Gene Note

Vascular cell expression patterns of *Arabidopsis* bZIP group I genes

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Abstract The bZIP transcription factors are involved in various aspects of plant development. Studies of bZIP group I genes in several species have indicated that they may play a role in vascular development. In order to elucidate the functions of *Arabidopsis* bZIP group I genes in vascular development, the expression pattern of seven *AtbZIP* group I genes, *AtbZIP18*, *29*, *30*, *51*, *52*, *59*, and *69*, were examined in relation to vascular development using promoter::reporter lines of transgenic *Arabidopsis* plants. *AtbZIP18*, *51*, *52*, and *59* were preferentially expressed in developing vascular cells including differentiating vessels and their precursor cells. *AtbZIP18*, *52*, and *59* showed partially overlapping expression in vascular cells of cotyledons, and partially overlapping expression of *AtbZIP51*, *52*, and *59* was observed in root vascular cells. These results suggest that the segenes may have partially redundant functions in vascular development.

Key words: Arabidopsis bZIP group I, bZIP transcription factor, vascular development.

The bZIP transcription factors are involved in various aspects of plant development, such as pathogen defense (Thurow et al. 2005), light responses (Ulm et al. 2004), seed maturation (Bensmihen et al. 2005) and flower development (Wigge et al. 2005). In several plant species, some members of the bZIP group I genes appear to play a role in vascular development. Tomato VSF-1 is expressed in vascular tissues and activates the expression of a gene encoding a structural cell wall protein (Ringli and Keller 1998; Torres-Schumann et al. 1996). The rice RF2a and RF2b genes were isolated as activators of phloem-specific gene expression (Dai et al. 2004; Yin et al. 1997). Antisense suppression lines and dominant negative mutants of RF2a show aberrant vascular tissue development (Dai et al. 2003; Petruccelli et al. 2001; Yin et al. 1997). These results suggest that some Arabidopsis bZIP group I genes may also play roles in regulating vascular development. However, the spatial and temporal expression of the AtbZIP group I genes has not been reported, and it has not been possible, therefore, to evaluate whether they play a role in the process of vascular development. In this study, as a first step toward understanding the potential roles of the AtbZIP group I genes in vascular development, we examined the expression pattern of AtbZIP group I genes in transgenic

Arabidopsis plants transformed with promoter::reporter constructs.

The AtbZIP family has been subdivided into ten groups (A-I, and S) on the basis of the amino acid sequence similarities of the bZIP domains (Jakoby et al. 2002). The AtbZIP group I contains 13 members (Figure 1) and the members share a characteristic lysine residue, which replaces the highly conserved arginine in the basic domain of members of other bZIP groups except group I (N-X₇–R to K-X₉–L, where X represents an amino acid) (Jakoby et al. 2002) (Figure 2, bold characters). This amino acid substitution may alter the binding specificity to the nucleotide sequences of *cis*-elements, because this substitution has been correlated with a higher affinity for non-palindromic binding sites (Fukazawa et al. 2000). Phylogenetic analysis of AtbZIP group I showed that seven members of the group, AtbZIP18, 29, 30, 51, 52, 59 and 69, together with VSF-1, RF2a, and RF2b form a distinctive subgroup (Figure 1). The amino acid sequences in the bZIP domain of seven AtbZIP group I proteins share 70-85% identity with bZIP domains of VSF-1, RF2a, and RF2b (Figure 2), although no remarkable sequence similarities can be found between other regions of the proteins. We therefore focused on these seven genes, AtbZIP18, 29, 30, 51, 52, 59 and 69,

^a Present address: Biotechnology Division, Korea Forest Research Institute, 44-3 Omokchundong, Suwon, 441-350, Republic of Korea Abbreviations: bZIP, basic region-leucine zipper; GUS, β -glucuronidase; NLS, nuclear localization signal; YFP, yellow fluorescent protein. This article can be found at http://www.jspcmb.jp/

in this study.

To characterize the expression patterns of the genes, promoter expression analyses were conducted using the GUS reporter, or YFP fused to the SV40 nuclear localization signal (NLS). The upstream sequences



Figure 1. Phylogenetic tree of *Arabidopsis* bZIP group I proteins, tomato (VSF-1) and rice (RF2a, RF2b) bZIP proteins. Amino acid sequences were aligned using the CLUSTAL W (1.8.3) program and the tree was constructed with TreeView software.

