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SHORT COMMUNICATION

EXB1/WRKY71 transcription factor regulates both shoot branching and responses to abiotic stresses

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ABSTRACT

As the sessile organisms, plants evolve different strategies to survive in adverse environmental conditions. The elaborate regulation of shoot branching is an important strategy for plant morphological adaptation to various environments, while the regulation of reactive oxygen species (ROS), salicylic acid (SA) and jasmonic acid (JA) is pivotal for plant responses to biotic and abiotic stresses. Recently, we have demonstrated that Arabidopsis EXB1, a WRKY transcription factor, is a positive regulator of shoot branching as a cover story in Plant Cell. Here we show that WRKY23, an EXB1 close member, has a redundant role in control of shoot branching. We further show that *EXB1* is induced by H_2O_2, ABA or mannitol treatments, suggesting that *EXB1* may also play roles in plant responses to abiotic stresses. RNA-sequencing (RNA-seq) analysis using 4EnhpEXB1-EXB1GR inducible line indicates that the genes involved in oxidative stress, oxidation reduction, SA or JA signaling pathway are regulated by EXB1 induction in a short time. We suggest that EXB1/WRKY71 transcription factor may play pivotal roles in plant adaptation to environments by both morphological and physiological ways.

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Unlike animals, plants cannot escape from adverse environmental conditions by relocation. Plants evolve different strategies to adapt to various environmental changes. Control of shoot branching is one of the excellent strategies in response to environmental stimuli by forming proper plant morphology,[1] while the regulation of producing reactive oxygen species (ROS), salicylic acid (SA) and jasmonic acid (JA) is another important strategy to adapt to environments by changing the physiological status of plant cells.[2] Branches are derived from the axillary meristems (AMs) in leaf axils. AMs first develop into axillary buds by producing a few leaves. Some axillary buds can further develop into shoot branches, while other axillary buds just stay dormant. Thus, the initiation of AMs and axillary bud outgrowth are the 2 important steps that determine the final number of plant branches.[3,4]

The initiation of AMs and axillary bud outgrowth were regulated elaborately. The *REGULATOR OF AXILLARY MERISTEMS 1* (*RAX1*) is a key regulator in control of AM initiation. *RAX1* functions redundantly with *RAX2* and *RAX3*.[5,6] *RAX1*, *RAX2* and *RAX3* encode closely related R2R3 MYB transcription factors. The function of *RAX* genes in AM formation appears to be conserved throughout plant kingdom. *BLIND* (*BL*) is orthologous to *RAX* genes and *bl* mutants display defects of AM initiation in tomato and pepper.[7,8] By screening an activation tagging Arabidopsis mutant collection we obtained a dominant mutant *exb1-D* which producing many more branches than the wild-type control. We demonstrate that EXB1 positively regulates shoot branching by affecting both axillary meristem initiation and the bud outgrowth. Furthermore, we reveal that EXB1 facilitate AM initiation by directly regulating *RAX* genes at the transcriptional level and also promote bud outgrowth by repressing auxin pathway.[3]

EXB1 encodes a WRKY transcription factor previously named WRKY71.[3] WRKY family is one of the largest transcription factor families and there are 74 members in Arabidopsis genome.[9] WRKY28 (EXB2), WRKY8 (EXB3), WRKY48 (EXB4) and WRKY57 (EXB5) are closely related to EXB1 in the phylogenetic tree and are proved to be redundant to EXB1. WRKY23 is also located in the EXB1 clade of the WRKY phylogenetic tree (Fig. 1A). WRKY23 has been identified to regulate auxin signaling in the downstream of IAA14, AUXIN RESPONSE FACTOR 7 (ARF7) and ARF19.[10,11] WRKY23 has also been

