Available online at www.sciencedirect.com
SCIENCE DIRECT®
Plant Science xxx (2006) xxx–xxx
PLANT SCIENCE
www.elsevier.com/locate/plantsci

Enhancer trapping in woody plants: Isolation of the ET304 gene encoding a putative AT-hook motif transcription factor and characterization of the expression patterns conferred by its promoter in transgenic *Populus* and *Arabidopsis*

Sergei A. Filichkin [a], Qian Wu [b], Victor Busov [c], Richard Meilan [d], Carmen Lanz-Garcia [b], Andrew Groover [e], Barry Goldfarb [b], Caiping Ma [a], Palitha Dharmawardhana [a], Amy Brunner [a], Steven H. Strauss [a,*]

[a] *Department of Forest Science, Oregon State University, Corvallis, OR 97331, United States*
[b] *Department of Forestry, North Carolina State University, Raleigh, NC 27695-8008, United States*
[c] *School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI 49931, United States*
[d] *Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN 47907, United States*
[e] *Institute of Forest Genetics, Pacific Southwest Research Station, USDA Forest Service, Davis, CA 95616, United States*

Received 19 January 2006; received in revised form 12 March 2006; accepted 22 March 2006

Abstract

Enhancer trapping is a useful tool in isolation of novel genes and functional characterization of promoters directing tissue-specific expression in trees. Using an enhancer trap approach we isolated a novel gene *ET304* from *Populus* (*Populus*: aspens and cottonwoods). Both ET304 enhancer trap line and putative *ET304* promoter fused to a *GUS* reporter gene conferred strongly root-predominant expression patterns and directed expression to specific root tissue and cell types. GUS activity was detected in lateral root primordia, root apical meristem, elongation zone and cortex. *ET304* promoter sequence contained a canonic auxin response element (AuxRE) located upstream of the enhancer trap insertion site. In a synchronized lateral root induction system *ET304* promoter conferred an auxin-responsive expression in newly emerging lateral roots of both poplar and *Arabidopsis*. A detailed histochemical examination of poplar transgenics showed that *ET304* promoter was highly active in actively growing lateral roots, their primordia and to a lesser extent—in secondary meristems of aerial organs rich in free endogenous auxin. These results were consistent with the expression profiling of ET304 mRNA in various tissues of mature poplar trees. The analysis of *Populus* genomic sequence suggested that ET304 represents a large family of putative transcription factors containing a conserved AT-hook motif and DNA binding domain.

Keywords: *Populus*; Aspen; *Arabidopsis*; Enhancer trap; AT-hook; Lateral roots

1. Introduction

Gene trap [33] and enhancer trap (ET) [12,35] are powerful tools for isolation and characterization of tissue-specificity of novel plant genes. Enhancer trapping was successfully used in tagging of vascular-expressed genes in *Populus* [15]. We applied the ET approach for identification of new genes with root-predominant expression patterns in poplar trees.

Genus *Populus* (poplars, including aspens and cottonwoods) is considered to be the model taxon for tree genomics and biotechnology [5,6,9,34]. *Populus* is unique among woody plants genera in the following array of experimental attributes: (1) a small genome, approximately 480 Mbp, 4× larger than *Arabidopsis*, and 40× smaller than pine; (2) rapid juvenile growth, allowing fast phenotypic assessments; (3) facile clonal propagation allowing precise evaluation of genetic manipulations and wide distribution of stocks; (4) numerous genetic markers and maps to aid trait dissection and map-based gene cloning; (5) large collections of publicly available expressed

* Corresponding author. Tel.: +1 541 737 6578; fax: +1 541 737 1393.
E-mail address: steve.strauss@oregonstate.edu (S.H. Strauss).

0168-9452/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.plantsci.2006.03.011

sequence tags; (6) high-throughput transformation/regeneration [16]. The availability of the genomic sequence (http://genome.jgi-psf.org/poplar1/poplar1.home.html) makes *Populus* especially well suited for detailed comparative studies of new gene families.

Roots have fundamental biological significance for plant nutrient and water uptake, plant support, carbon sequestration [10]. The frequency and dominance of lateral roots are major determinants of root architecture and biomass accumulation below ground. Despite of the interest for phytoremediation and carbon sequestration, only a few genes that are differentially expressed during root development were studied in woody plants [4,6]. To our knowledge, no root predominant promoters with characterized tissue and cell type specificity have been isolated to date from *Populus* species.

We identified an individual *Populus* ET line with an insertion in a promoter region of *ET304* gene encoding a protein containing a conserved AT-hook motif and DNA binding domain. At least 19 gene models in *P. trichcarpa* genome predict proteins containing AT-hook motif adjacent to the conserved DNA biding domain similar to ET304.

