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**RESEARCH PAPER**

# Characterization of *Arabidopsis ZIM*, a member of a novel plant-specific GATA factor gene family

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## Abstract

**The *Arabidopsis* gene *ZIM* encodes a putative transcription factor containing a novel GATA-type zinc-finger domain with a longer spacer between its two sets of conserved cysteine residues (C-$X_2$-C-$X_{20}$-C-$X_2$-C). In *Arabidopsis*, ZIM and homologous proteins, ZML1 and ZML2, were identified as GATA factors containing the C-$X_2$-C-$X_{20}$-C-$X_2$-C motif, a CCT domain, and an uncharacterized conserved domain. Proteins that possess this domain structure were found exclusively in plants, indicating that they belong to a novel family of plant-specific GATA-type transcription factors. When *ZIM* was overexpressed using a CaMV 35S promoter in *Arabidopsis*, hypocotyls and petioles were elongated. The elongation phenotype was observed under all wavelengths of light tested and even in the presence of biosynthetic inhibitors of either brassinosteroid or gibberellin. In *ZIM*-overexpressing plants, *XTH33* which is predicted to function in cell wall modification was detected as an up-regulated gene by microarray analysis, and this could account for the elongation phenotype. Genes in *ZIM*-overexpressing plants were identified that were up-regulated in a tissue-specific manner, which suggests that transcriptional regulation by *ZIM* and its consequent effects are spatially controlled.**

Key words: *Arabidopsis*, cell elongation, GATA factor, microarray, transcription factor, zinc finger.

## Introduction

Transcription factors that control gene expression both temporally and spatially play important roles in the regulation of fundamental biological processes, such as development and differentiation. In sessile plants, transcriptional regulation is also important for adaptation to abiotic stresses such as drought, cold, and high salinity, and for protection from biotic stresses. Analysis of the *Arabidopsis* genome has revealed that approximately 6% of *Arabidopsis* genes encode transcription factors, a higher rate than that of *Drosophila melanogaster*, *Caenorhabditis elegans*, and yeast (The Arabidopsis Genome Initiative, 2000; Riechmann *et al*., 2000). Most plant transcription factors are putative, predicted from homology to known DNA-binding domains, and as a result little is known about their physiological and molecular functions.

Transcription factors are grouped based on their DNA-binding motifs. The zinc-finger, bZIP, bHLH, Myb, and MADS families are common to most organisms, while the AP2/EREBP and NAC families are specific to plants (Riechmann *et al*., 2000). Zinc-finger motifs are further subclassified by their specific zinc-binding structures, such as TFIIIA, GAL4, and GATA (Harrison, 1991). Dof and WRKY are additional zinc-finger subtypes which are specific to plants (Takatsuji, 1998). The GATA-type zinc-finger protein, which binds to the (A/T)GATA(A/G) sequence via a C-$X_2$-C-$X_{17}$-C-$X_2$-C motif, was originally identified in vertebrates (Evans and Felsenfeld, 1989). Subsequently, C-$X_2$-C-$X_{18}$-C-$X_2$-C and C-$X_2$-C-$X_{19}$-C-$X_2$-C motifs of this subtype were found in fungi and plants (Daniel-Vedele and Caboche, 1993; Teakle and Gilmartin, 1998). In addition, the *Arabidopsis* gene *ZIM* (*Zinc-finger protein expressed in Inflorescence Meristem*) was previously reported (Nishii *et al*., 2000), which is expressed in flowers and flower buds, coded for a novel GATA-type zinc-finger protein with a C-$X_2$-C-$X_{20}$-C-$X_2$-C motif.

In addition to its zinc-finger domain, ZIM possesses an acidic transcriptional activation domain and is localized to

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the nucleus, both hallmarks of many transcription factors (Nishii *et al*., 2000; Shikata *et al*., 2003). In this report, transgenic plants which overexpressed *ZIM* were generated and analysed in order to elucidate the roles of this potential transcription factor *in vivo*. Because of the large amount of redundancy among transcription factor families, a gain-of-function, rather than a loss-of-function, approach might be more likely to yield a visible perturbation in gene expression patterns (Riechmann and Ratcliffe, 2000; Zhang, 2003). In addition to physiological characterization, a comprehensive survey of gene expression was undertaken on the obtained overexpression plants using microarrays, which might reveal the underlying molecular mechanism.

## Materials and methods

### *Plant material and growth conditions*

*Arabidopsis thaliana* ecotype Columbia was used in this study. Plants were grown on soil in a growth chamber at 22 °C under long day conditions (LD, 16/8 h light/dark).

