Supplemental Data

Title: Sorghum DW1 positively regulates BR signaling by inhibiting the nuclear localization of BIN2

Authors: Ko Hirano, Mayuko Kawamura, Satoko Araki-Nakamura, Haruka Fujimoto, Kozue Ohmae-Shinohara, Miki Yamaguchi, Akihiro Fujii, Hiroaki Sasaki, Shigemitsu Kasuga, Takashi Sazuka

Supplementary figures: S1 to S8 Supplementary tables: S1 to S2



0.2

Fig. S1. Phylogenetic analysis of D2 homologs using the NJ method. SbD2 (Sobic.003G030600) and OsD2 (LOC_Os01g10040) are shown in red and blue, respectively. Bootstrap values were obtained by 100 bootstrap replicates.



0.1

Fig. S2. Phylogenetic analysis of BR6ox homologs using the NJ method. SbBR6ox (Sobic.001G172400) and OsBR6ox (LOC_Os03g40540) are shown in red and blue, respectively. Bootstrap values were obtained by 100 bootstrap replicates.



0.1

Fig. S3. Phylogenetic analysis of BRI1 homologs using the NJ method. SbBRI1 (Sobic.003G277900) and OsBRI1 (LOC_Os01g52050) are shown in red and blue, respectively. Bootstrap values were obtained by 100 bootstrap replicates.





Fig. S4. Phylogenetic analysis of BIN2 homologs using the NJ method. SbBIN2 (Sobic.003G24800), OsBIN2 (LOC_Os05g11730), and AtBIN2 (AT4G18710) are shown in red, blue, and green, respectively. Bootstrap values were obtained by 100 bootstrap replicates.



Fig. S5. Interaction of OsDW1-OsBIN2 in a Y2H assay. The growth of yeast strain AH109 transformants is shown. BIN2s were used as bait, and DW1s were used as prey. –TL, synthetic complete medium lacking Trp and Leu; –TLH A, synthetic complete medium lacking Trp, Leu, His, and Ade. OsDW1-01g and OsDW1-03g refer to LOC_Os01g01390 and LOC_Os03g16400, respectively.



Fig. S6. Transient expression of AtDW1, AtBIN2, and AtBZR1 in *Arabidopsis* protoplasts. (a) AtDW1 localizes on the plasma membrane and in the cytosol. (b) AtBIN2 localizes to the nucleus and the cytosol. (c) Simultaneous expression of AtDW1 and AtBIN2 inhibits nuclear localization of AtBIN2. (g) AtBZR1 localizes to the nucleus and the cytosol. (h) Simultaneous expression of AtBZR1 and AtBIN2 inhibits nuclear localization of AtBZR1. (i) AtDW1, AtBIN2, and AtBZR1 co-expression results in AtBZR1 localizing to the nucleus. *Arabidopsis* protoplasts were transformed with either AtBIN2-GFP, AtBZR1-GFP, AtDW1-GFP, and co-transformed with AtBIN2-FLAG, AtDW1-HA, or AtBIN2-FLAG/AtDW1-HA. (d-f, j-l) Chloroplast autofluorescence of *Arabidopsis* protoplasts are shown as the red channel.



Fig. S7. Phylogenetic analysis of DW1 homologs using the neighbor-joining method. Bootstrap values were obtained by 100 bootstrap replicates.



Fig. S8. Phylogenetic analysis of DW1 homologs using the maximum parsimony method. Bootstrap values were obtained by 100 bootstrap replicates.

