed Maturation Genes by a Trihelix Transcriptional Repressor in Arabidopsis Seedlings.



**Supplemental Figure 1. Regulatory motifs in the 2S3 promoter.**

*Cis*-acting elements important for the regulation of seed storage proteins were defined using the PLACE program (Higo et al., 1999). The sequence of the 49-bp region (MJ49) used as bait in yeast one-hybrid screening is indicated in the lower panel. The G-box (CACGTG) is shaded and other *cis*-elements are boxed. Numbers indicate nucleotide positions relative to the transcription initiation site (+1) as defined by the 5' UTR represented in the TAIR database.

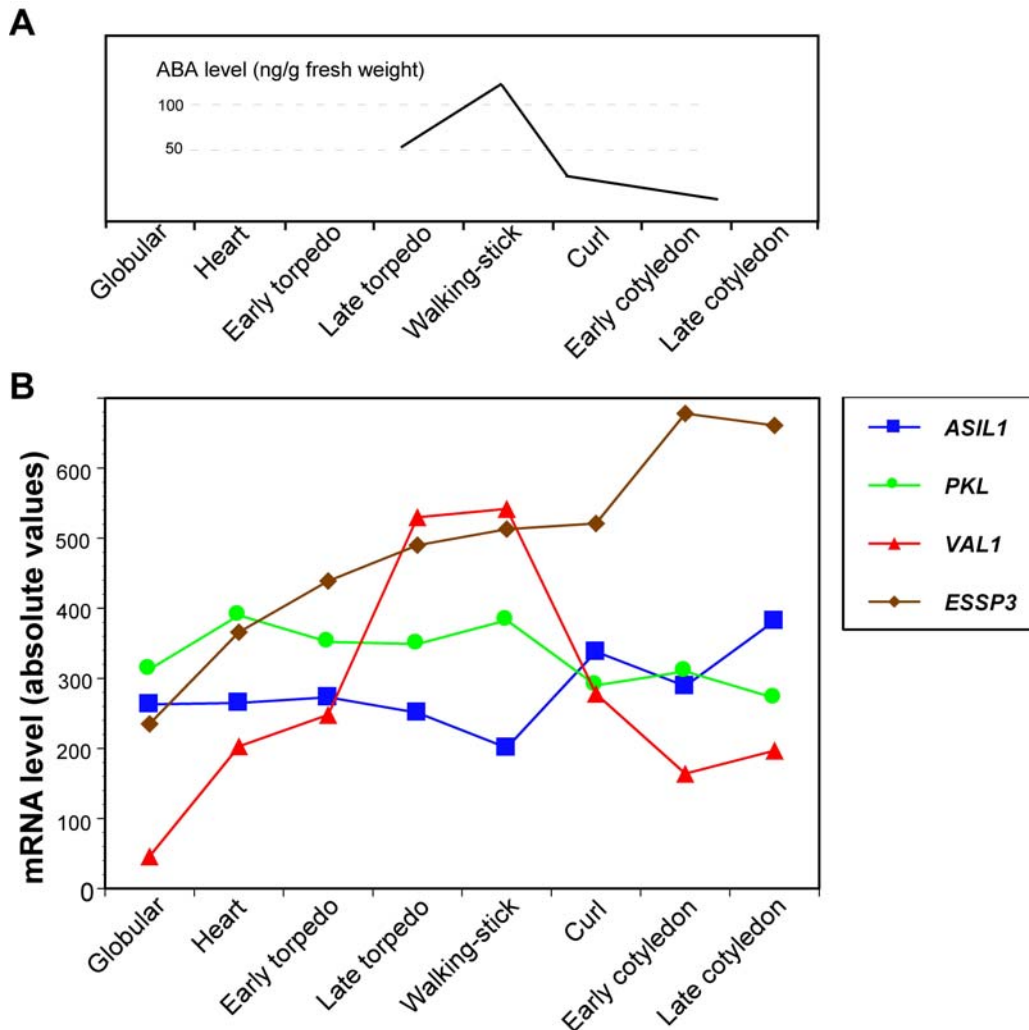
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ASIL1 (1) MEDDDEIQSIPSPGDSLSLSPQAPPSP-FILPTNDVTVAIVKKPQPGLSSQSPSMNA
SIP1 (1) MSDDDLVAS-PSSNNNSSS--LPPSPSPPPSTNTELAIP--P-P-----P-----
-----
Gly-rich
ASIL1 (56) LALVVHTPSVTGGGSGNRNRRGGGGGSGGGGCRDDCWSEEAATKVLIEAWGDRFS
