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# Identification of a root-specific glycosyltransferase from *Arabidopsis* and characterization of its promoter

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A set of Ds-element enhancer trap lines of *Arabidopsis thaliana* was generated and screened for expression patterns leading to the identification of a line that showed root-specific expression of the bacterial *uidA* reporter gene encoding  $\beta$ -glucuronidase (GUS). The insertion of the Ds element was found to be immediately downstream to a glycosyltransferase gene At1g73160. Analysis of At1g73160 expression showed that it is highly root-specific. Isolation and characterization of the upstream region of the At1g73160 gene led to the definition of a 218 bp fragment that is sufficient to confer root-specific expression. Sequence analysis revealed that several regulatory elements were implicated in expression in root tissue. The promoter identified and characterized in this study has the potential to be applied in crop biotechnology for directing the root-specific expression of transgenes.

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## 1. Introduction

The ability to drive gene expression in a tissue-specific manner is an important factor in transgenic crop biotechnology. In many cases, high-level expression may be required in certain tissues such as seeds and may be achieved by the use of a strong tissue-specific promoter such as a seed storage protein promoter (Cramer *et al* 1999). In other cases, expression of the transgene that confers the desired trait comes at a cost to the plant, and that cost may be minimized if expression of the transgene is restricted to those tissues where it is required. This applies, for example, to genes that confer tolerance to abiotic stresses (Xiong *et al* 2002) or in the engineering of male sterility where pollen development is inhibited by the expression of toxic proteins (Mariani *et al* 1991). In still other cases, it may be of interest to express antimicrobial or pesticidal proteins in certain tissues of the plant which are the sites of infection or attack but not in

other tissues such as the fruit, which may be required for human consumption.

Promoters that confer root-specific expression are of interest for engineering resistance against root nematodes (Huang *et al* 2006), or for improving tolerance to abiotic stress such as water or salt stress, by the expression of a gene that confers tolerance in a part of the plant such as the root, which is especially important with respect to that stress. Root-specific promoters also have the potential for application in the engineering of plants to improve uptake of nutrients through root tissues.

We identified and characterized a glycosyltransferase gene from *Arabidopsis* which shows root-specific expression. This suggests a tissue- or cell type-specific function. A 305 bp fragment comprising 218 bp of the promoter region of the gene confers efficient root-specific expression of a  $\beta$ -glucuronidase (GUS) reporter. The promoter sequence identified in this study has the potential

**Keywords.** Crop biotechnology; enhancer traps; nematode resistance; root expression; tissue-specific

Abbreviations used: ARF, auxin response factor; CTAB, cetyltrimethyl ammonium bromide; GAPC, glyceraldehyde phosphate dehydrogenase isoform C1; GUS,  $\beta$ -glucuronidase; MS, Murashige–Skoog; OSE, organ-specific element; PCR, polymerase chain reaction; TAIL-PCR, thermal asymmetric interlaced PCR

for application in directing the selective expression of genes in the root.

## 2. Materials and methods

### 2.1 Plant material and growth conditions

Strains of *Arabidopsis* used were derived from ecotypes Landsberg-er and Columbia-O. The plants were grown at 21°C in an illuminated growth chamber (Conviron) under cool, white fluorescent tubelights at an intensity of 8000 lux and a 16 h/8 h light/dark cycle. For growth under sterile conditions, seeds were surface sterilized with ethanol and plated on Murashige–Skoog (MS) agar medium + 2% sucrose supplemented with the appropriate antibiotic where necessary (Sundaresan et al 1995).

### 2.2 Plasmid and recombinant DNA methods

For Southern analysis of the ETC60 line, plant genomic DNA was isolated according to the cetyltrimethyl ammonium bromide (CTAB) protocol (Murray and Thompson 1980). The DNA pellet was air dried and resuspended in 100–200  $\mu$ l TE and stored at –20°C until use. For Southern hybridization, approximately 4  $\mu$ g of genomic DNA was digested with *Eco*R1 enzyme, electrophoresed and blotted onto a nylon membrane and probed using transposon sequences (Sundaresan et al 1995). The blots were washed sequentially at 65°C with 2 $\times$  SSC, 0.5 $\times$  SSC, 0.2 $\times$  SSC and finally 0.1 $\times$  SSC each time for 20 min and autoradiographed. The template DNA for polymerase chain reaction (PCR) amplification was isolated from *Arabidopsis* plants using