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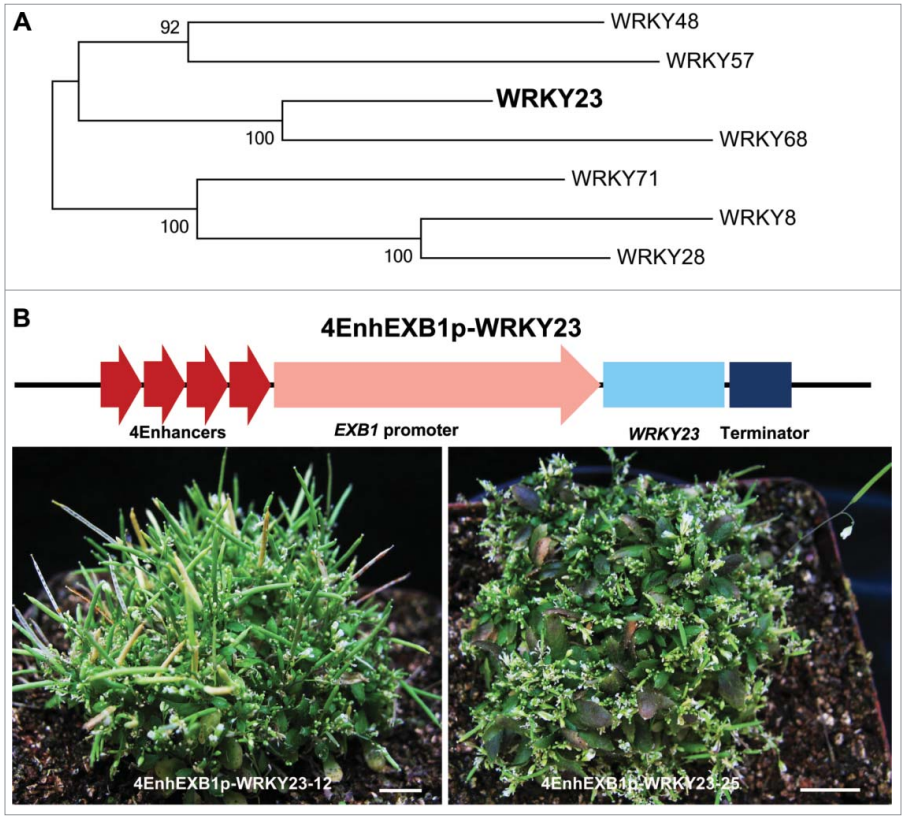
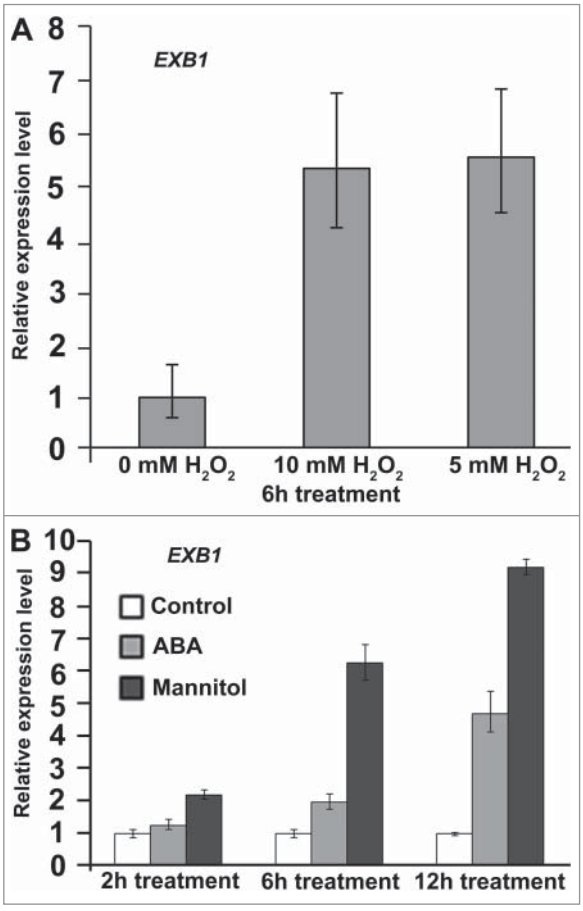