Isolated *ET304* promoter directed strong auxin-dependent expression of the reporter gene during initiation and development of adventitious roots.

Promoters that direct cell-specific expression or silencing of target genes in roots will help to clarify the roles played by genes that control root development. A collection of root promoters with distinct expression patterns, similar to those generated for *Arabidopsis* [27], is needed for such applications. Studies of promoters active during lateral root primordium (LRP) initiation have to date employed cell-cycle [20] or cell type-specific [27] promoters transcriptionally fused to β-glucuronidase (*GUS*) [20,27] or green fluorescent protein (*GFP*) [3,24] reporter genes.

Because of the novelty and potential biotechnological application of *ET304* promoter, its structure and behavior were described in detail, including expression patterns in transgenic poplar and *Arabidopsis* and in response to the synchronous auxin induction during lateral root development.

2. Materials and methods

2.1. Plant material, bacterial strains and growth conditions

The female hybrid aspen clone INRA 717-1B4 (*Populus tremula* × *P. alba*) was used for the production of all transgenic poplars, including the collection of 674 enhancer trap lines [15]. Enhancer trap line 304 (ET304) was isolated as described previously [44]. The Columbia 0 ecotype was used for all *Arabidopsis* transformations.

For tissue type expression analysis, samples were collected from wild *P. trichocarpa* and field grown *P. trichocarpa* clone Nisqually-1; both located at Corvallis, Oregon, USA. Male flowers and female post-pollinated catkins were collected in March. Female and male newly initiated inflorescence buds were collected in June. Vegetative tissues leaf, roots, shoot apex, axillary buds, xylem and phloem were collected between April and August. Xylem and phloem (including the cambium) were sampled from 2-year-old actively growing tree. Seeds from wild *P. trichocarpa* were germinated at 25 °C by placing surface-sterilized-seeds on filter paper soaked with sterile water in the Petri dishes. After 24 h incubation in the dark seedlings were exposed to light and harvested at 24 and 43 h time points post-imbibition.

Agrobacterium tumefaciens strain C58/pMP90 (GV3101), a disarmed derivative of the nopaline C58 strain [23], was transformed by the freeze–thaw method [22]. *Escherichia coli* strain Top 10 (Invitrogen Co., San Jose, CA) was used in all cloning procedures. In vitro-grown poplar plantlets served as the source of explants. Micro-cuttings were cultured on hormone-free 1/2 strength Murashige–Skoog (MS) medium [29]. Shoot cultures were maintained on MS medium and grown at 25 °C under a 16 h light photoperiod. Light was provided by fluorescent tubes (TL70, F25T8/TL735, Philips, Atlanta, GA) at a photon flux density of 45 μeinstein m^{-2} s^{-1}. Vitamins, growth regulators, and antibiotics were filter-sterilized and added to the media after autoclaving. All media were adjusted to a pH 5.6–5.8 before autoclaving, except Luria–Bertani (LB) broth [43] and induction medium (IM) for *Agrobacterium* [16], which were adjusted to pH 7.0 and 5.0, respectively. *Arabidopsis* was grown in a Percival growth chamber AR66LC8 (Percival Scientific Inc., Perry, IO) at 18 °C and 85% relative humidity under a 16 h photoperiod. Plant transformation procedures followed are described elsewhere [11].

In the auxin-induction experiments, *Arabidopsis* seeds were germinated and grown on MS-sucrose (1%) medium supplied with either 10 μM naphthaleneacetic acid (NAA) (Sigma, St. Louis, MO) or 10 μM *N*-1-naphthylphthalamic acid (NPA). Poplar plantlets were grown hydroponically in propagation medium (0.25 g l^{-1} MES, 0.1 g l^{-1} myo-inositol, 2.15 g l^{-1} MS, 0.2 g l^{-1} L-glutamine, 20 g l^{-1} sucrose, 1 mg l^{-1} nicotinic acid, 1 mg l^{-1} pyridoxine hydrochloride, 1 mg l^{-1} calcium pantothenate, 1 mg l^{-1} thiamine hydrochloride, 1 mg l^{-1} L-cysteine, 0.5 mg l^{-1} biotin, pH 5.8) containing 20 μM NAA or 20 μM NPA.

In auxin-induction experiments the 3–5 mm long stem explants of *pET304::GUS* transgenic plantlets were placed on callus induction medium (CIM), CIM media without NAA, or CIM media supplied with 10 μM NAA and 10 μM NPA. After 3 weeks incubation in dark at 25 °C, explants were stained for GUS and photographed as described below.

