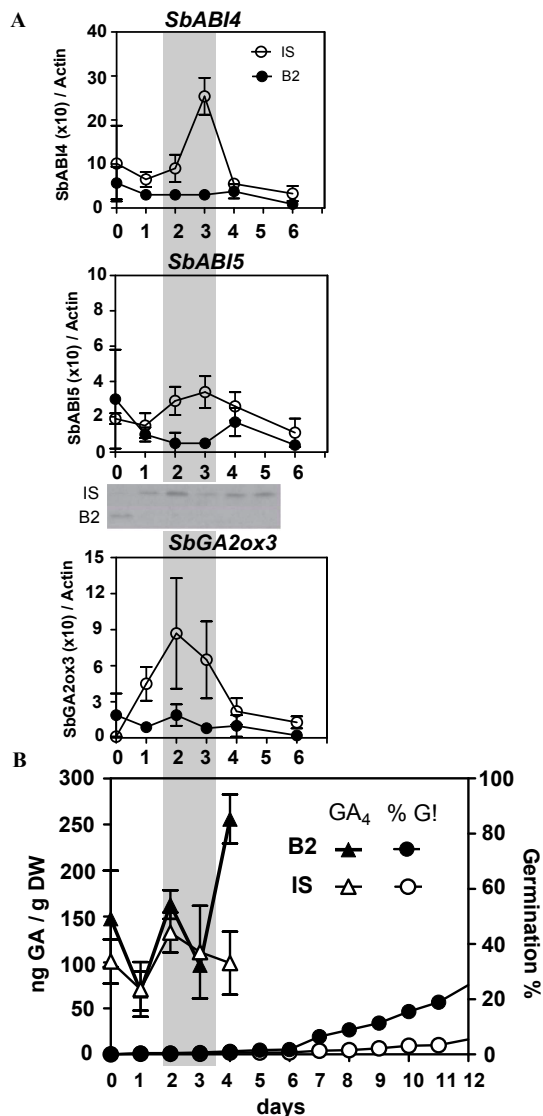


## Supplementary Data

*In vitro* binding of *Sorghum bicolor* transcription factors ABI4 and ABI5 to a conserved region of a *GA 2-OXIDASE* promoter: possible role of this interaction in the expression of seed dormancy

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Figure S1



Adapted from Rodríguez *et al.*, 2012; 2009.

**Figure S1.** A, Embryonic transcript levels for *SbABI4*, *SbABI5* and *SbGA2ox3* genes during 20°C incubation of immature grains for RedlandB2 (sprouting susceptible, full symbols) and IS9530 (sprouting resistant, empty symbols). Bars indicate the SEM. *SbABI5* protein accumulation (detected by western blot) during grain incubation, are shown below *SbABI5* transcript levels for both lines. B, Evolution of germination percentage (circles) and embryonic content of GA<sub>4</sub> (triangles) during incubation time for RedlandB2 (full symbols) and IS9530 (empty symbols) immature seeds, incubated at 20°C. The grey box indicates temporal coincidence for all components. Results taken from Rodríguez *et al.* (2012; 2009).

## Figure S2

### *SbGA2ox3* probe (242 bp)

GGGCGCCGTGGGAAAACCTGCTATTGGCCGGCGTG  
CCATCGCCTCGTATCGTATGCGTCTCCCATGGAGC  
CATGCATGCAGCGCGGCGGGCCGACCTGCCACCG  
GTGGATCGCGTCCGGACACGTCGCGCCCCGGACC  
CCGACTGCCCTGGTCCATCGGCACCGTGCACCC  
AAAGCGATCCCACCCCGATGCATCCTTTTCGTCT  
CCCTGTCAGCTGGGTCCCATCCAGCCAGGTGCCG  
CC

### *SbGAMyb* probe (183 bp)

TCTGGGAAGAACCAACAACCATCGGTCCGAAGTT  
CAAGTTCTTCTGCTGGCACACCTTGTGAGACTAC  
TACGGTGGTTAGCCCAGAGTTTGATATGGGCCAG  
GAATATTGGGAAGAACAGCCTGGTTCTTTCCTCA  
GTGAATATGCTCATTTTAGTGAAATTCTTTGACC  
GAATCCACTCCT