Figure 1. WRKY23 Had Redundant Function with EXB1. (A) The phylogenetic tree of EXB1/WRKY71, WRKY8, WRKY28, WRKY48, WRKY57, WRKY23 and WRKY68 was generated based on the full-length protein sequences using Neighbor-Joining method by MEGA 6. (B) Top, the schematic representation of 4EnhEXB1p-WRKY23 construct; Bottom, the phenotypes of 2 independent lines of 4EnhEXB1p-WRKY23 transgenic plants.



found to regulate plant embryo and root development by affecting auxin distribution through the control of flavonol biosynthesis.[10,12] However no findings have demonstrated the roles of WRKY23 in shoot branching. To reveal the functions of *WRKY23* in shoot branching, we first generated the construct 4EnhEXB1p-WRKY23 by using the 4 CaMV 35S enhancers and 2700-bp-long *EXB1* promoter (4EnhEXB1p) to overexpress *WRKY23* gene (Fig. 1B). The 4EnhEXB1p-WRKY23 transgenic plants produced many more branches than the wild-type control (Fig. 1B). The WRKY23 overexpression plants even can produced thousands of branches in a single plant, indicating that WRKY23 had high capacity to promote plant shoot branching (Fig. 1B).

WRKY transcription factors have been reported to participate in responses to various biotic or abiotic stresses.[9] In order to test whether EXB1 might also be involved in the regulation of plant responses to

Figure 2. EXB1 Was Induced by H_2O_2, ABA or Mannitol Rapidly. (A) The relative expression level of *EXB1* in wild-type plants at 6 hours after treated with 10 mM or 5 mM H_2O_2; (B) The relative expression level of *EXB1* in wild-type plants at 2 hours, 6 hours and 12 hours after treated with 100 mM ABA or 300 mM mannitol. The expression level of *EXB1* in mock-treated plants was set to 1.0. The error bars represent the SD of 3 replicates.

Table 1. The transcript alteration of genes in response to oxidative stress by EXB1 induction.

Gene ID	Gene product	Value of mock-treated plants	Value of DEX-treated plants	Log2(fold change)	p_value
AT1G14540	PER4	2.06828	4.63742	1.2	0.0231123
AT1G21520	AT1G21520	6.35587	83.2197	3.7	0
AT5G19890	AT5G19890	0.11012	1.27155	3.5	0.0000828
AT1G16420	MC8	0.09403	0.9566	3.3	0.000208394
AT1G52560	AT1G52560	0.09277	0.64057	2.8	0.0229653
AT4G08780	AT4G08780	7.62037	41.3088	2.4	2.47E-11
AT4G08770	Prx37	19.5044	103.508	2.4	2.22E-16
AT5G05340	PRX52	6.88871	20.509	1.6	0.00000491
AT2G38390	AT2G38390	6.05359	17.4199	1.5	0.0000504
AT4G36430	AT4G36430	4.42661	12.6194	1.5	0.000131116
AT5G06720	ATPA2	3.27123	8.0539	1.3	0.00369748
AT5G39610	ATNAC6	1.89284	4.38157	1.2	0.0198265
AT4G23190	CRK11	7.61116	17.0319	1.2	0.000103328
AT2G18150	AT2G18150	6.50389	13.8397	1.1	0.00219204
AT3G01420	DIOX1	8.68423	18.2481	1.1	0.00033264
AT3G49120	ATPCB	206.322	429.775	1.1	0.000609777
AT2G37130	AT2G37130	70.8261	144.86	1.0	0.000295334
AT3G49960	AT3G49960	1.00424	0.16773	−2.6	0.00166279
AT1G30870	AT1G30870	0.48317	0.09791	−2.3	0.0283957
AT5G05410	DREB2	16.8563	3.79822	−2.1	1.14E-08
AT5G67400	RHS19	0.70408	0.16062	−2.1	0.0112083
AT1G61120	GES	0.23429	0.06097	−1.9	0.0280802
AT4G26010	AT4G26010	1.1279	0.32219	−1.8	0.0156379
AT5G37770	TCH2	217.941	68.1689	−1.7	1.68E-09
AT1G05240	AT1G05240	0.8244	0.27342	−1.6	0.0446952
AT1G05250	AT1G05250	0.74497	0.24794	−1.6	0.0491903
AT2G41480	AT2G41480	1.8151	0.60604	−1.6	0.0198361
AT4G21830	ATMSRB7	2.48263	0.89057	−1.5	0.0258255
AT3G12580	HSP70	2.38277	0.91385	−1.4	0.00509735
AT4G11290	AT4G11290	25.1435	11.3171	−1.2	0.000426591
AT1G44970	AT1G44970	14.5406	6.60913	−1.1	0.00129019
AT4G16270	AT4G16270	6.41129	2.93899	−1.1	0.0154319