Arabidopsis transformation was performed by a modified floral dip method [8]. Kanamycin-resistant plants were selected by incubating plants for 10–14 days on 1% (w/v) Phytagar (Invitrogen) plates containing 4.3 g l^{-1} MS salts, 3% (w/v) sucrose, 25 mg l^{-1} kanamycin, and 200 mg l^{-1} timentin.

2.2. Vector construction

The pBSGUSNOS vector was assembled using the *E. coli* β-D-glucuronidase (*uidA*) gene and 3′-polyadenylation signal (terminator) from the nopaline synthase gene (*NOS*) of *A. tumefaciens*. A 2.1 kbp *GUS-tNOS* cassette was amplified using

the following primers: 5′-GGGCCCATGGTCCGTCCTGTAGAAACCC-3′ and 5′-GGTACCGCGGCCGCGATCTAGTAACATAGATGACACCGCG-3′ (*Apa*I and *Not*I restriction sites are underlined).

The fragment of putative *ET304* promoter encompassing approximately 3 kbp of the sequence upstream of ET304 open reading frame was amplified from 717-1B4 genomic DNA using the following PCR primers: 5′-GGGGGGGATCCGAACACTCCCCAGATTCACAAGTACTTTAGAG-3′ and 5′-GGGGATCGATGGTTGTCAAAGGATCACAAACAAGATGCGTC-3′ (*BamH*I and *Cla*I restriction sites are underlined). The sequence of obtained PCR fragment was nearly identical to the genomic sequence upstream of the gene eugene3.00130441_1 [461–1399] LG_XIII (*ET304*) from chromosome 13 of *P. trichocarpa* (clone Nisqually-1) (not shown). The PCR fragment was digested with *BamH*I and *Cla*I restriction endonicleases and cloned into the corresponding sites of pBSGUSNOS. Approximately, 5.1 kbp *Not*I fragment containing the *pET304::GUS::tNOS* cassette was cloned into the pART 27 binary vector [13].

A *Ds* transposon-based enhancer trap transformation vector used for poplar transformation was described previously [15].

2.3. Amplification of genomic DNA

The presence of the transgene in both poplar and *Arabidopsis* lines was confirmed by PCR using *npt II*-specific primers (5′-ATCCATCATGGCTGATGCAATGCG-3′ and 5′-CCATGATATTCGGCAAGCAGGCAT-3′) and *GUS*-specific primers (5′-TGGGCATTCAGTCTG-3′ and 5′-GTGATATCGTCCACCCA-3′) to amplify 253 and 509 bp of T-DNA insertion, respectively.

2.3.1. RT-PCR

Total cellular RNA from poplar tissues was isolated using Qiagen Plant mini RNA isolation kit according to the manufacturer's protocol (Qiagen Inc., Valencia, CA, USA). Isolated RNA was treated with RNase-free DNase (Ambion, Austin, TX, USA). cDNA was synthesized using 1 μg of total RNA, poly(A) oligonucleotide and Invitrogen first strand cDNA synthesis kit (Invitrogen Co., Carlsbad, CA, USA) according to the manufacturer's protocol.

Two hundred and eighteen base pairs ET304 (eugene3.00130441, chromosome 13) and 238 bp ET304-like (gw1.X.5544.1, chromosome 10) cDNA fragments were amplified using primer pairs 5′-GTGGTAGGAGGCAGTGTGG-3′/5′-ACTGGCATTGATGTTGCTG-3′ and 5′-GCCTGATCCCTCATCTATGC-3′/5′-TAGGCGTCATGCCCTAATTGCTGA-3′, respectively.

2.3.2. Real time RT-PCR

RNA was isolated using Qiagen kit as described above and its integrity, concentration and rRNA ratio (28S/18S) were estimated using Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Palo Alto, CA, USA). Prior to cDNA synthesis, total RNA samples were treated with RQ1 RNase-free DNAse I according to the manufacturer's protocol (Promega, Madison, WI, USA) and desalted using Microcon-100 spin columns (Millipore Corporation, Bedford, MA, USA).

Reverse transcription reactions were performed in 20 μl reactions containing 0.6 μg total RNA, 200 U SuperScript™ II RNase H⁻ reverse transcriptase and 5 μM random hexamer primers according to the manufacturer's protocol (Invitrogen). Reactions excluding enzyme were used as negative controls. ET304 PCR primers 5′-CCAATGAAAAAGCCAGATCTAGGT-3′ and 5′-CCACTCTCGGTGGCTTCTCT-3′ were designed for quantitative RT-PCR using ET304 cDNA sequence and Primer Express software (Perkin-Elmer Biosystems, Norwalk, CT, USA). *Populus* ubiquitin cDNA was amplified as internal standard using primers 5′-TGTACTCTTTTGAAGTTGGTGT-3′ and 5′-TCCAATGGAACGGCCATTAA-3′. Each primer was used at a concentration of 200 nM and primer pairs amplified a single product as shown by dissociation curve analysis of the amplicons. Amplification efficiencies were 99.8% and 99.6%, for ET304 and ubiquitin, respectively.