### *AtEm6* promoter probe (186 bp)

AGTTAAAGAACACGCGGCGAAGAAGTACGGCCA  
CTACCACGTTCAAAGTATACGTCAGCTAATAAAG  
TCAGACACGTGGCATGTCACCAAGAAAGAAGAG  
CATACTGATGACGTACCGATTGTCCCTGAGTCAC  
CACGTCGAACATCCACGATGCAACACAACCAAA  
ACGCCCTCCGGATTGA

### *SbGA2ox3* probe a (131 bp; intact ABRE and CE)

GGGCGCCGTGGGAAAACCTGCTATTGGCCGGCGTG  
CCATCGCCTCGTATCGTATGCGTCTCCCATGGAGC  
CATGCATGCAGCGCGGCGGGCCGACCTGCCACCG  
GTGGATCGCGTCCGGACACGTCGCGCCC

### *SbGA2ox3* probe b (131 bp; mutated ABRE)

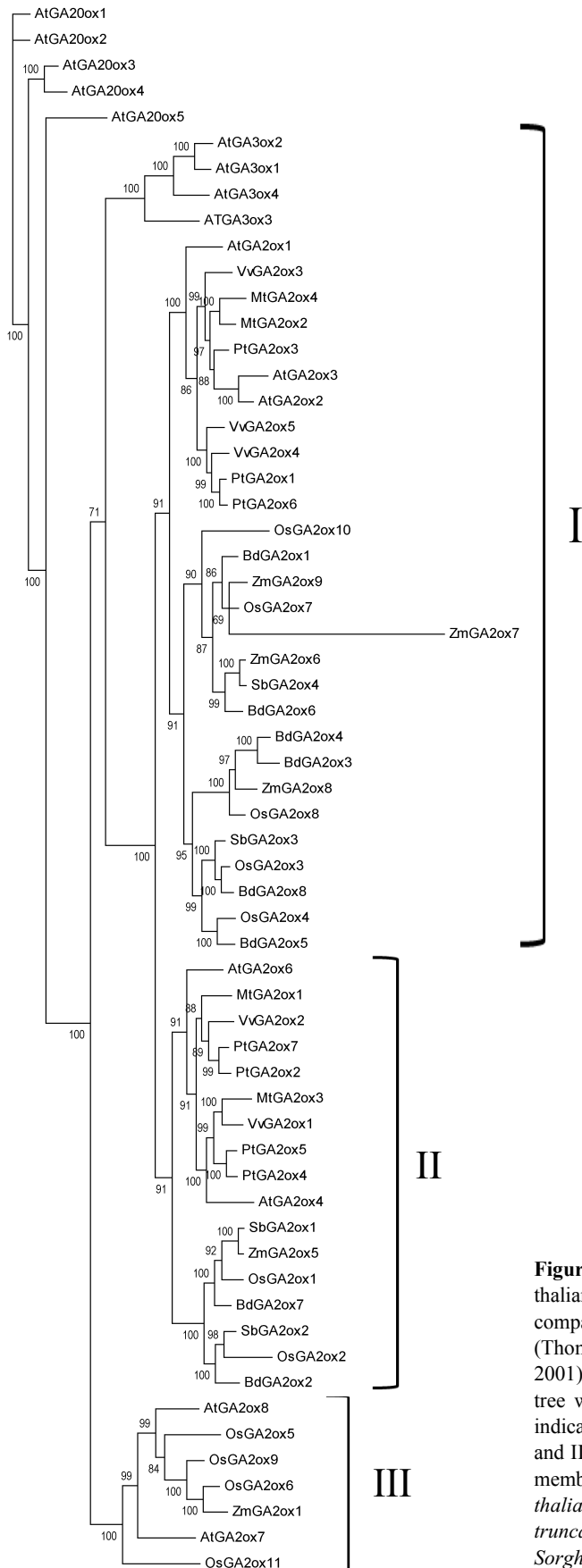
GGGCGCCGTGGGAAAACCTGCTATTGGCCGGCGTG  
CCATCGCCTCGTATCGTATGCGTCTCCCATGGAGC  
CATGCATGCAGCGCGGCGGGCCGACCTGCCACCG  
GTGGATCGCGTCCGGACAATTCGCGCCC

### *SbGA2ox3* probe c (114 bp; mutated CE and no ABRE included)

GGGCGCCGTGGGAAAACCTGCTATTGGCCGGCGTG  
CCATCGCCTCGTATCGTATGCGTCTCCCATGGAGC  
CATGCATGCAGCGCGGCGGGCCGACCTGCTCCG  
GTGGATCGCGT

**Figure S2.** Complete sequences of probes used in EMSA experiments. Mutated bases are highlighted in red in probes b and c.

