



Supplementary Information for

A shared *cis*-regulatory module activates transcription in the suspensor of plant embryos

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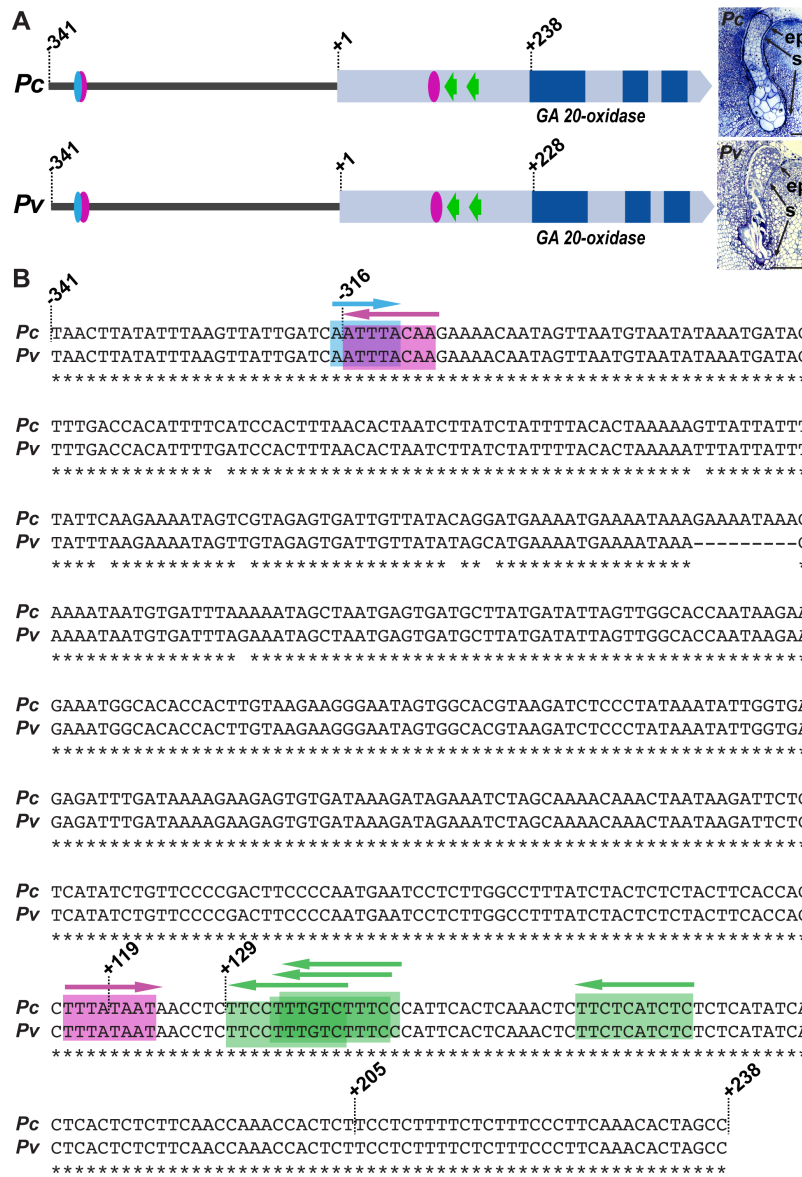


Fig. S1. Conservation of suspensor *cis*-regulatory elements in the upstream region of *GA 20-oxidase* in SRB and Common Bean. (A) Conceptual representation of SRB and Common Bean *GA 20-oxidase* genes and location of functional suspensor *cis*-regulatory motifs. Green arrows, purple ovals and blue ovals indicate the 10-bp motif, Region 2 motif and Fifth motif, respectively. Dark blue boxes represent exons. Light blue boxes represent UTRs and introns. Numbers indicate nucleotide positions relative to the SRB transcription start site (+1). Suspensor images were reproduced from Fig. 1B and D. (B) Nucleotide sequence alignment of the SRB and Common Bean *GA 20-oxidase* upstream regions. Nucleotides conserved in SRB and Common Bean are indicated by asterisks. Green, purple and blue boxes indicate the 10-bp motif, Region 2 motif and Fifth motif, respectively. Arrows indicate the orientation of the motifs. ep, embryo proper; Pc, *Phaseolus coccineus*; Pv, *Phaseolus vulgaris*; s, suspensor. (Scale bar: 50 μ m.)