### *Northern hybridization analysis*

Total RNA was prepared by using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) or Sepasol-RNA I (Nacalai Tesque, Kyoto, Japan). RNA was electrophoresed on a 1.0% agarose/formaldehyde gel and transferred to a nylon membrane (Hybond-N, Amersham Biosciences, Piscataway, NJ, USA). Probes were prepared by RT-PCR amplification using the following primer sets; ZIM249 (5′-ATCGATTGGTACATTGAGGGATCTC) and ZIM-T3 (5′-CGGTTACGGTTTCTATAATACATGTGATG) for *ZIM*, ZML1-F (5′-TGGAATTCTGCACATTGGTGTAGCG) and ZML1-R (5′-AAGAGCTCAAACTTGTCCCTGGAAGG) for *ZML1*, ZML2-F (5′-CCGAATTCTGCACATTAGAGAAGCTC) and ZML2-R (5′-TTGAGCTCACTGATCTCCTTGTTCAC) for *ZML2*, XTH33-F (5′-AGAGATGATTGGACGATCCAGACG) and XTH33-R (5′-GCAGGCATGACTTTGTATCTTGGC) for *XTH33*, and ACT8a (5′-ATGAAGATTAAGGTCGTGGCA) and ACT8b (5′-TCCGAGTTTGAAGAGGCTAC) for *ACT8*. Full length *ZIM* cDNA (DDBJ accession number AB035310, At4g24470) was also used as a probe for *ZIM*. The probe used for *SEP3* was prepared from a cDNA identified during the construction of the *Arabidopsis* equalized cDNA library microarray. The *SEP3* cDNA fragment corresponds to the region containing the I and C domains and the 3′-UTR sequence. Probes were $^{32}$P-labelled using Strip-EZ DNA (Ambion, Austin, TX, USA), and hybridizations were performed according to standard procedures (Church and Gilbert, 1984).

### *Production of* ZIM*-overexpressing plants*

The *ZIM* coding region was amplified by PCR from *ZIM* cDNA as a template with 5′-*Bam*HI (5′-CCGGATCCTCTCTATATCCTTCA-ATCAATGTG) and 3′-*Sac*I (5′-CCCGAGCTCTATCTTCTGATT-CTTATCACAG) primers. The PCR product was digested with *Bam*HI and *Sac*I, and ligated to the corresponding restriction site of pBI121 (Jefferson *et al*., 1987) replacing the β-glucuronidase gene.

The construct was introduced into *Agrobacterium tumefaciens* strain C58C1 rif/pGV2260, and then into *Arabidopsis* ecotype Columbia by vacuum infiltration (Bechtold *et al*., 1993). Transgenic lines were selected on germination medium (GM; Valvekens *et al*., 1988) agar plates containing 50 mg l$^{-1}$ kanamycin.

### *Measurements of hypocotyl length*

Seeds were surface-sterilized and sown on GM agar plates lacking sucrose. Plates were placed in the dark at 4 °C for 3 d. Germination was induced by a white light exposure for 2 h and plates were placed in the dark at 22 °C for 1 d. Seedlings were grown under continuous white (160 μmol m$^{-2}$ s$^{-1}$), red (10 μmol m$^{-2}$ s$^{-1}$), far-red (20 μmol m$^{-2}$ s$^{-1}$), or blue (6 μmol m$^{-2}$ s$^{-1}$) light, or in the dark for 5 d. The hypocotyl length was measured with imaging analysis software (Image-Ana LITE, Omron, Kyoto, Japan).

### *Application of phytohormone biosynthesis inhibitors*

Eight-day-old seedlings on GM agar plates lacking sucrose were transferred to GM agar plates with 1% sucrose containing paclobutrazol (Wako, Osaka, Japan) or Brz220 (Sekimata *et al*., 2002) following 8 d growth. The petiole lengths of the third and fourth leaves were measured. Brz220 was kindly provided by Dr Asami (RIKEN, Wako, Japan).

### *Microarray analysis*

Total RNA from shoot apices and leaves of 20-d-old plants was prepared as described above, then purified by LiCl precipitation. An *Arabidopsis* equalized cDNA library microarray (Ando *et al*., 2004) and an oligonucleotide microarray (Agilent Arabidopsis 1 Microarray, Agilent Technologies, Palo Alto, CA, USA) were used. Hybridization was performed once per microarray.

For fluorescence labelling of the *Arabidopsis* equalized cDNA library microarrays, 30 μg of total RNA was reverse transcribed with Cy-3 and Cy-5 labelled dUTP (Amersham Biosciences) using Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA). The labelled probes were purified using a PCR Purification Kit (Qiagen) and used for microarray hybridization.

Each microarray was preincubated in a prehybridization solution (4× SSC, 1% BSA, 0.25% SDS) at 37 °C for 1 h. For hybridization, the labelled probe was combined with 25 μg of poly(A)$^+$ RNA (Roche, Mannheim, Germany) and hybridization solution (4× SSC, 10× Denhardt's solution, and 0.25% SDS) to a final volume of 25 μl. This mixture was applied to the prehybridized microarray, covered with a cover slip (45×24 mm, Matsunami, Osaka, Japan) and incubated in a humid chamber at 65 °C for 16 h. After incubation, the microarray was washed with 1× SSC and 0.2% SDS, 0.2× SSC and 0.2% SDS at 55 °C, and 0.2× SSC at room temperature. After washing, the slides were dried by spinning at 240 *g* for 10 min.

The fluorescence intensities were measured using a ScanArray 4000XL (GSI Lumonics, Oxnard, CA, USA) and analysed using QuantArray (GSI Lumonics). The normalization of the two channels with respect to signal intensity was achieved by adjusting the level of signal intensities of internal control genes, *ACT2* and *ACT8*.