abiotic stresses, we first test the expression of *EXB1* using quantitative RT-PCR after treatment of wild-type plants with 5 mM and 10 mM H_2O_2, 100 mM ABA or 300 mM mannitol. The results showed that *EXB1* was induced rapidly after the treatment with H_2O_2, ABA or mannitol (Fig. 2), suggesting that EXB1 might also regulate plant adaptation to adverse environmental conditions physiologically.

To understand the molecular mechanism by which EXB1 regulates shoot branching and plant responses to stresses, we fused the coding sequence of *EXB1* to the sequence encoding the steroid-binding domain of the rat glucocorticoid receptor (GR)[13] and put the fusion under the 4EnhEXB1p to generate the construct 4EnhpEXB1-EXB1GR. 4EnhpEXB1-EXB1GR-13 transgenic plants were treated with dexamethasone (DEX) to induce EXB1 translocation into nucleus.[3] The samples were used to perform RNA-sequencing (RNA-seq) analysis. The results showed that 3114 genes were differentially expressed (fold change $\geq$ 2, $P < 0.05$) after the treatment with 30 μM for 4 hours. Among these genes, the expression level of *RAX* genes was rapidly increased after EXB1 induction, and the transcripts

Table 2. The transcript alteration of genes in SA signaling pathway by EXB1 induction.

Gene ID	Gene product	Value of mock-treated plants	Value of DEX-treated plants	Log2(fold change)	p_value
AT4G25560	AtMYB18	0.0591953	0.551923	3.2	0.0288016
AT5G54230	MYB49	0.476331	2.56793	2.4	0.000347191
AT2G14560	LURP1	50.5996	205.256	2.0	2.07E-09
AT5G22570	WRKY38	3.43386	13.3867	2.0	0.0000101
AT3G48920	AtMYB45	2.04465	6.3486	1.6	0.00366468
AT3G49690	RAX3	0.720147	2.11352	1.6	0.0116923
AT5G54610	ANK	4.91594	12.0211	1.3	0.00113844
AT4G23170	EP1	22.9788	49.4802	1.1	0.000166857
AT3G01420	DIOX1	8.68423	18.2481	1.1	0.00033264
AT5G44420	PDF1.2	39.1423	81.5765	1.1	0.00130954
AT2G14580	PRB1	9.5015	19.4753	1.0	0.00725166
AT2G36890	MYB38	4.35598	8.51135	1.0	0.0331253
AT5G13320	GDG1	2.22105	4.30273	1.0	0.0286926

Table 3. The transcript alteration of genes in JA signaling pathway by EXB1 induction.