Table S1. GA biosynthesis enzyme mRNA prevalence in the globular-stage suspensor and embryo proper. mRNA prevalence for each GA biosynthesis gene in SRB and Common Bean suspensor and embryo proper regions. RNA-Seq data were taken from GEO accession GSE57537. Numbers indicate average reads per kilobase per million (RPKM) of two biological replicates.

Enzyme	<i>Phaseolus vulgaris</i> Gene ID	SRB RPKM		Common Bean RPKM	
		Suspensor	Embryo Proper	Suspensor	Embryo Proper
<i>ent-kaurene synthase A</i>	Phvul.001G152100	1,934	234	1,097	109
<i>ent-kaurene synthase B</i>	Phvul.005G048500	501	27	671	28
<i>ent-kaurene oxidase</i>	Phvul.005G183600	3,783	333	3,645	163
<i>ent-kaurenoic acid hydroxylase</i>	Phvul.006G123500	26,915	1,220	24,713	932
<i>GA 20-oxidase</i>	Phvul.010G087500	34,805	517	31,799	473
<i>GA 3-oxidase</i>	Phvul.009G097100	3,265	56	3,757	37
<i>GA 2-oxidase</i>	Phvul.005G061000	0	0	21	10
<i>GA 2-oxidase</i>	Phvul.005G061200	0	0	3	0
<i>GA 2-oxidase</i>	Phvul.006G165000	0	7	7	7

Table S2. Probes used for SRB *in situ* hybridization. Probes were generated from cDNA clones made from micro-dissected suspensor and embryo proper regions of globular-stage SRB embryos 6 DAP (1). SRB EST sequences were compared to the *Phaseolus vulgaris* protein database (<http://phytozome.jgi.doe.gov>) using BLASTX, and the top *Phaseolus vulgaris* gene ID is displayed for each SRB EST (2).

Enzyme	GenBank Accession Number	SRB EST Length	<i>Phaseolus vulgaris</i> Gene ID
<i>ent-kaurene synthase A</i>	CA914587	415	Phvul.001G152100
<i>ent-kaurene synthase B</i>	CA899647	526	Phvul.005G048500
<i>ent-kaurene oxidase</i>	CA914551	466	Phvul.005G183600
<i>ent-kaurenoic acid hydroxylase</i>	CA914608	257	Phvul.006G123500
<i>GA 20-oxidase</i>	CA914823	582	Phvul.010G087500
<i>GA 3-oxidase</i>	CA915026	733	Phvul.009G097100

Table S3. Oligonucleotide sequences for generating constructs. Underlined nucleotides are incorporated restriction sites. Lower case nucleotides are mutated relative to the *GA 20-oxidase* upstream sequence.