For the oligonucleotide microarrays, 20 μg of total RNA was used. Preparation of fluorescent probe, hybridization, washing, and scanning were performed according to the manufacturer's protocols.

## Results

### *ZIM belongs to a novel group of GATA-type zinc-finger proteins*

The zinc-finger motif of ZIM displays a high degree of similarity to other GATA-type zinc-finger proteins, not only at the four conserved cysteine residues but also at flanking residues (Nishii *et al*., 2000). A sequence homology search revealed that there are ~30 *Arabidopsis* proteins with GATA-type zinc-finger domains. Phylogenetic analysis of these zinc-finger domains showed

that ZIM and two homologous proteins, ZML1 (ZIM-like 1) for At3g21175 and ZML2 (ZIM-like 2) for At1g51600, belonged to a different group from the other typical GATA-type proteins, including the characterized GATA factors, GATA-1 to -4 (Teakle *et al*., 2002) (Fig. 1A). The zinc-finger domains of ZIM, ZML1, and ZML2 possess 20 residues between the two cysteine pairs, while those of the other *Arabidopsis* GATA factors all have 18. This suggests that the C-$X_2$-C-$X_{20}$-C-$X_2$-C motif may recognize a distinct DNA sequence. ZIM displays 44% identity with both ZML1 and ZML2, which share 75% identity with each other. In addition to their similarity at the zinc-finger domains, the phylogenetic relationship among these proteins is also supported by the presence of two other highly conserved segments (Fig. 1B). One is the CCT domain, which has been observed in CONSTANS (CO), CO-like (COL), and TIMING OF CAB 1 (TOC1), and predicted to function in protein–protein interaction and nuclear localization (Robson *et al*., 2001). Another conserved domain present near the amino-terminus of ZIM has not been characterized to date.

Putative proteins with the C-$X_2$-C-$X_{20}$-C-$X_2$-C motif were found from The TIGR Gene Indices (http://www.tigr.org/tdb/tgi) (Quackenbush *et al*., 2000). In addition to the zinc-finger motif, these proteins in plants also possess both the CCT domain and the uncharacterized conserved domain (Fig. 1B). The amino-terminal region of ZIM is rich in acidic amino acid residues that are important to its function as a transactivation domain (Shikata *et al*., 2003). Although the sequences themselves are not conserved, the corresponding regions of ZIM-like proteins are also rich in acidic amino acid residues (Fig. 1B). The structural similarities of these proteins indicate that they belong to a novel family of GATA-type transcription factors. ZIM is a member of GATA factors found exclusively in plants.

### *Spatial expression analysis of* ZIM*,* ZML1*, and* ZML2

*ZIM* was originally isolated from *Arabidopsis* as a result of its pronounced expression during the reproductive phase, and *ZIM* mRNA was detected in flowers and flower buds (Nishii *et al*., 2000). To analyse the role of the *ZIM* gene, detailed expression analysis of *ZIM* in vegetative (14-d-old) and reproductive (30-d-old) phases was performed. *ZIM* mRNA was detected in all tissues examined, although relatively higher expression was observed in shoot apices, inflorescences (flowers and flower buds), and roots (Fig. 2). In the reproductive phase, *ZIM* was most strongly expressed in inflorescences, as observed previously (Nishii *et al*., 2000). The expression pattern of *ZML1* and *ZML2* was also characterized using probes specific for these genes. Overall, *ZML1* and *ZML2* displayed similar expression patterns to *ZIM* (Fig. 2), implying that they function redundantly or co-operatively.

### *Transgenic plants overexpressing* ZIM *show enhanced petiole and hypocotyl cell elongation*

Transgenic *Arabidopsis* plants were generated which expressed *ZIM* under the control of the cauliflower mosaic virus (CaMV) 35S promoter to provide an insight into the role of *ZIM*. Three independent lines homozygous for a single insertion of the T-DNA were obtained. Expression of *ZIM* in the transgenic plants (ZIM-ox) was much higher than in wild-type plants (Fig. 3A). Since the independent lines displayed roughly the same phenotype, line S11 was chosen for further analysis.

In ZIM-ox plants, the hypocotyls and petioles were increased in length (Fig. 3B, C). Observation by scanning electron microscopy indicated that the hypocotyl elongation was due not to increased cellular numbers but rather to cell elongation (Fig. 3D, E). In contrast to the petiole elongation, the leaf blades were, if anything, slightly smaller than that of the wild type (Fig. 3C). Under short day conditions (8/16 h light/dark), ZIM-ox plants showed a severe phenotype marked by much more elongated petioles with small leaf blades (data not shown).