All PCRs were performed in 25 μl volumes using Power SYBR Green PCR Master Mix and the ABI prism 7900 Sequence Detection System (PE-Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. cDNA prepared from 5 ng of total RNA was used as template. Reaction conditions for thermal cycling were 95 °C for 10 min, 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Two independent biological replicates were run for each time point, each with three technical replications. Results were analyzed using SDS 2.1 software (PE-Applied Biosystems).

Expression data were analyzed using the $2^{-\Delta\Delta C}{}_T$ method [26] with data normalized to ubiquitin as the internal control gene and relative to time point zero (prior auxin treatment).

2.4. Promoter motif search

Searches of known promoter motifs were performed using PLACE [18,19] and PlantCARE [25,36] databases via websites: http://www.dna.affrc.go.jp/htdocs/PLACE/ and http://intra.psb.ugent.be:8080/PlantCARE/, respectively.

2.5. Microscopy

For GUS staining, tissues were incubated for 6–12 h in 2 mM X-Gluc solution [43]. After staining root tissues were examined under a dissection microscope directly. Alternatively, root tissues were clarified by subsequent treatments with hydrochloric acid and sodium hydroxide [27,28] dehydrated in 20–50% (v/v) ethanol series, and stored in 40% (v/v) glycerol, 5% (v/v) ethanol solution. Whole root samples were mounted on glass slides in glycerol solution (described above) and photographed under a dissecting microscope.

For sectioning GUS-stained root tissues were fixed for 4 h at ambient temperature in 4% paraformaldehyde, and for 4 h in 4% formaldehyde, 50 mM phosphate buffer, pH 7. Samples were dehydrated in ethanol series and embedded in Technovit 7100 resin (Heraeus Kulzer, Wehrheim, Germany) according to manufacture's protocol. Six to seven micrometers sections were mounted on slides and examined using an Axioscope 20 (Carl

Table 1
Transformation frequencies and activity of *GUS* reporter gene driven by *ET304* promoter in primary transformants of *Populus*

Construct: *pET304::GUS*	Total number of individual Kan^R events confirmed by PCR	GUS staining			
		Root	Root tip	Leaf	Stem
Populus	40	36 (90%)	36 (90%)	3[a] (8%)	2[a] (5%)

[a] Weak GUS staining was observed with low frequency at random spots.

Zeiss Microimaging Inc., Thornwood, NY) with dark-field settings.

3. Results

3.1. Generation of Populus transgenics

Agrobacterium-mediated transformation with *pET304::GUS* vector yielded 40 kanamycin-resistant independent events (Table 1). Genomic DNA of all events tested positive for *NPTII* gene after PCR amplification. The majority of individual lines showed histochemical GUS staining associated predominantly with root tissues of young poplar plantlets grown in vitro (Table 1). In addition to roots, a small proportion of the *pET304::GUS* plants showed weakly stained spots on their leaves and stems.

3.2. GUS expression driven by the pET304 promoter in Populus

Examination of 40 independent poplar *pET304::GUS* transgenic lines demonstrated that GUS activity was consistently localized in young adventitious roots, mainly in RAM, lateral root (LR) tips, elongation zones and to a lesser extent around junctions of the main and lateral root. GUS staining was especially intense in emerging lateral root primordia (LRP) (Fig. 1). Older roots frequently displayed ''mosaic'' GUS staining in small-dispersed groups of cells. Root caps and root hairs typically showed little or no GUS staining. The longitudinal sections of junctions between the main and lateral root showed GUS patterning in the cortex of LR and in individual cells surrounding the vasculature of the main root. These cells were apparently associated with pericycle and/or endodermis layers.

In addition to roots, weak GUS staining was associated with meristems of axillary vegetative buds of the majority of 6-month-old *pET304::GUS* lines grown in green house conditions (Fig. 1). The weak GUS activity in lateral buds of in vitro grown plantlets was not consistent among all transgenic lines and was frequently observed only after shoot apex removal (data not shown).

3.3. Comparison of GUS patterns in enhancer trap line ET304 and pET304::GUS transgenic lines

The individual clones of enhancer trap line ET304 were grown in tissue culture propagation medium without hormones as described in Section 2. Intense GUS activity was mainly restricted to emerging LRP and root tips of adventitious roots (Fig. 1). GUS activity observed throughout the elongation zone was associated with cells and with cells from the cortex, endodermis and pericycle. GUS staining was also evident at the junctions between the main and lateral roots. The overall GUS staining pattern of the original ET304 line was essentially similar to that of *pET304::GUS* lines, with the exception of more intense staining in ET304 main root cortices. Enhancer trap line ET304 also had a characteristic feature that was commonly observed in poplar and *Arabidopsis pET304::GUS* transgenics; intensive GUS staining was localized in a distal portion of the root apical meristem, contrasting with weaker staining in the root cap.