**Figure S3**



**Figure S3.** Phylogenetic relationships for GA2oxs. *Arabidopsis thaliana* GA3oxs and GA2oxs were included for outgroup comparison. Full-length proteins were aligned using ClustalW (Thompson *et al.*, 1997). MrBayes (Huelsenbeck & Ronquist, 2001) was used to construct a Bayesian inference phylogenetic tree with 1,500,000 generations. Bootstrap support values are indicated. GA2oxs were divided into three different groups: I, II and III. Members of I and II groups catabolize C19-GAs, while members of III group catabolize C20-GAs. At: *Arabidopsis thaliana*; Bd: *Brachypodium distachyon*; Mt: *Medicago truncatula*; Os: *Oryza sativa*; Pt: *Populus trichocarpa*; Sb: *Sorghum bicolor*; Vv: *Vitis vinifera*; Zm: *Zea mays*.

## Figure S4

```
IS9530      -505  GGGCGCCGTGGGAAAAC TGCTATTGGCCGGCGTGCCATCGCCTCGTATCGTATGCGTCTC
RedlandB2  -505  GGGCGCCGTGGGAAAAC TGCTATTGGCCGGCGTGCCATCGCCTCGTATCGTATGCGTCTC
*****

IS 9530
RedlandB2  CCATGGAGCCATGCATGCAGCGCGGCGGGCCGACCTGCCACCGGTGGATCGCGTCCGGAC
CCATGGAGCCATGCATGCAGCGCGGCGGGCCGACCTGCCACCGGTGGATCGCGTCCGGAC
*****

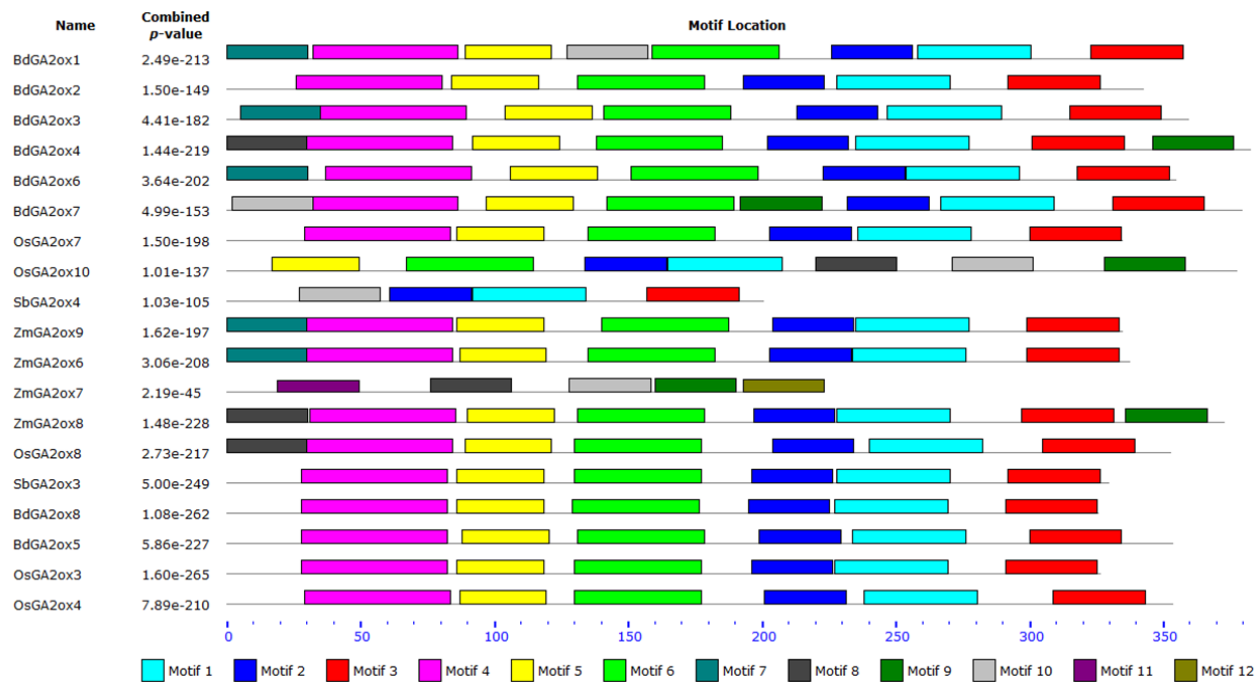
IS9530
RedlandB2  ACGTCGCGCCCCGGACCCCGACTGCCCTGGCTCCATCGGCACCGTGCACCCAAAGCGATC
ACGTCGCGCCCCGGACCCCGACTGCCCTGGCTCCATCGGCACCGTGCACCCAAAGCGATC
*****

IS9530
RedlandB2  CCACCCCGATGCATCCTTTTCGTCTCCCTGTCAGCTGGGTCCCATCCAGCCAGGTGCCG
CCACCCCGATGCATCCTTTTCGTCTCCCTGCCAGCTGGGTCCCATCCAGCCAGGTGCCG
*****

IS9530      CC   -263
RedlandB2   CC   -263
**
```