the Nucleon Phyt PURE kit (Amersham) following the manufacturer's protocol. Thermal asymmetric interlaced (TAIL)-PCR was carried out using the degenerate primer AD2 in combination with the nested primers Ds5-1, Ds5-2 and Ds5-3 for primary, secondary, and tertiary amplification reactions, respectively (Liu and Whittier 1995). Genomic DNA from the promoter region of At1g73160 was amplified using gene-specific primers carrying appropriate restriction sites (*Hind*III and *Sal*I), subcloned into the blunt end cloning vector pMOS (Amersham) following the manufacturer's instructions and sequenced. The cloned fragment was excised using *Hind*III and *Sal*I and further subcloned into the binary GUS reporter vector pBI101 (Clontech).

### 2.3 Plant transformation and analysis of GUS expression

Promoter::GUS constructs in the binary vector pBI101 were mobilized into the *Agrobacterium* strain AGL1 by triparental mating using *Escherichia coli* HB101 (pRK2013) as a helper. Transformation of *Arabidopsis in planta* was carried out using the method of Bechtold et al (1993). Transformants were selected by plating seeds on MS plates containing 2% sucrose and 50 mg/l kanamycin. GUS expression was analysed as described in Sundaresan et al (1995).

### 2.4 cDNA isolation and expression analysis

RNA was isolated from the roots and leaves using Trizol (Invitrogen) following the manufacturer's protocol. For cDNA synthesis, 1  $\mu$ g total RNA was treated with RNase-free DNase, heated to inactivate the DNase and reverse transcribed using oligo(dT)<sub>12-18</sub> and Superscript II (Invitrogen) in a volume of 20  $\mu$ l. The primers GLTF

**Table 1.** Sequence of primers used in this study

Primer name	Sequence
AD2	5'-NGTCGA(G/C)(A/T)GANA(A/T)GAA-3'
Ds5-1	5'-CCGTTTACCGTTTTGTATATCCCG-3'
Ds5-2	5'-CGTTCCGTTTTTCGTTTTTTACC-3'
Ds5-3	5'-GGTCGGTACGGAATTCTCCC-3'
M13 forward	5'-CGCCAGGGTTCCAGTCACGAC-3'
GLTF	5'-AATGCACCCGAAAGTCTATTTGC-3'
GLTR	5'-AAAACCCTAGGCCCATCTCTTAC-3'
GAPC1	5'-CTTGAAGGGTGGTGCCAAGAAGG-3'
GAPC2	5'-CCTGTTGTCGCCAACGAAGTCAG-3'
U1H	5'-TACCAAGCTTGACAATTTCTCTGAACGGAA-3'
D1S	5'-TGAAGTCGACGGTAATGATTGTTGTGAATCTGAATG-3'
U2H	5'-AGCAAGCTTGGTTCATGAACATTGACTGTG-3'
U3H	5'-AAACCGGAAAGCTTATTAGGTGTGATATCCCG-3'

and GLTR were used to amplify a 345 bp 3' portion of the At1g73160 cDNA. The reverse transcription reaction was diluted 1.5-fold and 1  $\mu$ l was used for PCR in a 20  $\mu$ l reaction. Reaction conditions were 94°C for 2 min, followed by 35 cycles of 94°C for 15 s, 55°C for 20 s, and 72°C for 30 s. Glyceraldehyde phosphate dehydrogenase isoform C1 (GAPC) was taken as a control and amplified under the same conditions for 25 cycles using the primers GAPC1 and GAPC2, which gave a 550 bp product.