3.4. Expression pattern of pET304::GUS in Arabidopsis

To further characterize the root-specific expression pattern directed by the *pET304* promoter, the *pET304::GUS* construct was transformed into *Arabidopsis.* Seedlings of a total of 76 independent kanamycin-resistant T_1 lines were stained for GUS. Sixty-eight out of 76 (90%) of the transgenic lines demonstrated a root-predominant GUS expression pattern (Table 2). Approximately, 10% of the examined lines had no visible GUS staining in any tissues, suggesting that they were selection escapes, the transgene was silenced, and/or that the T-DNA insert was rearranged.

Expression of the GUS-reporter gene was examined in detail in different tissues of transgenic *Arabidopsis*. Intense GUS

Table 2
Transformation frequencies and patterns of GUS expression in *Arabidopsis* transgenic lines of T_1–T_3 generations

Generation of *pET304::GUS Arabidopsis* transgenics	Total number of Kan^R events/lines	Root	Root apical meristem	Lateral root	Leaf (excluding stipules)	Stem	Stipules
T_1 primary transformants	76	68 (90%)	68 (90%)	68 (90%)	0 (0%)	0 (0%)	68 (90%)
Segeregating T_2, lines grown on Kan media	11	11 (100%)	11 (100%)	11 (100%)	0 (0%)	0 (0%)	11 (100%)
Homozygous T_3 lines	3 (100%)	3 (100%)	3 (100%)	3 (100%)	0 (0%)	0 (0%)	3 (100%)