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of the ATG translation start of each gene (1997 kb for AtbZIP18 (At2g40620), 1044 kb for AtbZIP29 (At4g38900), 1971 kb for AtbZIP30 (At2g21230), 830 kb for AtbZIP51 (At1g43700), 1000 kb for AtbZIP52 (At1g06850), 1920 bp for AtbZIP59 (At2g31370), and 1000 kb for AtbZIP69 (At1g06070)), together with 9 bp of the coding region of each gene, were amplified from Arabidopsis (Columbia) genomic DNA by PCR with gene-specific primer sets (Table 1). The DNA fragments were subcloned into the pENTR/D/TOPO vector (Invitrogen) and then integrated by site-specific recombination into the pBGGUS or pBGYN binary vectors (Kubo et al. 2005) in which the GATEWAY cassette (Invitrogen) was fused to the 5' ends of the GUS and the YFP-NLS fragments, respectively. These constructs were introduced into Agrobacterium tumefaciens strain C58C1 and then transformed into Arabidopsis plants using the floral dip method (Clough and Bent 1998). Transformants harboring the transgenes were screened by resistance to bialaphos. Histochemical GUS staining was performed according to Pyo et al. (2004). More than ten independent transgenic Arabidopsis lines of each gene were analyzed and all lines showed almost similar patterns of GUS staining.

During primary vascular development, procambial cells, the precursors of vascular cells are detected as elongated cells that are distinct from the nearly homogeneous surrounding ground tissue cells (West

		NLS Leucine zipper	
AtbZIP18	141	LAELWVVDPKRAKRIIANRQSAARSKERKARYILELERKVQTLQTEATTLSAQLSLFQRD	2
AtbZIP29	387	LAEMAMSDPKRVKRILANRQSAARS K ERKMRYIVELEHKVQTLQTEATTLSAQLTLLQRD	4
AtbZIP30	363	LAEIVMADPKRVKRILANRVSAARS K ERKTRYMAELEHKVQTLQTEATTLSAQLTHLQRD	4
AtbZIP51	187	LAELALLDPKRAKRILANRQSAARS K ERKIRYTGELERKVQTLQNEATTLSAQVTMLQRG	2
AtbZIP52	141	LSELWNIDPKRAKRILANRQSAARS K ERKARYIQELERKVQSLQTEATTLSAQLTLYQRD	2
AtbZIP59	194	LAELALIDPKRAKRIWANRQSAARS K ERKTRYIFELERKVQTLQTEATTLSAQLTLLQRD	2
AtbZIP69	203	LSELALIDPKRAKRIWANRQSAARS K ERKMRYIAELERKVQTLQTEATSLSAQLTLLQRD	2
RF2a	162	LAELALVDPKRAKRIWANRQSAARS K ERKMRYIAELERKVQTLQTEATTLSAQLALLQRD	2
RF2b	125	LSDLAAIDPKRAKRILANRQSAARS K ERKARYITELERKVQTLQTEATTLSAQLTLFQRD	1
			-
VSF-1	290	LAEIAVLDPKRAKRILANRLSAARS K ERKTRYISELEHKVQKLQTETTTLSTQVTILQKN	3
VSF-1	290	LAEIAVLDPKRAKRILANRLSAARS K ERKTRYISELEHKVQKLQTETTTLSTQVTILQKN * **** *** *** ********* ** *** *** **	3
VSF-1	290	LAEIAVLDPKRAKRILANRLSAARS K ERKTRYISELEHKVQKLQTETTTLSTQVTILQKN * **** *** *** *** ******** ** *** ***	3
VSF-1	290	LAEIAVLDPKRAKRILANRLSAARSKERKTRYISELEHKVQKLQTETTTLSTQVTILQKN * **** *** *** *** *** *** *** *** ***	3
VSF-1 AtbZIP18	290	LAEIAVLDPKRAKRILANRLSAARSKERKTRYISELEHKVQKLQTETTTLSTQVTILQKN * **** *** *** *** **** *** *** *** **	3
VSF-1 AtbZIP18 AtbZIP29	290 201 447	LAEIAVLDPKRAKRILANRLSAARSKERKTRYISELEHKVQKLQTETTTLSTQVTILQKN * **** *** *** *** *** *** *** *** ***	3 2 4
VSF-1 AtbZIP18 AtbZIP29 AtbZIP30	290 201 447 423	LAEIAVLDPKRAKRILANRLSAARSKERKTRYISELEHKVQKLQTETTTISTQVTILQKN * **** *** *** *** *** *** *** *** ***	3 2 4 4
VSF-1 AtbZIP18 AtbZIP29 AtbZIP30 AtbZIP51	290 201 447 423 247	LAEIAVLDPKRAKRILANRLSAARSKERKTRYISELEHKVQKLQTETTTISTQVTILQKN * **** *** *** *** **** *** *** *** **	3 2 4 2
VSF-1 AtbZIP18 AtbZIP29 AtbZIP30 AtbZIP51 AtbZIP52	290 201 447 423 247 201	LAEIAVLDPKRAKRILANRLSAARSKERKTRYISELEHKVQKLQTETTTISTQVTILQKN * **** *** *** *** **** *** *** *** **	3 2 4 2 2
VSF-1 AtbZIP18 AtbZIP29 AtbZIP30 AtbZIP51 AtbZIP52 AtbZIP59	290 201 447 423 247 201 254	LAEIAVLDPKRAKRILANRLSAARSKERKTRYISELEHKVQKLQTETTTISTQVTILQKN * **** *** *** *** ********* ** *** **	244222
AtbZIP18 AtbZIP29 AtbZIP30 AtbZIP51 AtbZIP52 AtbZIP59 AtbZIP69	290 201 447 423 247 201 254 263	LAEIAVLDPKRAKRILANRLSAARSKERKTRYISELEHKVQKLQTETTTISTQVTILQKN * **** *** *** *** ********* ** *** **	3 2 4 4 2 2 3
AtbZIP18 AtbZIP29 AtbZIP30 AtbZIP51 AtbZIP52 AtbZIP59 AtbZIP69 RF2a	290 201 447 423 247 201 254 263 222	LAEIAVLDPKRAKRILANRLSAARSKERKTRYISELEHKVQKLQTETTTISTQVTILQKN * ************************************	3 2 4 4 2 2 2 3 2
AtbZIP18 AtbZIP29 AtbZIP30 AtbZIP51 AtbZIP52 AtbZIP59 AtbZIP69 RF2a RF2b	290 201 447 423 247 201 254 263 222 185	LAEIAVLDPKRAKRILANRLSAARSKERKTRYISELEHKVQKLQTETTTISTQVTILQKN * ************************************	3 2 4 4 2 2 2 3 2 2