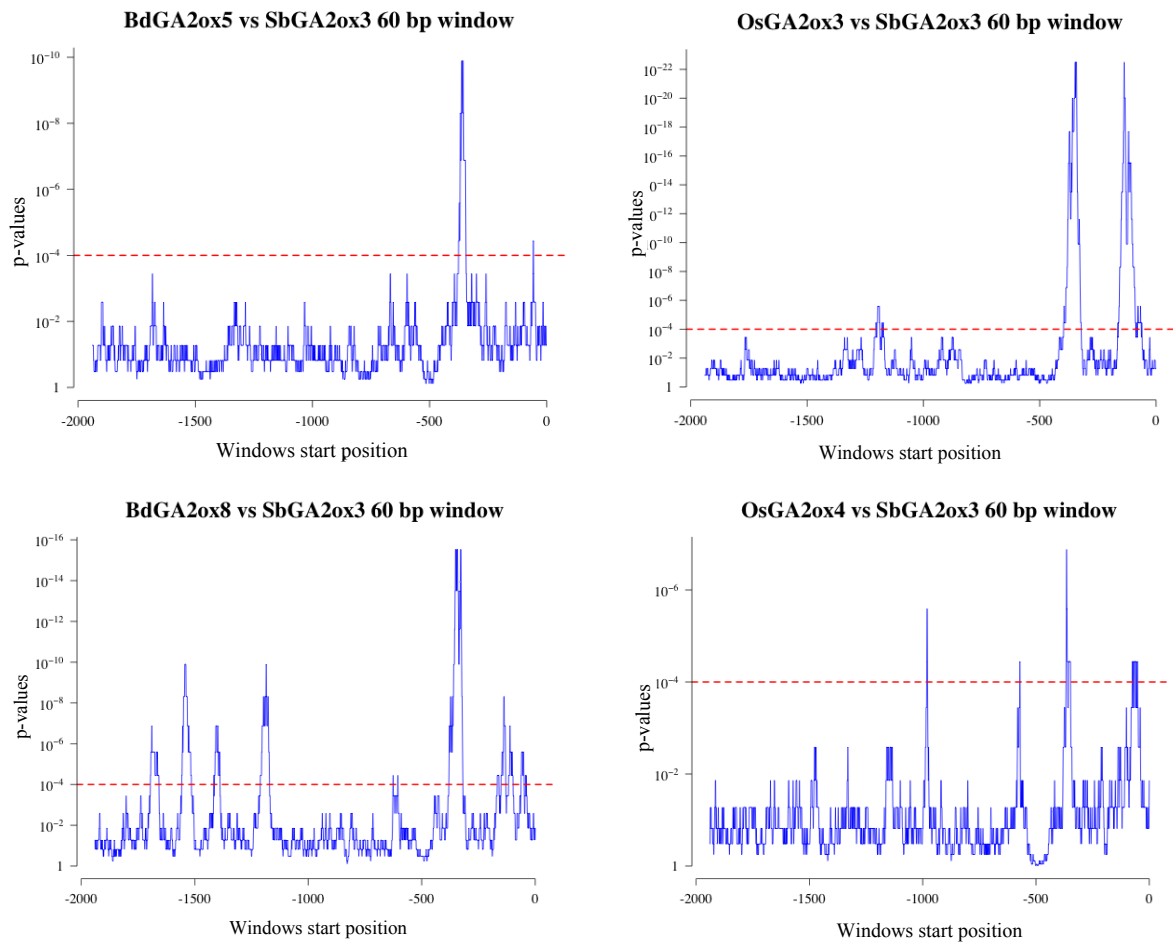
**Figure S4.** *GA2ox3* probe sequence alignment for *S. bicolor* genotypes IS9530 and RedlandB2. Sequences were completely aligned with Clustal W software (Thompson *et al.*, 1997).