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AT4G25620.1 5.80E-07 G. max Glyma.17G262500.1 3.60E-75 Glyma.01G220800.1 2.70E-73 Glyma.01G220800.1 1.80E-19 Glyma.11G022800.1 5.80E-19 Glyma.16G180600.1 3.00E-17 Glyma.05G209100.1 5.20E-17 Glyma.05G209100.1 5.20E-17 Glyma.08G015800.1 8.80E-11 S. lycopersicum Solyc12g095780.1.1 7.00E-56 Solyc03g025290.2.1 6.00E-20 P. persica Prupe.5G102900.1 1.20E-67 Prupe.5G102900.1 1.20E-67 Prupe.2G298300.1 4.30E-20 C. sinensis orange1.1g013739m 1.90E-74 orange1.1g012593m 3.80E-20 C. papaya evm.model.supercontig_36.87 1.10E-44 evm.model.supercontig_107.82 3.10E-20 S. purpurea SapurV1A.0776s0090.1 8.40E-73 SapurV1A.0998s0120.1 1.60E-17 SapurV1A.0998s0120.1 1.60E-17 SapurV1A.0998s0120.1 1.60E-17 SapurV1A.0790s08008.1 1.20E-67		AT5G52430.1	1.60E-16
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orange1.1g012593m 3.80E-20 C. papaya evm.model.supercontig_36.87 1.10E-44 evm.model.supercontig_214.15 1.30E-23 evm.model.supercontig_107.82 3.10E-20 S. purpurea SapurV1A.0776s0090.1 8.40E-73 SapurV1A.0554s0040.1 1.50E-67 SapurV1A.0862s0140.1 4.80E-20 SapurV1A.0098s0120.1 1.60E-17 SapurV1A.0790s0080.1 1.20E-15 P. trichocarpa Potri.002G001100.2 1.40E-72 Potri.012G144200.1 1.70E-20 Potri.001G102200.1 7.80E-18 Potri.003G129400.1 1.10E-16 gymnosperm MA_16066p0010 1.19E-53 MA_10429574p0010 1.65E-23		orange1.1g010808m	1.40E-22
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evm.model.supercontig_107.82 3.10E-20 S. purpurea SapurV1A.0776s0090.1 8.40E-73 SapurV1A.0554s0040.1 1.50E-67 SapurV1A.0862s0140.1 4.80E-20 SapurV1A.098s0120.1 1.60E-17 SapurV1A.0790s0080.1 1.20E-15 P. trichocarpa Potri.002G001100.2 1.40E-72 Potri.012G144200.1 1.70E-20 Potri.001G102200.1 7.80E-18 Potri.003G129400.1 1.10E-16		evm.model.supercontig_214.15	1.30E-23
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SapurV1A.0098s0120.1 1.60E-17 SapurV1A.0790s0080.1 1.20E-15 P. trichocarpa Potri.002G001100.2 1.40E-72 Potri.005G260000.1 4.40E-69 Potri.012G144200.1 1.70E-20 Potri.001G102200.1 7.80E-18 Potri.003G129400.1 1.10E-16		SapurV1A.0862s0140.1	4.80E-20
SapurV1A.0790s0080.1 1.20E-15 P. trichocarpa Potri.002G001100.2 1.40E-72 Potri.005G260000.1 4.40E-69 Potri.012G144200.1 1.70E-20 Potri.001G102200.1 7.80E-18 Potri.003G129400.1 1.10E-16 gymnosperm P. abies MA_16066p0010 MA_10429574p0010 1.65E-23		SapurV1A.0098s0120.1	1.60E-17
P. trichocarpa Potri.002G001100.2 1.40E-72 Potri.005G260000.1 4.40E-69 Potri.012G144200.1 1.70E-20 Potri.001G102200.1 7.80E-18 Potri.003G129400.1 1.10E-16 gymnosperm P. abies MA_16066p0010 1.19E-53 MA_10429574p0010 1.65E-23		SapurV1A.0790s0080.1	1.20E-15
Potri.005G260000.1 4.40E-69 Potri.012G144200.1 1.70E-20 Potri.001G102200.1 7.80E-18 Potri.003G129400.1 1.10E-16 gymnosperm P. abies MA_16066p0010 1.19E-53 MA_10429574p0010 1.65E-23	P. trichocarpa	Potri.002G001100.2	1.40E-72
Potri.012G144200.1 1.70E-20 Potri.001G102200.1 7.80E-18 Potri.003G129400.1 1.10E-16 gymnosperm P. abies MA_16066p0010 1.19E-53 MA_10429574p0010 1.65E-23		Potri.005G260000.1	4.40E-69
Potri.001G102200.1 7.80E-18 Potri.003G129400.1 1.10E-16 gymnosperm Image: Comparison of the system of the sy		Potri.012G144200.1	1.70E-20
Potri.003G129400.1 1.10E-16 gymnosperm Image: Constraint of the state of the stat		Potri.001G102200.1	7.80E-18
gymnosperm P. abies MA_16066p0010 1.19E-53 MA_10429574p0010 1.65E-23		Potri.003G129400.1	1.10E-16
gymnosperm P. abies MA_16066p0010 1.19E-53 MA_10429574p0010 1.65E-23			
P. abies MA_16066p0010 1.19E-53 MA_10429574p0010 1.65E-23	gymnosperm		
MA_10429574p0010 1.65E-23	P. abies	MA_16066p0010	1.19E-53
		MA_10429574p0010	1.65E-23

Supplemental Table 1. Dw1 homologs used to create phylogenetic trees.

lycophyte		
S. moellendorffii	Sm446486	5.40E-24
	Sm447750	1.20E-23

bryophyte

P. patens	Pp3c9_7640V3.1	3.40E-19
	Pp3c15_6950V3.1	1.40E-18

^{a.} tblastn was conducted using Sobic009G229800.1 (SbDW1) as query.