Figure 2. Amino acid sequence alignment of *Arabidopsis* bZIP group I proteins (AtbZIP18, 29, 30,51, 52, 59, 69), tomato (VSF-1) and rice (RF2a, RF2b) bZIP proteins. Asterisks (*) indicate that the residues in that column are identical in all sequences in the alignment. Replacement of the highly conserved arginine residue by lysine in the basic region of the group I family members is represented in bold.

Table 1. Primer sequences used in this study. Underlines represent additional recognition sequence of topoisomerase I for TOPO DNA recombination reaction.

 Primer	Sequence
AtbZIP18PF	5'- <u>CACC</u> GAACACTATTACTGTTTCCATGAGATTGCA-3'
AtbZIP18PR	5'-ATCCTCCATATCTGGATCGTCCTTCTTCTT-3'
AtbZIP29PF	5'-CACCGTACATGACAACATAAGGAGGACAGAATC-3'
AtbZIP29PR	5'-ATCACCCATTTTAGATCGGATAATGCAGTT-3'
AthZIP30PF	5'-CACCATCTCCATCAGCACATTTGGAGGAAATGTC-3'
AtbZIP30PR	5'-ACCACCCATTTTGATTTTCTGAGATTCCGA-3'
Ath7IP51PF	5'-CACCGTGCTATGAGTCTAGGAAGATTGGTTCAGA-3'
AtbZIP51PR	5'-TCCTTCCATTGATTTTTTTTTTTTTCCCGGCG-3'
Ath7IP52PF	5'-CACCAGCTGGTAAAAGTAAGCATCATTAGGTTTT.3'
AtbZIP52PR	5'-TTTCTCCATTTTTTTAAGAGAATCTGAGAGATG-3'
Ath7IP59PF	5'-CACCA ATGATGCTA ATGGTCCCTGATCGCCATTL3'
AtbZIP59PR	5'-CTTATCCATTTCTACTGACTTATCACCAAA-3'
Ath7ID60DE	5' САСССАСТАСТТАТА АСАСССТТСАСА ААТАСА А 3'
AtbZIP69PR	5'-CTTATCCATTTCAAGAACTTGACCTAAACC-3'

and Harada 1993, Jürgens 1994). Subsequently, the procambial cells differentiate into vascular cells. In the cotyledons, strong expression of GUS activity driven by the AtbZIP18 promoter was detected in elongated cells that did not have visible secondary cell wall formation (Figure 3A, B arrows). This result suggested that the AtbZIP18 promoter was expressed in procambial cells. GUS staining was also observed in mature veins with clear secondary cell wall thickenings and mesophyll cells of the cotyledons. However, GUS activity was not detected in rosette leaves, hypocotyls, or roots (data not shown). AtbZIP51:: GUS expression was predominantly observed in developing xylem cells of the roots (Figure 3C), and was also detected in the root meristem (data not shown). GUS activity was not detected in organs other than the root.