**Figure S5**



**Figure S5.** Domain architecture of group I monocot GA2oxs proteins, showing the similarity in domain composition and location. SbGA2ox4 appears as a truncated version of SbGA2ox3.

**Figure S6**



**Figure S6.** Pair wise comparison plots between SbGA2ox3 and each M3 member promoter (OsGA2ox3, OsGA2ox4, BdGA2ox5 and BdGA2ox8) performed with EARS tool. Red dashed line indicates the selected cut off p-value (0.0001), suggesting the existence of significant peaks 1 and 2 in all the comparisons.

**Table S1.** GA2ox proteins sequence accessions, naming terminology and data base used for eight plant species.

Specie	ID code	GA2ox name	Database
<i>Arabidopsis thaliana</i>	ath:AT1G78440	GA2ox1	KEGG
	ath:AT1G30040	GA2ox2	
	ath:AT2G34555	GA2ox3	
	ath: AT1G47990	GA2ox4	
	ath: AT1G02400	GA2ox6	
	ath: AT1G50960	GA2ox7	
	ath:AT4G21200	GA2ox8	
	ath:AT1G15550	GA3ox1	
	ath:AT1G80340	GA3ox2	
	ath:AT4G21690	GA3ox3	
	ath:AT1G80330	GA3ox4	
	ath:AT4G25420	GA20ox1	
	ath:AT5G51810	GA20ox2	
	ath:AT5G07200	GA20ox3	
	ath:AT1G60980	GA20ox4	
ath:AT1G44090	GA20ox5		
<i>Brachypodium distachyon</i>	bdi:100826640	GA2ox1	KEGG
	bdi:100841118	GA2ox2	
	bdi:100835624	GA2ox3	
	bdi:100836239	GA2ox4	
	bdi:100831936	GA2ox5	
	bdi:100821200	GA2ox6	
	bdi:100842224	GA2ox7	
	bdi:100845998	GA2ox8	
<i>Medicago truncatula</i>	MTR_1g086550	GA2ox1	KEGG
	MTR_2g019370	GA2ox2	
	MTR_2g033270	GA2ox3	
	MTR_4g096840	GA2ox4	
<i>Oryza sativa</i>	LOC_Os05g06670	GA2ox1	Rice Genome
	LOC_Os01g22920.1	GA2ox2	
	LOC_Os01g55240.1	GA2ox3	
	LOC_Os05g43880.1	GA2ox4	
	LOC_Os07g01340.1	GA2ox5	
	LOC_Os04g44150.1	GA2ox6	
	LOC_Os01g11150.1	GA2ox7	
	LOC_Os05g48700.1	GA2ox8	
	LOC_Os02g41954.1	GA2ox9	
	LOC_Os05g11810.1	GA2ox10	
	LOC_Os04g33360.1	GA2ox11	
<i>Populus trichocarpa</i>	POPTR_550316	GA2ox1	KEGG
	POPTR_409194	GA2ox2	
	POPTR_758699	GA2ox3	
	POPTR_765885	GA2ox4	
	POPTR_723959	GA2ox5	



	POPTR_822902 POPTR_245496	GA2ox6 GA2ox7	
<i>Sorghum bicolor</i>	Sb09g004520 Sb03g013450 Sb03g035000 Sb09g006490	GA2ox1 GA2ox2 GA2ox3 GA2ox4	KEGG
<i>Vitis vinifera</i>	vvi:100258599 vvi:100248676 vvi:100244543 vvi:100264177 vvi:100248356	GA2ox1 GA2ox2 GA2ox3 GA2ox4 GA2ox5	KEGG
<i>Zea mays</i>	zma:100281860 zma:100285694 zma:100280480 zma:100272456 zma:100192514 zma:100284370	GA2ox1 GA2ox5 GA2ox6 GA2ox7 GA2ox8 GA2ox9	KEGG