Hypocotyl elongation is generally inhibited by light. Photoreceptor mutants such as *phyA*, *phyB*, and *cry1* fail to inhibit elongation in far-red, red, and blue light, respectively (Reed *et al*., 1993; Nagatani *et al*., 1993; Parks and Quail, 1993; Ahmad and Cashmore, 1993). On the other hand, mutants such as *hy5* which function after light signal integration from different photoreceptors develop elongated hypocotyls in any wavelength of light (Koornneef *et al*., 1980). This was the case with the ZIM-ox plant, which developed the elongated hypocotyl phenotype under all light wavelengths of light tested; red, far-red, and blue as well as white light (Fig. 4). This phenotype did not result from a general increase in cell elongation, because ZIM-ox hypocotyls were as long as wild-type hypocotyls when grown in the dark. In addition, the ZIM-ox hypocotyl lengths were shorter under light conditions than in the dark, indicating that the response to light was still active. These results suggest that the overexpression of *ZIM* affects signal transduction following integration of the photoreceptor-mediated light signals.

Gibberellins (GAs) and brassinosteroids (BRs) are known to affect cell elongation (Hooly, 1994; Clouse and Sasse, 1998). To investigate whether ZIM-ox elongation was due to up-regulation of GA and/or BR pathways, the inhibitory effects on petiole elongation of the compounds paclobutrazol (PAC) and Brz220, that are biosynthesis inhibitors of GAs and BRs, respectively, were examined. In the presence of either PAC or Brz220, petiole elongation was inhibited in both wild-type and ZIM-ox plants (Fig. 5). This implies that *ZIM* overexpression does not result in constitutively active GA or BR signalling. ZIM-ox petioles, however, were still longer than the wild type in the presence of the inhibitors (Fig. 5). Together,