Construct	Forward Oligonucleotides (5' to 3')	Reverse Oligonucleotides (5' to 3')
D-4509	GTGACGTC <u>CCCCGGG</u> CTGCAGGAATTCG	GAGGGATCAACATGCTAGTGTTTG
D-2000	TCATGAATTCATTTATTTTATTCACAAGAAAGCA	CCAGTTGCAACCACCTGT
D-1500	ATTGAATCTGTAGCTCTAGGTAGATCTTCC	CCAGTTGCAACCACCTGT
D-925	GTTGGAATCTTATACTAATAATTAGATACTATTTTAA	CCAGTTGCAACCACCTGT
D-750	AATAGAATTCGATTAGAGCCTTTCATTGTATAAAG	CCAGTTGCAACCACCTGT
D-600	ATTGAATCTGTCTAGTGGTAGTGAAATAATGAG	CCAGTTGCAACCACCTGT
D-450	AAATGAATTCGAAAAAGAAACCTTAAAAAGAATT	CCAGTTGCAACCACCTGT
D-275	AAATGAATTCCTTGACCACATTTCAICCCAC	CCAGTTGCAACCACCTGT
GUS-only	GTTGGAATTCATGGTCCGCTCTGTAGAAA	CCAGTTGCAACCACCTGT
GOF1	AAATGAATTCGAAAAAGAAACCTTAAAAAGAATT	GATCCCCGGGTACCGAGCTCGGGCTAGTGTTTGAAGGGAA
GOF2	GCTGGAATTCATCGTCTATATATGCTAATAACTTATATT	GATCCCCGGGTACCGAGCTCGGGCTAGTGTTTGAAGGGAA
GOF3	GCTGGAATTCCTAACTTATATTTAAGTTATTGATCAATTTAC	GATCCCCGGGTACCGAGCTCGGGCTAGTGTTTGAAGGGAA
GOF4	GCTGGAATTCATTTACAAGAAAACAATAGTTAATGTA	GATCCCCGGGTACCGAGCTCGGGCTAGTGTTTGAAGGGAA
GOF5	AAATGAATTCCTTGACCACATTTTCATCCAC	GATCCCCGGGTACCGAGCTCGGGCTAGTGTTTGAAGGGAA
GOF6	AAATGAATTCGAAAAAGAAACCTTAAAAAGAATT	GATCCCCGGGTACCGAGCTCGTAAAGGTGGTGAAGTAGAGAGTAG
GOF7	AAATGAATTCGAAAAAGAAACCTTAAAAAGAATT	GATCCCCGGGTACCGAGCTCGTCTTTTATCAAATCTCTACCA
GOF8	GCTGGAATTCATCGTCTATATATGCTAATAACTTATATT	GATCCCCGGGTACCGAGCTCGGAGGTTATTATAAAGGTGGTGAA
GOF9	GCTGGAATTCATCGTCTATATATGCTAATAACTTATATT	GATCCCCGGGTACCGAGCTCGAGAGTGGTGGTTGAAGAGA
M1	GCTGGAATTCATCGTCTATATATGCTAATAACTTATATT	GGTTCggcgcccGGTGGTGAAGTAGAGAGTAGATAAA
M1	CACCgggcccgcgAACCTCTTCCTTTGTCTTTCC	GATCCCCGGGTACCGAGCTCGGGCTAGTGTTTGAAGGGAA
M2	GCTGGAATTCATCGTCTATATATGCTAATAACTTATATT	TGAGTGATATGAGAtctctctctcGAGTTTGAGTGAATGitecctcacctctcGAGGTTATTATAAAGGTGGTGAA
M2	TCTCATATCACTCACTCTCTTCAACCAACCACCTCTAaaaaaaaaaaaaaaaaaaCTTCAACACTAGCCCCGAG	GATCCCCGGGTACCGAGCTCGGGCTAGTGTTTGAAGTTTTTTTTTTTTAGAGTGGTTGGTTGAAGAGA
M3	GCTGGAATTCATCGTCTATATATGCTAATAACTTATATT	GATCCCCGGGTACCGAGCTCGGGCTAGTGTTTGAAGTTTTTTTTTTTTAGAGTGGTTGGTTGAAGAGA
M5	GCTGGAATTCATCGTCTATATATGCTAAGccaggATATTTCctggcTTGATCAATTTACAAGAAAAACAA	GATCCCCGGGTaccgagctcgGGCTAGTGTTTGAAGGGAA
M6	GCTGGAATTCATCGTCTATATATGCTAaaaaaaATATTTaaaaaaTTGATCAATTTACAAGAAAAACAA	GATCCCCGGGTaccgagctcgGGCTAGTGTTTGAAGGGAA
M7	GCTGGAATTCATCGTCTATATATGCTAAGccaggATATTTCctggcTTGATCAattataaaGAAAAACAATAGTTAATGTAATATAATG	GATCCCCGGGTaccgagctcgGGCTAGTGTTTGAAGGGAA
M8	GCTGGAATTCATCGTCTATATATGCTAATAACTTATATTTAAGTTAATGATCAATTTAATAAG	GATCCCCGGGTaccgagctcgGGCTAGTGTTTGAAGGGAA

References

1. Weterings K, *et al.* (2001) Regional localization of suspensor mRNAs during early embryo development. *The Plant cell* 13(11):2409-2425.
2. Altschul SF, *et al.* (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic acids research* 25(17):3389-3402.