Supplemental Table 2. Details of primers used in this study

Primer name	Sequence (5' to 3')	Purpose
SbD2_RT_F	CTACGATGAGCCCTACAGGTTC	RT-PCR (Os01g0103800)
SbD2_RT_R	ACCAAGTGATGGAGAAAGATGG	
SbBR6ox_RT_F	TTATAGCGAGGATATAGGTATCATAGG	RT-PCR (Os03g0270700)
SbBR6ox_RT_R	TTCTGTTTAAATTTGAAAATTGATGG	
SbBRI1_RT_F	CTGCCCAGGGGTTAGTAGTG	RT-PCR
SbBRI1_RT_R	TGTACCATCAGAAGGGAATGC	
SbDW1_RT_F	TGCATTGAATTCGAGCTGTC	RT-PCR (SbDW1 BL treatment)
SbDW1_RT_R	ATTTCACTGCTCCTGCGAAC	
SbDW1_RT_F	GTCTTCAGTTGGGAGCAGCCCTGGCACCAG	RT-PCR (organ expression)
SbDW1_RT_R	ATTGGCACCAGATTGACCATTTCCACGAGC	
SbUbiquitin_RI_F		RT-PCR (sorghum ubiquitin)
SDDW1_AD_F	GUGGATUCATATGTUTUCAGTIGGG	Y2H
SDUV1_AD_R		X2LL
SUBINZ_BD_F		12H
OsDW1-01g AD E		V2H (Oc01c0103800)
$O_{SDW} = O_{I} $		1211 (050190105800)
OsDW1-01g_AD_K		V2H (Oc03c0270700)
$O_{SDW} = O_{SDW} = O_{S$	GGCTCGAGTTACTGTAGTTGCCTTGC	1211 (030390270700)
OsBIN2 BD F		Y2H
OsBIN2 BD R	GCGTCGACTTAGCTCCCAGTATTGAAG	
AtDW1 AD F	GCGAATTCATGGGCTCAGAGCAGGATC	Y2H
AtDW1 AD R	GGCCCGGGTCAGGCTTTCCTGTGTCTGTTC	
AtBIN2 BD F	GCGAATTCATGGGCTCAGAGCAGGATC	Y2H
AtBIN2 BD R	GGCCCGGGTCAGGCTTTCCTGTGTCTGTTC	
SbDW1-GFP_F	CACCATGTCTTCAGTTGGGAGCAGCC	transient assay construction
SbDW1-GFP_R	ATGCAATTGTCTTGCCAATGCACTCC	2
SbBIN2-GFP_F	TACAACTACATCTAGATGGAGGCGCCGCCGGGACC	transient assay construction
SbBIN2-GFP_R	TGCTCACCATACTAGTGCTCCCAACATGCCCGAAGT	
SbBZR1-GFP_F	TACAACTACATCTAGAATGACGTCGGGGGGCGGCC	transient assay construction
SbBZR1-GFP_R	TGCTCACCATACTAGTCTTGGCGCCGACGCCAAG	
SbBIN2-FLAG_F	CACCATGGAGGCGCCGCCGGGACCGG	transient assay construction
SbBIN2-FLAG_R	GCTCCCAACATGCCCGAAGTTGACAC	
SbDW1-HA_F	GGCCAGTGCCAAGCTTTGCAGCGTGACCCGGTCG	transient assay construction
SbDW1-HA_R	CGTATGGGTAACTAGTATGCAATTGTCTTGCCAATG	
AtBIN2-GFP_F	TACAACTACATCTAGATGGCTGATGATAAGGAGAT	transient assay construction
AtBIN2-GFP_R	TGCTCACCATACTAGTAGTTCCAGATTGATTCAAGA	
AtBZR1-GFP_F		transient assay construction
		1
AtBIN2-FLAG_F		transient assay construction
		transient append as struction
		transient assay construction
		turne in at an an a star at a st
	TAGAACTACATCTAGAATGGGGCTCAGAGCAGGAT	transient assay construction
AtDW1-GFP_R	TGCTCACCATACTAGTGGCTTTCCTGTGTCTGTTC	