SIP1 (41) -----PTIQP---ASTR-----PAAFPAREDCWSEAAHTLVEAWGSHYL
-----
Helix 1
*****
ASIL1 (112) EPGKGTILKQQHWEVAEIVNKS--QCKYPKTDIOCKNRIDTVKKKYKQEKAKIAS
SIP1 (78) ELKRCNLRQKYWQEVANAVNALHGHTKKQYRTDIOCKNRIDTLKKKYKIEKARVSQ
-----
Helix 2 Helix 3
ASIL1 (166) GDG--PSKWVFFKKLESIGGTTTFIASKASEKAPMGGALGNSRSSMFKRQTKGN
SIP1 (134) SHCRYVSPWPFNGLDDLIG--DNFKSS-----PAPVTVAPRRKTPPMLLPLPSAV
-----
*****
ASIL1 (220) QIVQQQEQKRGSDSMRWHFRKR--SASETESESDPEPEASPEESAESLPPLOPIQP
SIP1 (183) PVGPRS--KRPAASMEDTVSRRNFSAMAAAAAASEESDEEESE-----TSSP
-----
ASIL1 (274) LSFHMPKRLKVDKSCGGGSGVGDVARAILGFTIAYEKAETAKLKLMAELEKERMKF
SIP1 (231) AAITLAGARKEE-SCALAECCSRLEAIGRFABIYERVEDAKQROMVELEKQRMQF
-----
ASIL1 (330) AKEMELQRMQ-FLKTQLEITQNNQEEERSRQRCGERRIVDDDDDRNGKNNGNVSS
SIP1 (286) AKDLEIQRMKLIMESQVQLEKLRKAKSN-S-QAGE-----
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**Supplemental Figure 2. Alignment of the amino acid sequences of ASIL1 and SIP1.**