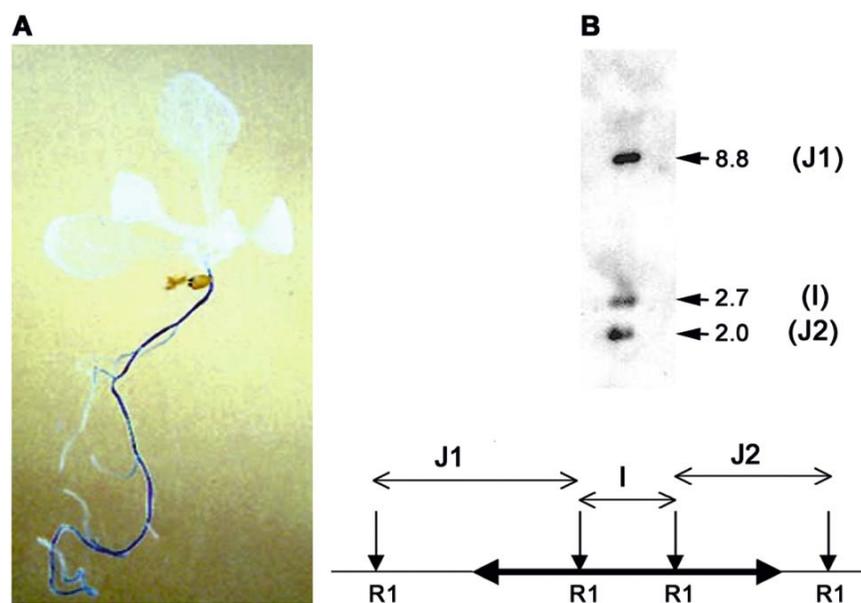
### 3. Results and discussion

Enhancer traps have been extensively used in bacteria, animals and plants to identify and study genes based on their expression pattern as well as to provide cellular markers (Bellen 1999). We employed enhancer detection to screen for genes that are expressed specifically in plant roots. A collection of approximately 400 independent enhancer trap lines of *Arabidopsis* were generated according to the protocol described by Sundaresan *et al* (1995). The enhancer trap lines carry an engineered Ds transposon bearing a *uidA* (GUS) reporter gene. The enhancer trap lines were used to screen for the expression pattern of the GUS gene in seedlings at the 4-leaf stage.

Out of the 130 lines screened for the expression pattern of the GUS reporter gene, one line, ETC60, was found to show root-specific expression (figure 1A). To assess the copy number of the transposon in ETC60, F3 seeds obtained from the primary F2 transposant were germinated on plates containing kanamycin to score for the dominant neomycin

phosphotransferase II selection marker present on the transposon. Out of 52 seedlings tested, 34 were kanamycin resistant (Kan<sup>R</sup>) and 18 were sensitive to kanamycin (Kan<sup>S</sup>). The Kan<sup>R</sup>:Kan<sup>S</sup> ratio was not significantly different from a value of 3:1, which would be expected for a single-copy insertion ( $0.25 > P > 0.1$ ). Southern analysis of the line using transposon sequences as a probe confirmed the presence of a single insertion (figure 1B).

To determine the genomic location of the Ds element in ETC60, sequences flanking the insertion were amplified by TAIL-PCR (Liu and Whittier 1995). The products obtained in the secondary and tertiary TAIL-PCR reactions are shown in figure 2A. The amplified products from the secondary and tertiary TAIL-PCR reaction were cloned into the vector pTOPO-II (Invitrogen) and sequenced. The sequence indicated identity to a portion of BAC T18K17 (Genbank Accession AC010556) comprising 194 bases from 58626 to 58819. The TAIL-PCR clone was used as a probe in Southern analysis of genomic DNA from ETC60 and wild-type following digestion with *Eco*R1 (figure 2B). The difference in mobility of the hybridizing fragment between wild-type and ETC60 confirmed that the TAIL-PCR clone represented DNA flanking the transposon insertion in ETC60. The position and orientation of the Ds insertion is shown in figure 2C. The Ds element in ETC60 is inserted in the intergenic region between At1g73160, which encodes a putative glycosyltransferase, and At1g73170, which encodes a putative ATPase. The two genes are separated by 4 kb and are transcribed in opposite directions, convergently.



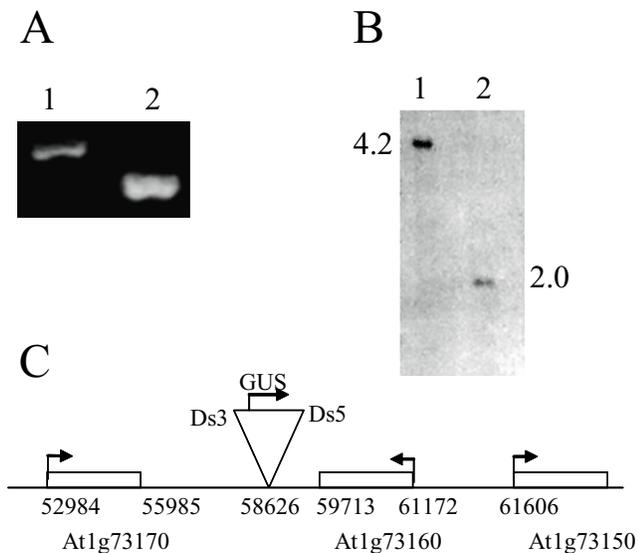
**Figure 1.** Characterization of the *Arabidopsis* enhancer trap line ETC60. **(A)** Root-specific GUS reporter gene expression. **(B)** Southern blot of *Eco*R1 digested genomic DNA of ETC60 probed with the DsE transposon. The 2.7 kb band represents an internal fragment (I) and the 2.0 and 8.8 kb bands represent the two junction fragments (J1, J2) indicative of a single-copy insertion. R1: *Eco*R1 site.