AtbZIP52:: GUS was specifically expressed in the developing vasculature throughout the plant and showed the strongest expression of the seven genes investigated here (Figure 3D-F). In the cotyledon, the expression of AtbZIP52::GUS gene was found in elongated cells that did not have visible secondary cell wall formation (Figure 3D, E, arrows). This result suggested that the AtbZIP52 promoter was expressed in procambial cells of the cotyledon. In roots, the expression of GUS activity was found in the vascular tissue, especially in cells with or without secondary cell wall thickenings located between two mature protoxylems (Figure 3F). However, GUS activity was undetectable in mature vasculature (Figure 3D, F; see the hypocotyls and root, respectively). The GUS activity derived from *AtbZIP52::GUS* was also detected in the root meristem (data not shown). These results suggested that the AtbZIP52 promoter was active in procambial cells or developing xylem cells of



Figure 3. Expression patterns of *AtbZIP18*, *51*, *52*, and *59* in *Arabidopsis* plants. (A, B) Expression of the *AtbZIP18* promoter::GUS in cotyledons. (C) Expression of the *AtbZIP51* promoter::GUS in cotyledons (D, E) and roots (F). (G, H, J) Expression of the *AtbZIP59* promoter::GUS in cotyledons (G), leaf primodia (H), and roots (J). (I) Expression of the *AtbZIP59* promoter::YFP-NLS in roots. Arrows in D and E indicate procambial cells with GUS activity. Bars: (A, D, G, H, J) 200 μ m; (B, C, E, F) 50 μ m; (I) 100 μ m.

the roots. Expression of *AtbZIP59::GUS* was observed in the vasculature throughout the plants, and GUS activity in cotyledons was detectable along the veins with secondary cell wall formation (Figure 3G). GUS activity was also detected in the leaf primordia of *AtbZIP59:: GUS* plants (Figure 3H). In roots, *AtbZIP59* promoter activity was found in the steles including developing xylem cells (Figure 3I, J) and root tips (Figure 3J).

In *AtbZIP30::GUS* and *AtbZIP29::GUS* plants, expression was not detected in vascular tissues, but was found in the stipules and the root meristem for *AtbZIP30::GUS*, and in the quiescent center of the root for *AtbZIP29::GUS* (data not shown). In *AtbZIP69::GUS* transformants, GUS activity was below the limit of detection in any of the tissues and organs.

In conclusion, AtbZIP18, and 52 were found to have partially overlapping patterns of expression in the procambial cells, and 18 and 59 were in the xylem cells of cotyledons, while AtbZIP51, 52, and 59 showed partially overlapping expression in xylem cells of the roots. These overlapping but somewhat distinctive expression patterns suggest that the AtbZIP18, AtbZIP51, AtbZIP52 and AtbZIP59 genes may have redundant functions, in part, in vascular development. For instance, three Arabidopsis class III homeodomainleucine zipper genes (HD-Zip III), PHABULOSA (PHB), PHAVOLUTA (PHV) and REVOLUTA (REV/IFL1/ AVB1), show overlapping expression in the adaxial domains of lateral organs, in vascular bundles, and in apical meristems (McConnell et al. 2001; Emery et al. 2003; Prigge et al. 2005). These genes act redundantly to provide adaxial identity of lateral organs and to regulate tissue pattern formation in vascular bundles (Prigge et al. 2005). It has also been suggested that bZIP group I proteins form homo- or hetero-dimers for transcriptional regulation of their target genes (Dai et al. 2003, 2004; Petruccelli et al. 2001; Torres-Schumann et al. 1996). Thus, Arabidopsis bZIP proteins may regulate their target genes through interactions with other bZIP members or with other regulatory proteins. Functional characterization of AtbZIP group I genes in vascular development by genetic and reverse genetic approaches, together with isolation of co-factors and target genes, will be required in future studies in order to fully understand the role of these factors in plant vascular development.