Gene ID	Gene product	Value of mock-treated plants	Value of DEX-treated plants	Log2(fold change)	p_value
AT2G34600	TIFY5B	9.53484	1.03625	−3.2	0.000000345
AT1G72520	LOX4	15.1331	1.64327	−3.2	0
AT4G11280	ACS6	104.144	12.0035	−3.1	0
AT1G19640	JMT	1.42277	0.161271	−3.1	0.0000465
AT1G32640	RD22BP1	104.287	16.5214	−2.7	0
AT5G42650	AOS	69.7379	11.2917	−2.6	0
AT1G17380	JAZ5	23.6471	4.03	−2.6	2.88E-11
AT1G17420	LOX3	24.9445	4.26703	−2.5	0
AT3G23250	ATMYB15	21.882	4.00251	−2.5	1.91E-10
AT3G50060	MYB77	82.2341	19.4365	−2.1	8.29E-13
AT1G28480	GRX480	6.93256	1.65924	−2.1	0.00078703
AT3G15210	ATERF-4	92.9679	24.0022	−2	1.56E-11
AT2G02990	RNS1	5.97335	1.49915	−2	0.00042616
AT3G25780	AOC3	75.5138	19.3517	−2	2.32E-11
AT1G61120	GES	0.234291	0.060973	−1.9	0.0280802
AT1G19180	JAZ1	116.687	31.28	−1.9	3.58E-11
AT4G23600	JR2	11.4154	3.30733	−1.8	0.0000113
AT4G37260	MYB73	88.5454	27.6989	−1.7	0.0000105
AT2G24850	TAT3	6.55538	2.32079	−1.5	0.00067809
AT3G52400	SYP122	61.575	21.9425	−1.5	0.00000012
AT1G20510	OPCL1	70.4878	24.4484	−1.5	8.29E-08
AT1G74430	MYB95	12.0119	4.60133	−1.4	0.00257587
AT5G13930	TT4	83.3234	31.2281	−1.4	0.000000296
AT5G24780	VSP1	62.3915	23.2181	−1.4	0.00000055
AT1G54040	TASTY	2.71165	1.07254	−1.3	0.0194946
AT5G13220	TIFY9	18.6439	7.46426	−1.3	0.00469042
AT4G05100	AtMYB74	0.759768	0.322459	−1.2	0.116597
AT5G07690	MYB29	39.86	17.8935	−1.2	0.0000415
AT5G60890	ATMYB34	9.95635	4.55748	−1.1	0.0041261
AT5G61420	AtMYB28	81.5909	36.9272	−1.1	0.00026045
AT5G64900	PROPEP1	10.7115	4.92772	−1.1	0.0226213
AT5G24770	VSP2	47.7935	21.5581	−1.1	0.0000757
AT3G45140	LOX2	53.0825	26.9621	−1	0.00040705
AT3G17860	TIFY6B	7.76129	3.90539	−1	0.0239613
AT2G06050	OPR3	58.2752	29.0943	−1	0.00092024

of genes involved in auxin pathways were also significantly altered.[3]

Our further analysis showed that the expression levels of genes related to the plant responses to oxidative stress (Table 1), SA signaling pathway (Table 2), and JA signaling pathway (Table 3) were also altered significantly (fold $\geq$ 2 or $\leq$ 0.5, $p \leq 0.05$) after EXB1 induction. These results further suggested that EXB1 may be involved to plant physiological responses to different stresses. Previous studies have demonstrated that WRKY8, WRKY28, WRKY48 and WRKY57 also participate in plant responses to biotic or abiotic stresses.[14-17] A recent published article has demonstrated that WRKY71, WRKY8 or WRKY28 can accelerate flowering in *Arabidopsis* as well,[18] suggesting that EXB1 might increase the possibility of successful reproduction under environmental stresses by speeding up the life cycle. These findings suggested that EXB1/WRKY71 family transcription factors may facilitate plant adaptation morphologically and physiologically through promoting shoot branching,[3] and speeding up the life cycle[18] in response to different environmental cues.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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