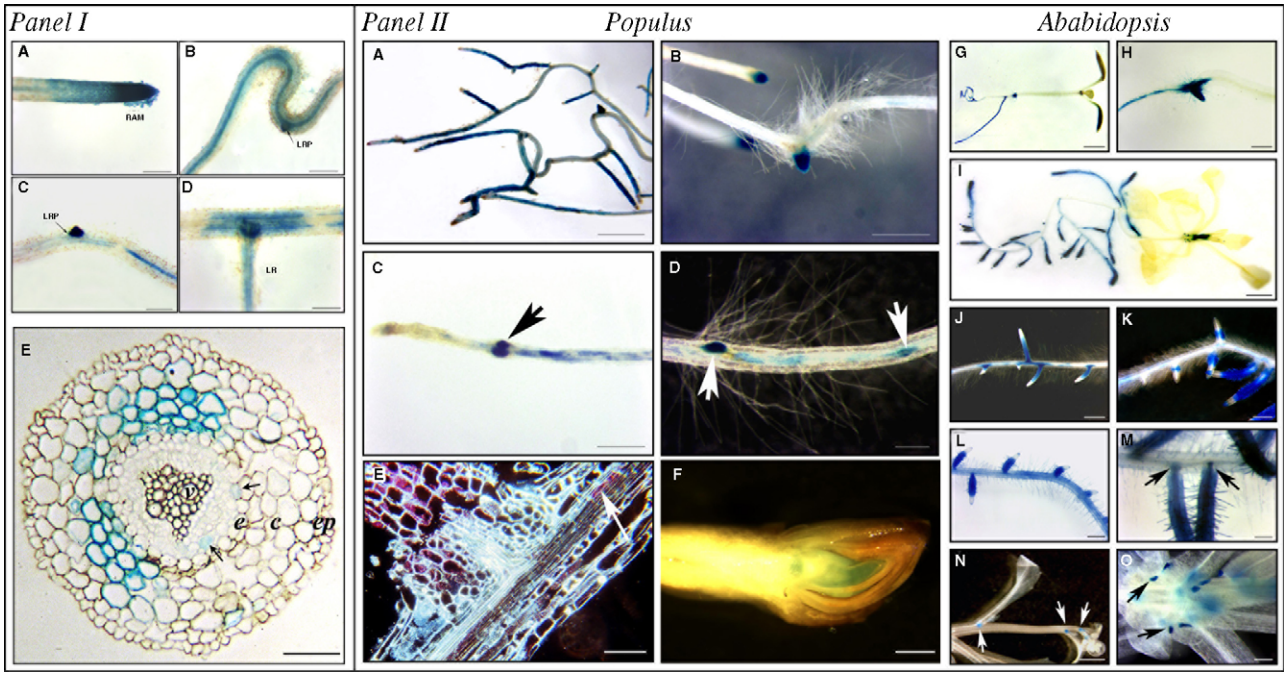

Fig. 1. Panel I: Histochemical localization of GUS activity in adventitious roots of the poplar enhancer trap line ET304. A: Root tip; B and C: emerging lateral root primordia (LRP); D: junction between the main root and the lateral root (LR); E: a cross-section of ET304 lateral root showing GUS staining in the cells of endodermis (*e*), pericycle (arrows), and cortex (*c*); vascular bundle (*v*), epidermis (*ep*). Whole root mounts in A–D were clarified after GUS staining using sodium hydroxide. Young lateral root of the ET304 line was fixed and 7 μM sections across the cluster of cells showing GUS staining were prepared as described in Section 2. Scale bars = 0.3, 0.5, 0.4, 0.3, and 0.05 mm for A–E, respectively.Panel II: Expression of the *GUS* reporter gene driven by *ET304* promoter in transgenic *Populus* and *Arabidopsis*. A–F: *Populus*. A: General view of GUS expression pattern in the adventitious roots of approximately 8 weeks old *pET304::GUS* transgenics grown in vitro. GUS staining is localized predominantly in lateral roots. The intensive staining is evident in apical meristem and elongation zone of lateral roots. GUS activity is also present at the lateral root junctions. B: GUS staining of LR tips. C and D: GUS staining of newly emerging (C) and established (D) lateral root primordia. LRP are shown by arrows. Dispersed clusters of cells expressing GUS are visible in C. E: A longitudinal section of the lateral root junction. Dark field microscopy. GUS staining is visualized as purple color. Arrow indicates GUS staining in the vasculature of the main root. F: Weak GUS staining in axillary vegetative bud from a 6-month-old green house grown *pET304::GUS* line. Scale bars = 3.0, 0.6, 0.4, 0.2, 0.09 and 1 mm in A–F, respectively. G–O: *Arabidopsis*. G: Intense GUS staining is evident in lateral roots but not in a primary root (except RAM). H: GUS staining of a single adventitious LRP emerging at the junction of hypocotyl and primary root in 10 days old seedlings. I: General view of a 15 days old seedling. GUS staining is associated with root tips, distal portions of RAM and elongation zones of the main and lateral roots. J–M: The junctions between the vascular bundle of the main root and elongation zone of the lateral root exhibit a continuous pattern of GUS staining (indicated by arrows). The characteristic pattern of low GUS expression in proximal parts of root tips is evident in J–L. A continuous pattern of GUS staining between vasculature of lateral root and main root junctions is visible in M (shown by arrows). N: GUS staining in zones surrounding secondary meristems (indicated by arrows) in leaf axils of 18 days old plant. O: Intense GUS staining of stipules (shown by arrows). Diffuse GUS staining is also visible around secondary meristems. All seedlings were germinated and grown on MS-sucrose medium without hormones. Scale bars = 1.7, 0.5, 1.5, 0.6, 0.3, 0.5, 2.8, and 0.2 mm for G–O, respectively.

staining was observed in elongation and apical zones of lateral roots, emerging LRP, tips of the primary root, and the RAM (Fig. 1). Strong GUS activity was evident in adventitious root primordia emerging at the junction of the hypocotyl and primary root. In contrast, the distal portion of RAM showed weaker GUS staining. In the elongation zone GUS staining appeared to be associated with cortical endodermis and/or pericycle cells.

In addition to root tissues, intense GUS staining was observed in stipules located on both sides of the leaf base. Weak, diffuse GUS staining was also associated with the shoot apex and areas located in leaf axils. These areas were likely to represent secondary, accessory meristems that later give rise to lateral flowering shoots [14,17] GUS staining in the zones surrounding secondary meristems diminished with time but remained clearly visible until full plant maturity (not shown). No significant GUS activity was detected in stems, leaves, inflorescences, siliques and other tissues (data not shown).

To demonstrate stable inheritance, the seeds from 11 independent events of T_1 generation were plated on kanamycin-containing medium. A random selection of 125 kanamycin-resistant (Kan^R) seedlings from 11 tested lines showed a similar root-predominant pattern after staining for GUS activity. The number of Kan^R and kanamycin-sensitive (Kan^S) seedlings was calculated at 10 days after germination (DAG). In six of the 12 lines studied, statistical analysis of T_2 progeny satisfied the χ^2 goodness of fit test at the 5% significance level, suggesting a simple Mendelian inheritance pattern in these lines (data not shown). Two independent T_3 lines that did not segregate in the presence of kanamycin were considered homozygous for the transgene locus and used for auxin induction experiments.