The insertion site of the Ds element is closer to At1g73160 than to At1g73170. To test the possibility that root-specific expression of the GUS reporter in ETC60 reflects the expression pattern of At1g73160, we examined the expression of At1g73160 in roots and aerial tissues by RT-PCR. Expression of At1g73160 was observed in roots but not in leaves (figure 3). The specificity of the amplicon was further confirmed by probing a blot containing the RT-PCR samples of root and leaf with a radioactively labelled probe made from a 345 bp amplicon of the At1g73160 gene obtained with primers GLTF and GLTR. A positive signal was obtained only with the root sample and not with the leaves (data not shown), which suggested that the root-specific expression pattern of ETC60 possibly reflects that of At1g73160. These observations are consistent with the microarray expression data given in the Genevestigator database (Zimmerman *et al* 2004), which suggest that At1g73160 is expressed specifically in roots. After completing the experimental part of this study, we noted that a recently updated genome annotation predicts an additional gene At1g73165, which lies between At1g73160 and At1g73170 and is close to the site of Ds insertion. The Ds insertion is 158 bp upstream of At1g73165, which is predicted to encode a CLV3-related protein ligand CLE1, 74

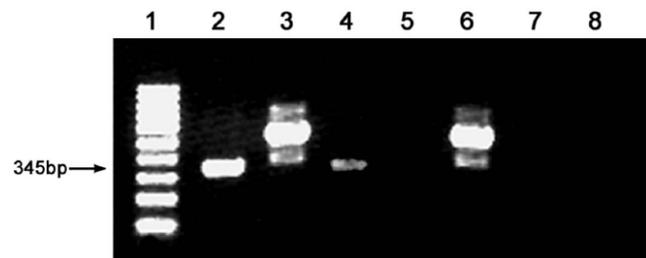
amino acids in length (Cock and McCormick 2001; Ni and Clark 2006). There is as yet no experimental evidence on the endogenous expression pattern of CLE1 in plants.

To isolate and characterize the region responsible for root-specific expression of At1g73160, we amplified and cloned a 614 bp region that encompassed the promoter of At1g73160. The amplified region included 527 bp of the DNA sequence between the annotated At1g73160 and At1g73150 genes plus the first 87 bp of the coding sequences of At1g73160. The primers used for amplification were U1H and D1S. The amplified region was cloned into the GUS reporter binary vector pBI101 (Clontech) resulting in a translational fusion of the GUS gene to the promoter of At1g73160. The resulting plasmid named pBI101U1 was introduced into the *Agrobacterium* strain AGL1 and used for *in planta* transformation of *Arabidopsis* plants.

T1 transformant seedlings were selected by germinating seeds on MS plates supplemented with sucrose (2%) and kanamycin (50 mg/l). Seventy-three independent transformants were identified, transferred to MS + kanamycin plates and grown till the 6-leaf stage. A single leaf and a section of the root were excised and stained for GUS expression. Thirty percent of the T1 plants showed root-specific GUS expression (figure 4; table 2), and the



**Figure 2.** Genomic location of Ds transposon insertion in ETC60. (A) TAIL-PCR on ETC60 genomic DNA. Lanes 1, 2: secondary and tertiary TAIL-PCR products, respectively. (B) ETC60 genomic Southern blot probed with flanking sequence to validate flanking DNA sequence cloned by TAIL-PCR. Lane 1: *Eco*R1 digested wild-type DNA; lane 2: *Eco*R1 digested ETC60 genomic DNA. (C) Schematic diagram showing the position and orientation of Ds insertion with respect to DNA coordinates of the BAC clone T18K17 from chromosome 1 of *Arabidopsis*.