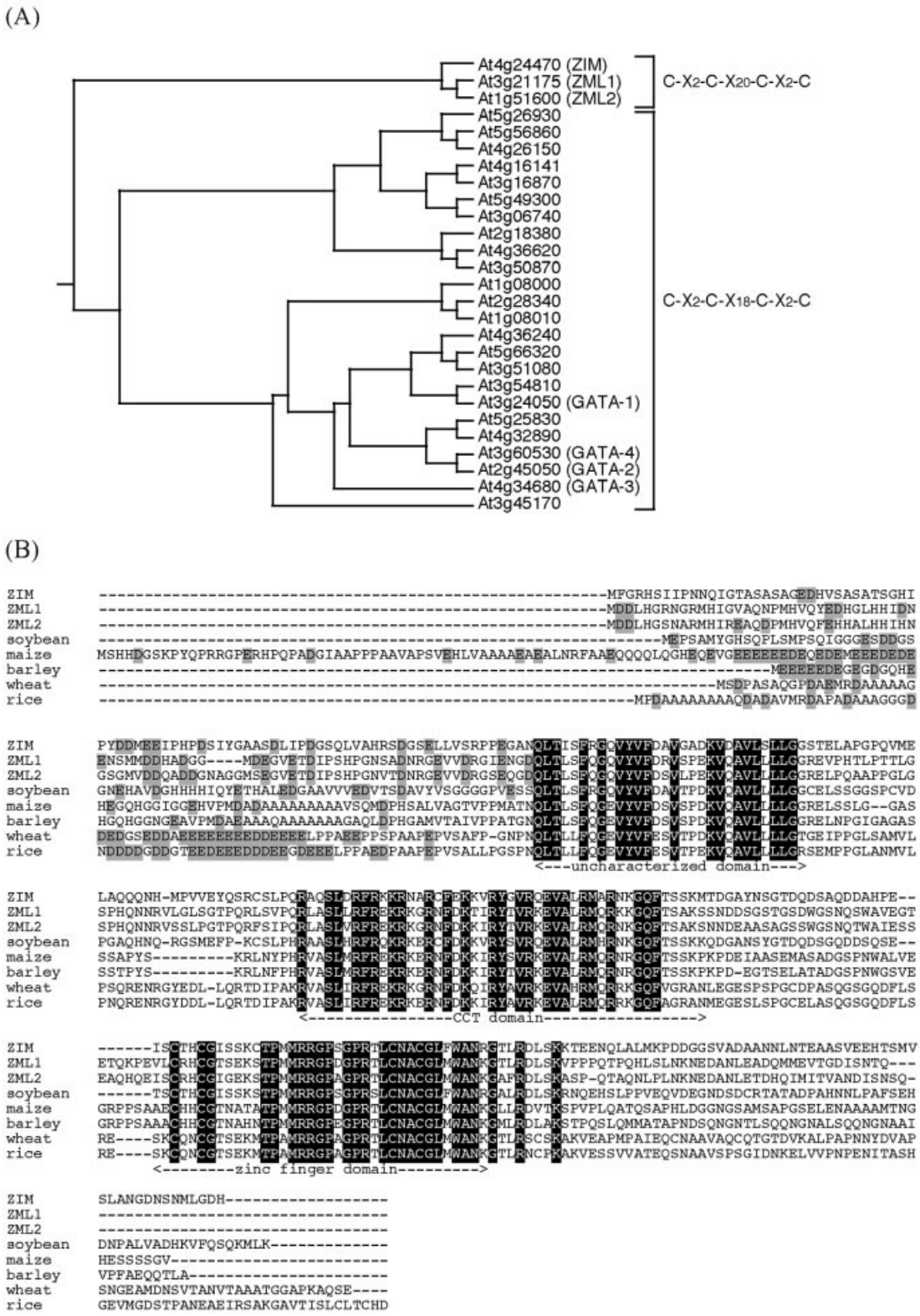


**Fig. 1.** Phylogenetic relationship within the *Arabidopsis* GATA-type zinc-finger family. (A) Cladegram of the *Arabidopsis* GATA-type zinc-finger domains. Segments corresponding to the zinc-finger domains in (B) were aligned by CLUSTAL W (http://clustalW.genome.ad.jp/). The tree was constructed by the Neighbor–Joining method. Motifs are shown on the right. (B) Alignment of ZIM-like protein sequences. ZIM, ZML1, and ZML2 are aligned with the amino acid sequences predicted for ESTs from soybean (The TIGR Gene Indices number TC152145), maize (TC181526), barley (TC71761), wheat (TC73736), and rice (TC117187). Acidic amino acid residues in amino-terminus and identical amino acids are indicated by grey and black boxes, respectively. The three conserved domains are indicated by arrows.

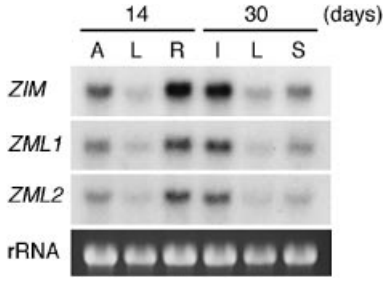


**Fig. 2.** Northern hybridization analysis of *ZIM*, *ZML1*, and *ZML2*. Total RNA (10 μg) was prepared from 14-d-old and 30-d-old plants grown under LD conditions. RNA blots were probed with gene-specific probes as described in the Materials and methods. Ethidium bromide staining of the 25S rRNA was used to indicate similar RNA loading in each lane. A, shoot apices; L, leaves; R, roots; I, inflorescences; S, stems.

these observations indicate that petioles of ZIM-ox plants are elongated by a mechanism independent of GAs and BRs.

In addition to the elongation phenotype, ZIM-ox plants displayed an upward leaf position (Fig. 3F). *Arabidopsis* has open rosette leaves during the day and directs its leaves upward at night and this leaf movement is controlled by the circadian clock (Barak *et al*., 2000). Although ZIM-ox exhibited defects in leaf opening, the position of the leaves at night was more upward, indicating that leaf movement itself was not impaired (data not shown).

### *Microarray analysis of gene expression in ZIM-ox*

Microarray analysis was performed to screen for putative ZIM target genes as well as for genes responsible for the ZIM-ox elongation phenotype, by comparing the expression patterns of wild-type and ZIM-ox plants. Gene expression in shoot apices and leaves were analysed separately, and the focus was on genes up-regulated in ZIM-ox, as ZIM is a transcriptional activator (Shikata *et al*., 2003). Among the 6400 clones in an *Arabidopsis* equalized cDNA library microarray (Ando *et al*., 2004), 42 genes were found to be up-regulated more than 2-fold in ZIM-ox; three of these genes were shoot-apex-specific, 38 were leaf-specific, and one was up-regulated in both shoot apices and leaves. The genes specifically up-regulated in shoot apices included *SEPALLATA3* (*SEP3*), which was found to be expressed in floral whorls 2–4, where it determines the identities of petals, stamens, and carpels (Mandel and Yanofsky, 1998; Pelaz *et al*., 2000). The remaining genes up-regulated in shoot apices were annotated as encoding putative proteins.

Another round of microarray analysis using a commercially available oligonucleotide microarray (Agilent Technologies, 14 000 clones) was performed using the leaf RNA preparation. On these arrays, over 600 genes showed more than 2-fold up-regulation in ZIM-ox compared with wild type, including 27 genes whose ratios were