ASIL1 amino acid sequence aligned with SIP1 using the AlignX program, part of the Vector NTI suite with default settings of parameters. The putative nuclear localization signals are indicated by asterisks. The regions rich in proline and hydrophobic residues (dash), triple helix domain (bold) and the conserved C-terminal  $\alpha$ -helical domain (double underlined) are also indicated. Identical amino acids in the two proteins are shown as white letters on a black background. Amino acids with weak similarity are indicated as black letters on a grey background. Amino acids with no similarity are shown as black letters on a white background.

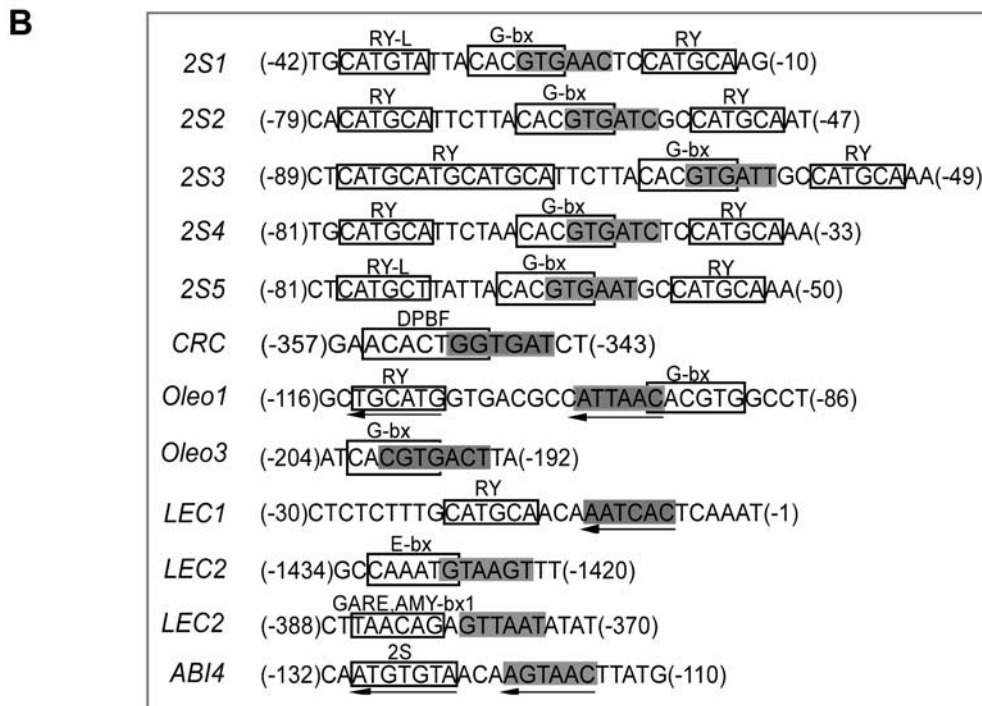


**Supplemental Figure 3. Expression profiles of *ASIL1* and known embryonic gene repressors *PKL*, *VAL1* and *ESSP3* in developing embryos corresponding to eight developmental stages.**

(**A**) ABA levels in developing seeds (Karssen et al., 1983; Vicente-Carbajosa and Carbonero, 2005); (**B**) Accumulation of *ASIL1*, *PKL*, *VAL1* and *ESSP3* mRNA in developing embryos. Affymetrix 22k microarray data from AtGenExpress (AtGE Development) (<http://jasp.weigelworld.org/expviz/expviz.jsp>; Schmid et al., 2005).

**A**

		Rice	Pea	Soybean	Bean
		GT2	GT1	GT2	SBF1
	* * * *				
ASIL1-bx	---TACACG <b>GTGATT</b> GCC----				
GT3a-bx	-----ATT <b>GTTACCA</b> T-----				
Rice GT1-bx	----CTAG <b>GTTAATT</b> TAT----	(+)			
Rice GT2-bx	---TGGCG <b>GTAATT</b> AACTG--	+		+	
Rice GT3-bx	---TCGAG <b>GTAAT</b> CCGCG--	+		+	
Soybean GT2-bx	CATCTACAG <b>TTACT</b> AGCTAGT			+	
Pea GT1-bxII	---GTGTG <b>GTTAAT</b> ATG----		+		
Pea GT1-bxII*	----GTGAG <b>GTAAT</b> AATCC---		+		
Pea GT1-bxII**	----TATGG <b>GTAACA</b> TTT---		+		
Pea GT1-bxIII	----TAGT <b>GAAA</b> ATGATA---		+		
Pea GT1-bxIII*	----GAGT <b>GTAAT</b> GTGT---		+		
Pea GT1-bxIII**	-----TT <b>GTAAG</b> TAACAG-		+		
Bean SBF-bx1	---TAAAA <b>GTTAAA</b> AC----				+
Bean SBF-bx2	-----CT <b>GTAAT</b> TAAATAT-				(+)
Bean SBF-bx3	---TATTG <b>GTTACT</b> AAA----				+



**Supplemental Figure 4. Putative ASIL1-binding sites and their close association with *cis*-acting elements required for seed-specific expression.**

(A) Sequence comparison of the *cis* elements of trihelix family proteins and their different binding abilities. Strong and weak binding affinities are indicated as + and (+), respectively; (B) Putative ASIL1-binding sites in the promoter regions of genes negatively regulated by ASIL1 as assayed by RT-PCR and microarray analysis. Putative ASIL1-binding DNA motifs are shaded and the previously identified positive seed-specific *cis*-elements analyzed using the PLACE program (Higo et al., 1999) are shown in rectangles. Arrows show when the correspondent sequence elements are on complementary strands.