**Figure 3.** Root-specific expression of At1g73160 transcript shown by RT-PCR. Lane 1: 100 bp DNA marker; lane 2: genomic DNA amplified with gene-specific primers GLTF and GLTR; lanes 3, 6: PCR with GAPC primers on root and shoot RT samples, respectively; lanes 4, 5: PCR with GLTF and GLTR primers on root and shoot RT samples, respectively; lanes 7, 8: PCR with GLTF and GLTR primers on samples of *Arabidopsis* root and shoot RNA, respectively, showing absence of amplification as a negative control.

**Table 2.** Analysis of expression patterns in promoter::GUS transformant lines

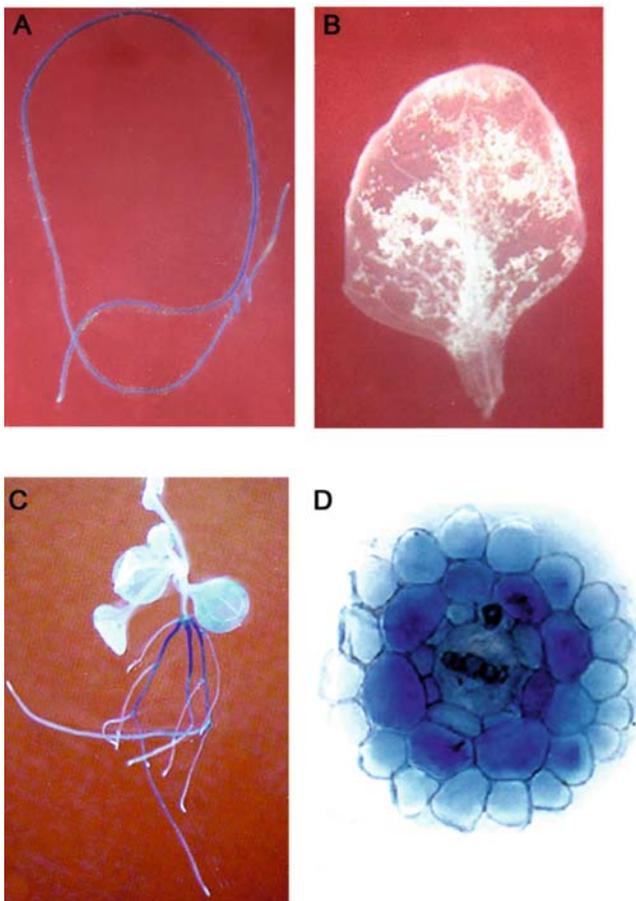
Promoter size (bp)	GUS expression in T1 transgenic plants		
	Root <sup>+</sup> Leaf <sup>+</sup>	Root <sup>+</sup> Leaf <sup>-</sup>	Root <sup>-</sup> Leaf <sup>-</sup>
614	39/73 (53%)	22/73 (30%)	12/73 (17%)
501	17/35 (48%)	15/35 (43%)	3/35 (9%)
305	17/39 (43.5%)	17/39 (43.5%)	5/39 (13%)

remainder showed either expression in both roots and leaves or in neither. Root-specific expression was also observed in the T2 generation (figure 4C). These observations indicated that the upstream region of At1g73160 is capable of directing root-specific expression, consistent with the expression pattern of At1g73160. Roots of T2 plants from a transformant line that showed root-specific expression were stained for GUS, embedded in 3% agarose and hand-sectioned. Sections were mounted on a slide and observed on a Zeiss Axioplan2 microscope. Expression was detected throughout the root and strong staining was observed in the root cortex and stele (figure 4D).

To further limit the promoter region responsible for root-specific expression, we generated two deletion derivatives of the 527 bp promoter. A 501 bp region comprising a 414 bp promoter sequence and 87 bp coding sequence was amplified using primers U2H in combination with D1S, and