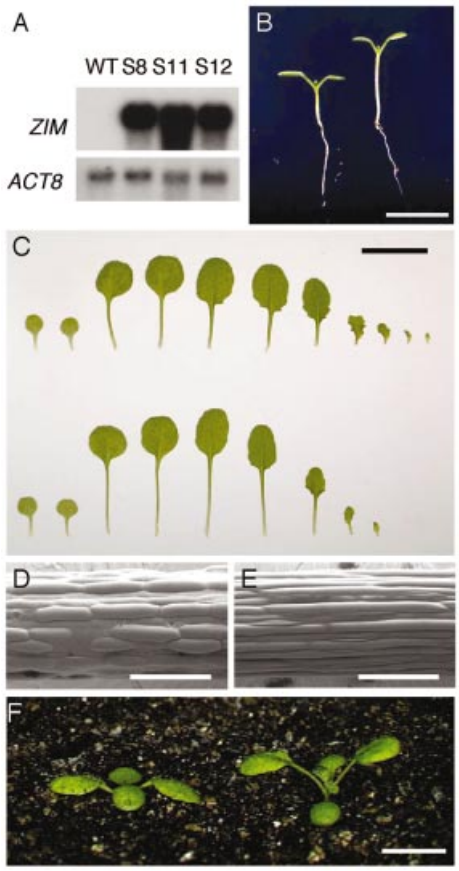


**Fig. 3.** Phenotypic analysis of *ZIM*-overexpressing plants. (A) Northern hybridization analysis of *ZIM* expression in transgenic plants. Total RNA was prepared from 16-d-old plants and 2 μg was loaded per lane. Lines S8, S11, and S12 were transgenic lines harbouring single copies of the homozygous transgene. *ACT8* was used to indicate similar RNA loading in each lane. (B–F) Phenotypes of ZIM-ox plants. Plants were grown under LD conditions. (B) Seven-day-old wild-type (left) and ZIM-ox (right) seedlings. (C) Cotyledons and rosette leaves of wild-type (upper) and ZIM-ox (lower) 17-d-old plants. (D, E) Epidermal cells of hypocotyls from wild-type (D) and ZIM-ox plants (E). (F) Ten-day-old wild-type (left) and ZIM-ox plants (right). Bars represent 1 mm (B), 1 cm (C), 200 μm (D, E), and 5 mm (F).

greater than 5-fold (Table 1). The most up-regulated gene was *XTH33* (30-fold), which encodes a putative xyloglucan endotransglucosylase/hydrolase. Other members of the *XTH* family, *XTH15*, *XTH16*, and *XTH30*, were also up-regulated by 5.1-, 3-, and 2.5-fold, respectively. Up-regulation of these genes could account for the observed cell elongation in ZIM-ox plants, as some *XTH* members have been found to play a role in cell wall modification (Rose *et al*., 2002).

In addition to the *XTH* genes, there were functionally related groups which were found to be up-regulated. For example, *EXL6* (At1g75930, 6.8-fold), At1g28570 (2.9-fold), At2g19060 (2.5-fold), At2g24560 (2.2-fold), At1g29670 (2.2-fold), At2g19010 (2.1-fold) and At1g74460 (2.1-fold), are all family II lipases with GDS(L) motifs (Arpigny and Jaeger, 1999), while

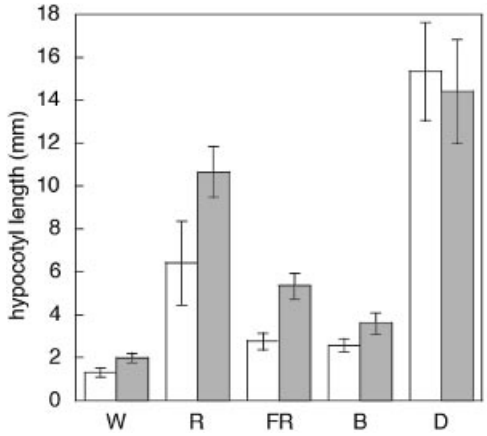


**Fig. 4.** Hypocotyl length under various wavelengths of light. Plants were grown under continuous white (W), red (R), far-red (FR), blue (B) light, or in the dark (D) for 5 d and hypocotyl lengths were measured. Open and grey bars represent wild type and ZIM-ox, respectively. Values are means ±SD of 15–24 plants.

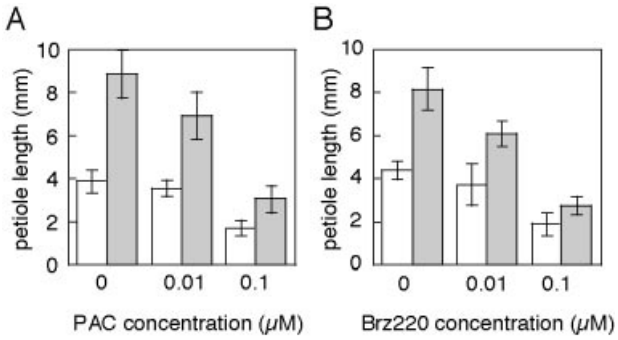


**Fig. 5.** Inhibition of petiole elongation by GA and BR biosynthesis inhibitors. Eight-day-seedlings were grown on GM plates with paclobutrazol (PAC) (A) or Brz220 (B) for 8 d. The petiole lengths of the third and fourth leaves were measured. Open and grey bars represent wild type and ZIM-ox, respectively. The means ±SD of six leaves in three plants are illustrated.

At5g63230 (5.8-fold), *BG2* (At3g57260, 5.7-fold), *BG3* (At3g57240, 4.8-fold), and At3g55430 (2.6-fold) encode β-1,3-glucanases (Dong *et al*., 1991).

To confirm the microarray data, northern blot analysis was performed for *SEP3* and *XTH33*. These genes were, as expected, found to be much increased in ZIM-ox plants (Fig. 6). Although *ZIM* is expressed abundantly in both shoot apices and leaves in ZIM-ox plants, *SEP3* was up-regulated specifically in shoot apices and *XTH33* specifically in leaves. These results indicate that the downstream targets of *ZIM* are regulated in a tissue-specific manner.