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References

- Bensmihen S, Giraudat J, Parcy F (2005) Characterization of three homologous basic leucine zipper transcription factors (bZIP) of the ABI5 family during Arabidopsis thaliana embryo maturation. *J Exp Bot* 56: 597–603
- Clough SJ, Bent AF (1998) Floral dip: a simplified method for

Agrobacterium-mediated transformation of *Arabidopsis thaliana*. *Plant J* 16: 735–743

- Dai S, Petruccelli S, Ordiz MI, Zhang Z, Chen S, Beachy RN (2003) Functional analysis of RF2a, a rice transcription factor. *J Biol Chem* 278: 36396–36402
- Dai S, Zhang Z, Chen S, Beachy RN (2004) RF2b, a rice bZIP transcription activator, interacts with RF2a and is involved in symptom development of rice tungro disease. *Proc Natl Acad Sci USA* 101: 687–692
- Emery JF, Floyd SK, Alvarez J, Eshed Y, Hawker NP, Izhaki A, Baum SF, Bowman JL (2003) Radial patterning of Arabidopsis shoots by class III HD-Zip genes id mediated by miRNA regulation. *Curr Biol* 13: 1768–1774
- Fukazawa J, Sakai T, Ishida S, Yamaguchi I, Kamiya Y, Takahashi Y (2000) Repression of shoot growth, a bZIP transcriptional activator, regulates cell elongation by controlling the level of gibberellins. *Plant Cell* 12: 901–915
- Jakoby M, Weisshaar B, Droge-Laser W, Vicente-Carbajosa J, Tiedemann J, Kroj T, Parcy F. bZIP Research Group (2002) bZIP transcription factors in *Arabidopsis*. *Trends Plant Sci* 7: 106– 111
- Jürgens GM (1994) Arabidopsis. In: Bard JBL (ed) *Embryos: Color Atlas of Development.* Wolfe Publishing, London, pp 7–22
- Kubo M, Udagawa M, Nishikubo N, Horiguchi G, Yamaguchi M, Ito J, Mimura T, Fukuda H, Demura T (2005) Transcription switches for protoxylem and metaxylem vessel formation. *Genes Dev* 19: 1855–1860
- McConnell JR, Emery J, Eshed Y, Bao N, Bowman J, Barton MK (2001) Role of PHABULOSA and PHAVOLUTA in determining radial patterning in shoots. *Nature* 411: 709–713
- Petruccelli S, Dai S, Carcamo R, Yin Y, Chen S, Beachy RN (2001) Transcription factor RF2a alters expression of the rice tungro bacilliform virus promoter in transgenic tobacco plants. *Proc Natl Acad Sci USA* 19: 7635–7640
- Prigge MJ, Otsuga D, Alonso JM, Ecker JR, Drews GN, Clark SE (2005) Class III homeodomain-leucine zipper gene family members have overlapping, antagonistic and distinct roles in Arabidopsis development. *Plant Cell* 17: 61–76
- Pyo H, Demura T, Fukuda H (2004) Spatial and temporal tracing of vessel differentiation in young Arabidopsis seedlings by the expression of an immature tracheary element-specific promoter. *Plant Cell Physiol* 45: 1529–1536
- Ringli C, Keller B (1998) Specific interaction of the tomato bZIP transcription factor VSF-1 with a non-palindromic DNA sequence that controls vascular gene expression. *Plant Mol Biol* 37: 977–988
- Torres-Schumann S, Ringli C, Heierli D, Amrhein N, Keller B (1996) In vitro binding of the tomato bZIP transcriptional activator VSF-1 to a regulatory element that controls xylem-specific gene expression. *Plant J* 9: 283–296
- Thurow C, Schiermeyer A, Krawczyk S, Butterbrodt T, Nickolov K, Gatz C (2005) Tobacco bZIP transcription factor TGA2.2 and related factor TGA2.1 have distinct roles in plant defense responses and plant development. *Plant J* 44: 100–113
- Ulm R, Baumann A, Oravecz A, Mate Z, Adam E, Oakeley EJ, Schafer E, Nagy F (2004) Genome-wide analysis of gene expression reveals function of the bZIP transcription factor HY5 in the UV-B response of Arabidopsis. *Proc Natl Acad Sci USA* 101: 1397–1402
- West M, Harada JJ (1993) Embryogenesis in higher plants: An overview. *Plant Cell* 5: 1361–1369

- Wigge PA, Kim MC, Jaeger KE, Busch W, Schmid M, Lohmann JU, Weigel D (2005) Integration of spatial and temporal information during floral induction in Arabidopsis. *Science* 309: 1056–1059
- Yin Y, Zhu Q, Dai S, Lamb C, Beachy RN (1997) RF2a, a bZIP transcriptional activator of the phloem-specific rice tungro bacilliform virus promoter, functions in vascular development. *EMBO J* 16: 5247–5259