3.5. Prediction of putative cis-acting elements in pET304 promoter

Two pairs of TATA and CAAT boxes – common *cis*-acting elements in eukaryotic promoter and enhancer regions – were

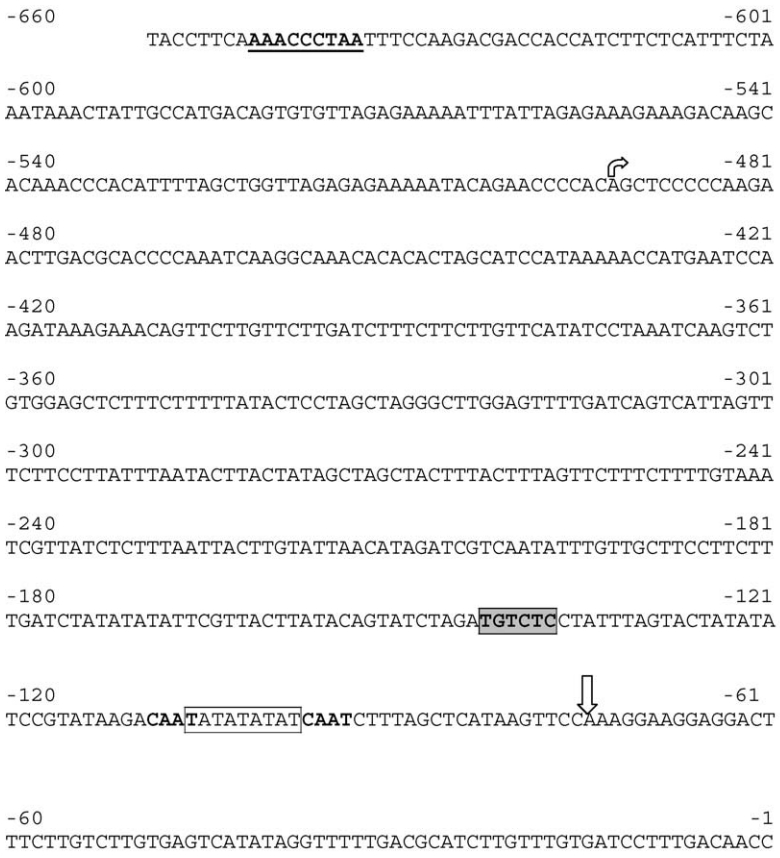

Fig. 2. Computer-predicted structure and putative regulatory sequences of *pET304* promoter. The canonical auxin response element TGTCTC is shown in bold in shaded box. Telomere motif AAACCCTAA (''telo-box'') required for activation of expression in the root primordia is shown in bold and underlined. The nucleotides of two predicted CAAT boxes are shown in bold. Two adjacent TATA boxes are boxed. Transcription initiation site is indicated by bent arrow. Enhancer trap insertion site of ET304 line truncating *pET304* promoter at position −75 is indicated by a vertical arrow.

identified using the PlantCARE database [25]. TATA boxes (at position −106 to −98) and CAAT boxes (at positions −97 to −94 and −109 to −106) were adjacent to each other and located in close proximity to the putative initiation codon (Fig. 2).

The computer search of known plant promoter motifs using PLACE database [18,19] indicated that the *pET304* sequence contains the canonic auxin response element TGTCTC [39,41] located at position −143 to −138 upstream of the start ATG codon. A putative telomere motif implicated in plant root development, AAACCCTAA (''telo-box''), was found between positions −641 and −632. The insertion site of the enhancer trap has truncated the *pET304* promoter in poplar line ET304 at position −75, placing TATA and CAAT boxes, auxin response element and ''telo-box'' upstream of the minimal 35S promoter. The sequence flanking the insertion site is described in detail in [44]).

3.6. Evaluation of ET304 promoter inducibility by auxin using a synchronized auxin-inducible lateral root formation system

Auxin is well established as a major factor regulating root development. The arrest of lateral root growth caused by *N*-1-naphthylphthalamic acid (NPA) – an inhibitor of acropetal auxin transport – can be rescued by addition of exogenous NAA [7]. A synchronous auxin-inducible system of LR formation based on these phenomena was recently developed and used for gene expression profiling in *Arabidopsis* [20,21]. To examine how the *pET304* promoter responded to auxin activation, we modified this LR induction system (LRIS) to examine GUS accumulation in LR of both poplar and *Arabidopsis pET304::GUS* transgenic lines.