## Discussion

ZIM belongs to a novel GATA-type zinc-finger family, along with ZML1 and ZML2, whose members share the highly conserved C-$X_2$-C-$X_{20}$-C-$X_2$-C motif, the CCT domain, and the uncharacterized domain (Fig. 1B). This family is widely distributed in plants. When *ZIM* was overexpressed under the control of the CaMV 35S promoter in *Arabidopsis*, clearly distinguishable phenotypes were observed. They provide insight into the role of *ZIM*. An overexpression approach is taken to avoid redundancy problems often experienced when studying with transcription factors. Indeed, neither *ZIM* T-DNA tagged lines nor antisense transgenic lines exhibited any apparently visible phenotype (data not shown). However, it should be noted that overexpression analysis does not necessarily get to the real biological function. Zhang (2003) described that overexpression phenotypes are usually affected either by the same function as the endogenous gene with higher activity (hypermorphs) or by a new function that is not present in the wild type (neomorphs). The phenotypes observed in *ZIM*-overexpressing plants might represent hypermorph phenotypes, since *ZIM* mRNA was detected in all the tissues tested (Fig. 2). *ZIM* overexpression in plants resulted in hypocotyl and petiole cell elongation, and upward positioning of leaves (Fig. 3). These observations in ZIM-ox plants are reminiscent of the responses of *Arabidopsis* to darkness. In ZIM-ox, however, hypocotyl elongation was still inhibited by light (Fig. 4), and expression of light-regulated genes which were up-regulated by light were not lower than that in the wild type (data not shown). These indicate that light signalling was active and that the *ZIM* overexpression affects the regulation of elongation and leaf positioning rather than the regulation of light response and signalling.

GAs and BRs positively regulate cell elongation, and the application of these molecules results in the elongation of hypocotyls, petioles, epicotyl, peduncles, and other tissues (Hooly, 1994; Clouse and Sasse, 1998). The *spy* mutant, in which GA signalling is constitutively active, displays enhanced hypocotyl and stem elongation (Jacobsen and Olszwski, 1993). Plants that overproduce BR or have constitutively active BR signalling demonstrate the elongation of both petioles and leaf blades (Choe *et al*., 2001; Wang *et al*., 2001, 2002). GAs and BRs are also suggested to be involved in the upward movement of leaves, although the underlying mechanism by which they regulate this process is unknown (Jacobsen and Olszwski, 1993; Gocal *et al*., 2001; Arteca and Arteca, 2001). The ZIM-ox plant, however, was sensitive to biosynthesis inhibitors of GAs and BRs, and displayed longer petioles than wild-type plants even in the presence of those inhibitors. This suggests that the elongation resulting from *ZIM* overexpression is independent of GAs and BRs.

Microarray analysis of ZIM-ox revealed a large degree of information regarding the underlying basis of the phenotype. Cell elongation is associated with cell-wall modification, and some members of the *XTH* family have been confirmed to function in cell elongation by loosening of the cell wall (Rose *et al*., 2002). These results showed

**Table 1.** *Up-regulated genes in* ZIM-ox

| Locus | Putative gene identification | ZIM-ox (a) | Wild type (b) | Ratio (a/b)[a] |
|---|---|---|---|---|
| At1g10550 | XTH33 | 3102.8 | 103.5 | 30.0 |
| At1g01680 | Hypothetical protein | 1281.5 | 83.2 | 15.4 |
| At2g23680 | Similar to cold acclimation protein WCOR413 | 333.8 | 35.1 | 9.5 |
| At1g15010 | Hypothetical protein | 4167.5 | 452.8 | 9.2 |
| At1g72240 | Hypothetical protein | 653.0 | 83.0 | 7.9 |
| At5g64750 | Putative protein | 1763.7 | 239.4 | 7.4 |
| At1g68690 | Protein kinase, putative | 885.8 | 128.8 | 6.9 |
| At1g75930 | Family II extracellular lipase 6 (EXL6) | 398.4 | 58.3 | 6.8 |
| At1g30900 | Putative vacuolar sorting receptor | 505.7 | 74.1 | 6.8 |
| At1g05340 | Unknown protein | 325.5 | 49.5 | 6.6 |
| At2g04450 | Putative mutT domain protein | 1153.0 | 177.9 | 6.5 |
| At3g48520 | Cytochrome $P_{450}$-like protein | 1232.2 | 195.8 | 6.3 |
| At3g55840 | Nematode resistance protein-like protein | 275.3 | 44.1 | 6.2 |
| At1g02340 | HFR1/REP1 | 754.4 | 126.2 | 6.0 |
| At1g24530 | Hypothetical protein | 2047.7 | 343.1 | 6.0 |
| At2g34930 | Putative disease resistance protein | 6799.3 | 1154.6 | 5.9 |
| At5g63230 | Glycosyl hydrolase family 17 | 1224.5 | 210.3 | 5.8 |
| At3g57260 | β-1,3-glucanase 2 (BG2) | 4255.9 | 746.6 | 5.7 |
| At2g45680 | Putative PCF2-like DNA binding protein | 350.7 | 62.0 | 5.7 |
| At2g41480 | Putative peroxidase | 395.1 | 70.6 | 5.6 |
| At1g30720 | Putative reticuline oxidase-like protein | 1553.3 | 277.7 | 5.6 |
| At1g76040 | Calcium-dependent protein kinase, putative (CPK29) | 253.8 | 45.9 | 5.5 |
| At3g45970 | Putative protein | 4336.1 | 841.3 | 5.2 |
| At2g27690 | Putative cytochrome P450 | 802.3 | 155.9 | 5.2 |
| At4g14130 | XTH15/XTR7 | 3508.0 | 683.5 | 5.1 |
| At2g30540 | Putative glutaredoxin | 1268.3 | 247.9 | 5.1 |
| At2g15180 | Hypothetical protein | 201.6 | 40.3 | 5.0 |

[a] Genes with ZIM-ox/wild type (a/b) ratio of 5.0 or greater are shown.