**Supplemental Table 3. Sequences of the oligonucleotides used in this study.**

Gene	AGI code	Oligos	Primers used for PCR and RT-PCR
<i>T-DNA</i>		LBb1	5'-GCGTGGACCGCTTGCTGCA-3'
<i>18S rRNA</i>	At3g41768	sense	5'-TCCTAGTAAGCGCGAGTCATCA-3'
		antisense	5'-AAGAACGGCCATGCACCACCAC-3'
<i>ASIL1</i>	At1g54060	sil-f1	5'-TCCATCTCCGGGAGATTCTTCCCTTTCAC-3'
		sil-f2	5'-AGCTTGAGAGTTTGATTGGTGG-3'
		sil-f3	5'-GCAGTCGACATGGAGGACGACGACGAGATTC-3'
		sil-f4	5'-CGCTGCAG ATGGAGGACG ACGACGAGAT-3'
		sil-f5	5'-CGGCATGCGGTTTGAATTTTCACT-3'
		sil-r1	5'-AGAACCTTTGTAGCTTCTTCGC-3'
		sil-r2	5'-CTATTCCAAGAGCTCCTCCCATAG-3'
		sil-r3	5'-AGCAGTTTCCGCCTTCTATAAGCTTCCG-3'
		sil-r4	5'-ATCGCTCGAGTCAGCTACTTACATTGCCGTTATTC-3'
		sil-r5	5'-CG TCT AGA GCT ACTT ACATTGCCGT-3'
		Gene	AGI code
<i>2S3</i>	At4g27160	sense	5'-AGCAAAACATGGCTAACAAGCTCT-3'
		antisense	5'-CTGGCATCTCTGTCTTGGACCT-3'
<i>ABI3</i>	At3g24650	sense	5'-GGAAACTGTGACGACTCTTCTGGT-3'
		antisense	5'-CTGAGGTGTCAAAGAACTCGTTGCT-3'
<i>ABI4</i>	At2g40220	sense	5'-GGGCAGGAACAAGGAGGAAGTG-3'
		antisense	5'-TCTCCTCCAAAAGGCCAAATGGT-3'
<i>ABI5</i>	At2g36270	sense	5'-ATGATCAAGAACCGCGAGTCTGC-3'
		antisense	5'-CGGTTGTGCCCTTGACTTCAAAC-3'
<i>CRC</i>	At4g28520	sense	5'-TTGACGTTTCAGTTGGCTCAGCAG-3'
		antisense	5'-TGCAGATAGTCTCCTCAAGGCCG-3'
<i>Em1</i>	At3g51810	sense	5'-GAGGCGAGGAAGGAGCAGTTAG-3'
		antisense	5'-TCCATCGTACTGAGTCCTCCTTTAC-3'
<i>FUS3</i>	At3g26790	sense	5'-CTCCGACGTATGATACTCCCGAAG-3'
		antisense	5'-CGCCTGTGTTTTCTAGCACGTACATT-3'
<i>LEC1</i>	At1g21970	sense	5'-ACCAGCTCAGTCGTAGTAGCC-3'
		antisense	5'-GTGAGACGGTAAGGTTTTACGCATGAT-3'
<i>LEC2</i>	At1g28300	sense	5'-ACGCAAACCTCTGTCCAAGAAGCTC-3'
		antisense	5'-CCATCTGCTCCACCGGGTAT-3'
<i>Oleo2</i>	At5g40420	sense	5'-GTTACGGTGGTGGCGTTACAA-3'
		antisense	5'-CAACCATTAAGCCGATCACCGA-3'
<i>RAB18</i>	At5g66400	sense	5'-CATGGCGTCTTACCAGAACCGTC-3'
		antisense	5'-TCCTCCCATCGGATTTCCGTAAT-3'
<i>ASIL1</i>	At1g54060	sense	5'-AGAGATGGAGTTGCAGAGAATGCAG-3'
		antisense	5'-TGCCGTTATTCTTGCCATTGCG-3'
<i>ACT2</i>	At3g18780	sense	5'-CTGTTGACTACGAGCAGGAGATGGA-3'
		antisense	5'-GACTTCTGGGCATCTGAATCTCTCA-3'
<i>18S rRNA</i>	At3g41768	sense	5'-TCCTAGTAAGCGCGAGTCATCA-3'
		antisense	5'-CGAACACTTCACCGGATCAT-3'
<i>Ef-1α</i>	At1g07920	sense	5'-TGAGCACGCTCTTCTTGCTTTCA-3'
		antisense	5'-GGTGGTGGCATCCAT^CTTGTTACA-3'
<i>UBQ10</i>	At5g05320	sense	5'-GGCCTTGATAATCCCTGATGAATAAG-3'
		antisense	5'-AAAGAGATAACAGGAACGGAAACATAGT-3'