a 305 bp region containing a 218 bp promoter sequence was amplified using U3H in combination with D1S. Each was used to make a translational fusion with GUS in pBI101 to give pBI101U2 and pBI101U3, respectively, followed by transformation into *Arabidopsis* as described above. Multiple, independent T1 transformants were identified and examined for GUS expression in excised leaf and root. In both pBI101U2 and pBI101U3 transgenics, we observed root-specific gene expression in 43% of T1 plants. Thus, the 305 bp fragment contains the sequences necessary to drive root-specific expression when fused to a reporter gene. The 527 bp region was analysed using the Plant CARE (Lescot *et al* 2002) and PLACE (Higo *et al* 1999) databases to identify putative regulatory elements that could contribute towards root-specific expression. The results are represented in table 3 and figure 5. Two potential TATA boxes were noted at positions -90 and -140, respectively, to the predicted translational start site (Joshi 1987). A putative transcriptional start sequence (AACATCA) occurred at -48. Also noted were seven motifs OSE1, OSE2, ASF1, RAV1, ARFAT, a core element of the SURE sequence and TAPOX1, which are associated with root expression. OSE1 and OSE2 are consensus sequence motifs of organ-specific elements (OSEs) found in promoters activated in infected cells of root nodules (Fehlberg *et al* 2005). ASF1 is a xenobiotic stress-activated transcription factor that binds to the TGACG motif and is expressed preferentially in root apical meristems (Klinedinst *et al* 2000). The TGACG motif is also found in association with as-1 in constitutive root promoters (Krawczyk *et al* 2002). RAV1 binds specifically to DNA with bipartite motifs of RAV1-A (CAACA) and RAV1-B (CACCTG). Expression levels of RAV1 were reported to be high in rosette leaves and roots (Kagaya *et al* 1999). The auxin response factor (ARFAT)-binding site is found in the promoters of primary/early auxin response genes of *Arabidopsis* (Inukai *et al* 2005). GAGAC forms a core of the sulphur-responsive element (SURE) found in the promoter of SULTR1;1 (high-affinity sulphate transporter) in *Arabidopsis* (Maruyama-Nakashita *et al* 2005). However, microarray analysis of the changes in gene expression in response to sulphur limitation suggests that At1g73160 does not appear to be regulated in response to sulphur (Zimmermann *et al* 2004). An overlapping auxin response factor (ARF) motif GAGACA is also found, and At1g73160 appears to show a slight reduction in expression in response to the auxin IAA (Zimmermann *et al* 2004). The root motif TAPOX1 'ATATT' is similar to the motif found in the root-specific tobacco *rolD* promoter and in the root-specific wheat peroxidase gene promoter (Elmayan and Tepfer 1995).

The sequences responsible for root-specific expression of At1g73160 were localized to a relatively small region comprising 305 bp (which includes 87 bp of the coding region). The intergenic region between At1g73150 and



**Figure 4.** Root-specific GUS expression in promoter::GUS transformants. (A, B) Portion of root and leaf of a T1 transgenic plant carrying 614 bp promoter of At1g73160 fused in frame to the GUS gene. (C) A T2 transgenic plant showing root-specific GUS gene expression. (D) Transverse section of transgenic plant root. GUS expression is concentrated in the cortex and vascular bundles.

**Table 3.** Putative root motifs found in the upstream region of At1g73160

Root motif	Sequence <sup>a</sup>	Position <sup>b</sup>	Reference
ASF1 MOTIF CAMV	TGACG (+)	-275 to -271	Krawczyk et al (2002)
ARFAT	TGTCTC (-)	-474 to -469	Ulmasov et al (1999)
OSE1	AAAGAT (+)	-75 to -70	Fehlberg et al (2005)
OSE2	CTCTT (-)	-175 to -171	Fehlberg et al (2005)
RAV1AAT	CAACA (+)	-49 to -45	Kagaya et al (1999)
SURE core	GAGAC (+)	-474 to -470	Maruyama-Nakashita et al (2005)
TAPOX1	ATATT (-)	-239 to -235	Elmayan and Tepfer (1995)

<sup>a</sup> '+' and '-' indicate the coding and non-coding strand, respectively.

<sup>b</sup> Coordinates are relative to the predicted translation start site.

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-527 GACAATTTCTCTGAACGGAAAATTTTAAGGTTTCTCTAGAGATTTTACGCACA GAGACAGGTAATTGGGGATTTCT
-452 GAAACAATGAATCGAAGATCGGGGAGATTAGGATCCGTGGTTCATGAACATTGACTGTGTTTGC AACGACGAAAC
-377 CCTAGGAAGCACAGCGTCGCAAGAATAGCGTAGACACACGCGCATTATCTCACGCGCCTAAAGAAACCCGATAAA
-302 TTAGGGGAATTACACATGTGTAAAGTGTGTGACGGAGTAATCACACGCCTACGTGTACAAAACAAAAATATCAAACCG
-227 GGAAAATAATTAGGTGTGATATCCCGGTTTAGTTACAAATCTCAGTTGGTT AAGAGGAGATTTGACTTTCAATTG
-152 AATGTAAAACCATATAAAAATTAGCTAAGCCATGTGCAATGTCATTGAGAAATGAACTTTGATATAATAACTCG
-77 AGAAAGATCCGAAATTTAAGCAAAGAATCAACATCAATAGTTGCACTCAATTCACAAAAATCAAATAAACCAAAA
-02 CAatggccttcaagaaccattcttcaagaacaactctttcttcttcttcttcttccacttcattttcattcagattca
+74 caacaatcattacc