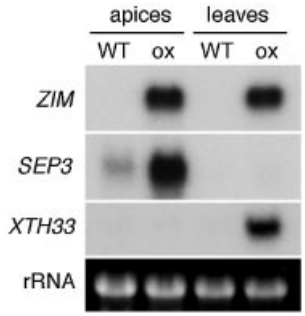


**Fig. 6.** Northern blot analysis of *XTH33* and *SEP3*. Total RNA was prepared from shoot apices and leaves of 20-d-old wild-type (WT) and ZIM-ox (ox) plants. 2 μg total RNA for *ZIM* and 10 μg for *SEP3* and *XTH33* were loaded per lane. Ethidium bromide staining of the 25S rRNA was used to indicate similar RNA loading in each lane.

that *XTH33*, *XTH15*, *XTH16*, and *XTH30* were up-regulated in ZIM-ox plants (Table 1). These genes are putatively responsible for the observed elongation phenotype. The expression of some *XTH* genes is induced by BRs and GAs. BRs have been shown to induce *XTH3*, *XTH4*, *XTH5*, *XTH17*, and *XTH22* (Xu *et al*., 1995; Yokoyama and Nishitani, 2001; Goda *et al*., 2002; Yin *et al*., 2002), and *XTH23* has been confirmed to be significantly induced by both BRs and GAs (Yokoyama and Nishitani, 2001). The *XTH* genes seem to share a diverse role in the responsiveness to phytohormones (Yokoyama and Nishitani, 2001). Interestingly, the *XTH* members up-regulated in ZIM-ox differ from those which have been reported to be downstream of BRs and GAs. This supports the finding that the mechanism of cell elongation in ZIM-ox is independent of the signalling pathways for GAs and BRs.

Northern analysis revealed that *SEP3* and *XTH33* are regulated in a shoot apex- and leaf-specific manner in ZIM-ox, respectively (Fig. 6). Most genes found to be up-regulated in ZIM-ox plants using the microarray were induced in either shoot apices or leaves, suggesting that gene expression is differentially regulated in these two tissues. The leaf phenotype of ZIM-ox also suggests that gene expression downstream of ZIM is regulated in a tissue-specific manner. ZIM-ox plants demonstrated preferential elongation of the petioles, while the leaf blades were smaller than those of the wild type (Fig. 3C). This indicates that the petioles and leaf blades possess distinct mechanisms of elongation. This is supported by physiological and genetic analyses which imply independent developmental regulatory networks in petioles and leaf blades (Gocal *et al*., 2001; Tsukaya *et al*., 2002).

How can ZIM-ox induce tissue-specific gene expression when overexpression by the 35S promoter will target high levels of ZIM protein to all tissues? This can be explained by the presence of repressors in some tissues. In leaves, CURLY LEAF (CLF) represses the expressions of *AGAMOUS* (*AG*) and *APETALA3* (*AP3*), which encode MADS proteins (Goodrich *et al*., 1997). The expression of

*SEP3*, which encodes an AG-like MADS protein, may be repressed in leaves by CLF or other factors. 35S::SEP3 plants, which are expected to overexpress *SEP3* in all tissues, show a dwarf phenotype, curled leaves, early flowering, and terminal flowers (Honma and Goto, 2001), whereas ZIM-ox plants, which overproduces *SEP3* exclusively in shoot apices, did not show such phenotypes. The difference in expression pattern between ZIM-ox and 35S::SEP3 may be the cause of these distinct phenotypes. The second explanation for tissue specificity is the presence of a differentially expressed interacting partner. ZIM, ZML1, and ZML2 all possess the CCT domain, which is found in the proteins CO, COL, and TOC1 and is predicted to be responsible for protein–protein interaction (Robson *et al*., 2001). To unravel the mechanism of tissue-specific regulation by ZIM fully, it will be helpful to investigate potential binding partners of ZIM which may act through the CCT domain. In addition to the explanation described above, the possibility of tissue-specific degradation of the ZIM protein should be taken into account since protein stability is often regulated differently in each tissue in plants.

In this report, the phenotypes resulting from *ZIM* overexpression have been mentioned in an attempt to understand the basic functions of ZIM, a member of a plant-specific GATA transcription factor. *ZIM* is most likely to function in cell elongation independently of GAs and BRs by regulating *XTH* family genes in a tissue-specific manner. This type of approach might serve to shed light on some of the general properties of this new plant-specific protein family and its characteristic C-$X_2$-C-$X_{20}$-C-$X_2$-C motif.

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