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**Figure 5.** Identification of root motifs in the At1g73160 promoter. Root motifs given in table 3 are shown in bold letters. Underlined sequences are putative TATA boxes. A putative transcription start site at -48 occurs within the sequence AACATCA, which matches the consensus given in Joshi (1987).

At1g73160 was only 434 bp, hence it was not surprising that the root-specific promoter region is small. A significant number of independent transgenic lines (about 40%) carrying the GUS reporter driven by the At1g73160 promoter showed root-specific expression, indicating that the promoter functions efficiently and confers root-specific expression in a large fraction of the transformants. Root-specific expression was also observed in T2 progeny of primary T1 transformants. Staining was observed throughout the length of the root but was stronger in the primary root compared with secondary roots. This was the case both in the ETC60 enhancer trap line and in the promoter::GUS fusion transformants. Within the root, strong staining was seen in the cortex and in the stele, indicating a broad pattern of expression covering most layers of the root.

At1g73160 is an intronless gene spanning 1460 bp. It shows strong similarity to two other *Arabidopsis* genes: At5g59070 (E-value:  $2 \times 10^{-94}$ ) and At4g19460 (E-value:  $1 \times 10^{-120}$ ) which, based on microarray analysis, also express in root tissue of *Arabidopsis thaliana* in addition to being expressed elsewhere (Zimmermann et al 2004). All three belong to family 1 of the glycosyltransferases. At4g19460 is predicted to be 2536 bp long with 2 introns, whereas At5g59070 is predicted to be 2092 bp long and contains one intron. Based on the digital northern data of the Genevestigator database

(Zimmermann et al 2004), At4g19460 is expressed in the shoot apex, flower, stamen, carpel, silique, seed and in adult leaves apart from root tissue. At5g59070 expresses in rosette leaves and stems apart from root tissue. In the case of At1g73160, expression is restricted predominantly to root tissue. As all three genes are expressed in root tissue, their upstream regions were analysed using the PLACE database (Higo et al 1999) to determine if they harboured any common regulatory elements that are known to confer expression in roots. Elements implicated in directing expression in roots were identified. These include ASF-1, RAV1AAT, Root motif TAPOX1, TELO, OSE1 and OSE2, ARFAT, SURE core sequence and SP8BF elements (figure 6). The TELO motif is found in the *Arabidopsis* eEF1A A1 gene promoter and is also found in the 5' region of genes encoding components of the translational apparatus. It is implicated in the activation of gene expression in root primordia and root meristems (Tremousaygue et al 1999). SP8BF is a nuclear factor that binds to the 5' upstream regions of three different genes coding for major proteins of sweet potato tuberous roots (Ishiguro and Nakamura 1994). The At1g73160 promoter sequence contained one site each for the Root motif TAPOX1, OSE1, OSE2, ASF1, RAV1, ARFAT and SURE core elements. At4g19460 harbours one site each for the ASF1, OSE1 and SURE core sequences, two

sites for SP8BF, three for RAV1AAT and five for TAPOX1 elements of which 3 are present within 500 bp upstream of the predicted translational start site. At5g59070 has one site each for the TAPOX1, RAV1, OSE1, TELO elements, two sites for ASF1, and three sites each for the OSE2 and SURE core elements. Thus, all three root-expressing glycosyltransferase family 1 members contain common regulatory DNA motifs, which are implicated in conferring root expression.

The occurrence of such elements (three for ASF1, four of each for RAV1 and Root motif TAPOX1) has also been noted within a 435 bp proximal region of the strong root-specific 1'2' promoter of the mannopine synthase (*MAS*) gene of *Agrobacterium tumefaciens* (Feltkamp *et al* 1995). The ASF1 motif has also been found in a cryptic root-specific promoter from *Arabidopsis* (Mollier *et al* 2000). However, another cryptic root-specific promoter from *Arabidopsis* appears to lack any known elements implicated in root-specific expression (Sivanandan *et al* 2005). Different motifs have been found in other root-specific promoters such as the tobacco RB7 promoter (Yamamoto *et al* 1991) and the related FaRB7 promoter from strawberry (Vaughan *et al* 2006). Other examples of root-specific promoters that have been described include the PHT1 family promoters from

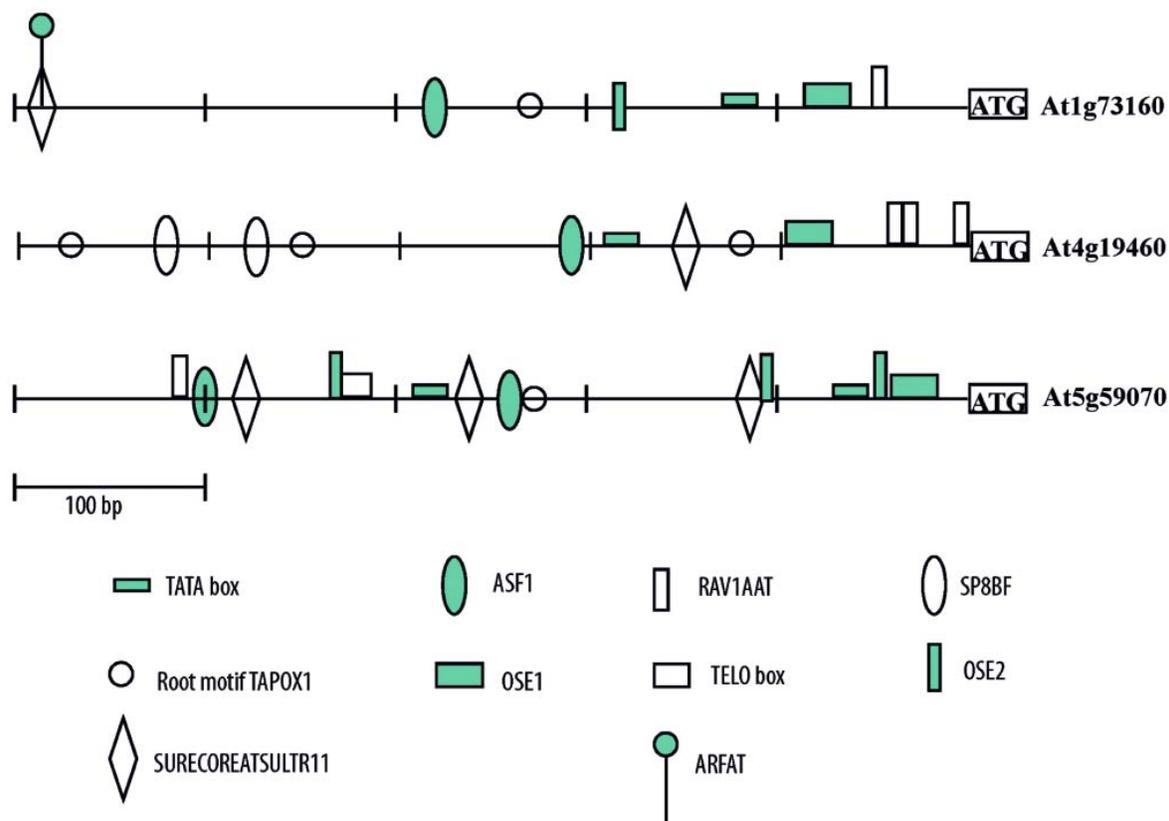
*Arabidopsis* (Mudge *et al* 2002; Karthikeyan *et al* 2002). A PHT1 promoter has also been shown to confer root-specificity in rice (Koyama *et al* 2005).

#### 4. Conclusions

We isolated and characterized a root-specific promoter from the At1g73160 gene of *Arabidopsis*, which encodes a root-specific glycosyltransferase. Sequences sufficient for directing efficient root-specific expression were localized to a 218 bp portion of the region upstream of the promoter. The isolation and characterization of root-specific promoters that could be used to engineer improved nutrient uptake, stress tolerance and resistance to soil-borne pathogens in a range of cultivated plant species is likely to be of continued interest.

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**Figure 6.** Location of putative root motifs in At1g73160 and in related glycosyltransferases At1g59070 and At4g19460 gene promoters in *Arabidopsis*.

and PV was a postdoctoral fellow of the Department of Biotechnology, New Delhi.

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