To block formation of LR seedlings of two homozygous *Arabidopsis pET304::GUS* lines were grown on MS medium containing 10 μM NPA inhibitor as described in Section 2. To induce synchronous LR initiation seedlings were then transferred for 3 days to medium supplied with 10 μM NAA. The number of initiated lateral roots and patterns of GUS expression were compared for NPA-treated control plants before and after auxin rescue (Fig. 3). No lateral roots or LRP formation was observed after NPA treatment. The GUS activity in NPA-treated control was detected only in a primary root tip. When multiple lateral roots were initiated after auxin treatment, strong GUS staining was observed both in RAM and in LRP (Fig. 4). This suggests that the expression directed by *pET304* was synchronized with LR initiation and development, and could be inhibited by NPA. In contrast, NPA was not able to inhibit entirely *pET304* activity in the RAM.

Similar results were obtained in experiments employing an NPA block of lateral root development for poplar

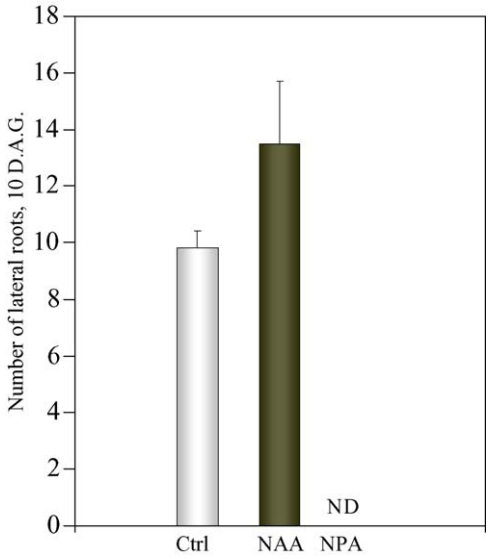

Fig. 3. The initiation of lateral roots growth using synchronized LRIS in *pET304::GUS Arabidopsis*. Each value represents the average number of LR per seedling. CTRL, seedlings were germinated and grown on hormone-free MS-sucrose medium. NPA, seedlings were germinated and grown in the presence of 10 μM NPA. NAA, seedlings were germinated and grown the presence of 10 μM NPA for 7 days followed by transfer on medium containing 10 μM NAA for 3 days. ND, not detected. Bars denote standard error of the mean for measurements of 10 seedlings.

pET304::GUS lines. *pET304* promoter activity was predominantly limited to apical meristems of roots developed prior to treatment with 20 μM NPA (Fig. 4). Only faint residual GUS staining remained visible in elongation zone of roots treated by NPA for 20 days. NAA treatment resulted in initiation of multiple LRP showing intense GUS staining. A dense chain of emerging LRP was connected by apparently continuous ''strings'' of GUS-expressing cells. These cells were apparently associated with cortical and/or possibly pericycle layers. The transfer of NPA-treated poplar roots to 20 μM NAA for 7 days resulted in synchronous activation of GUS expression in LRP and in cell clusters, as described above.

To evaluate auxin response of the *pET304* promoter in tissue culture the stem explants from three independent poplar *pET304::GUS* lines were placed on (a) hormone-free, (b) NAA- or (c) NAA/NPA-containing media as described in Section 2. No callus growth or GUS staining was detected in the explants grown on hormone-free and NAA/NPA-containing media (Fig. 4). In contrast, the explants grown in the presence of 10 μM NAA produced callus with intense GUS staining suggesting induction of *pET304* promoter by auxin. The plantlets consequently regenerated from these calli on SIM medium showed typical LR and RAM-specific GUS patterns described above (data not shown).

3.7. The relative levels of ET304 mRNA synthesis in response to auxin-induction

Acuumulation of ET304 mRNA in poplar roots was quantified before and after auxin induction using LRIS and the real time RT PCR. The time points after NAA treatment corresponded to 3, 6, 12, 24, 48 and 72 h post-induction. ET304 mRNA synthesis increased approximately two- and 1.5-fold at 6 and 12 h post-NAA induction, respectively (Fig. 5) and decreased to approximately pre-induction levels after 24 h of treatment. Detection of ET304 mRNA at zero time point was consistent with the observed basal GUS activity in root apical meristems formed prior to NPA treatment.

3.8. Expression profiling of ET304 and its putative poplar homolog

The analysis of *Populus trichocarpa* genome (http://shake.jgi-psf.org/Poptr1_1/Poptr1_1.home.html) indicated that at least 19 proposed gene models share structural (a single exon) and extensive sequence similarities with *ET304* (data not shown). Multiple sequence alignment (MSA) of ET304 (eugene3.00130441), ET304-like protein (gw1.X.5544.1) and *Arabidopsis* ESCAROLA (At1g20900), showed that these proteins share conserved AT hook motifs centered around GRP tripeptide, DNA binding domains of protein family pfam02178 and predicted nuclear localization signals (Fig. 6). We surveyed by RT PCR the expression of both ET304 and ET304-like mRNAs in 13 types of tissues from mature *P. trichocarpa* trees (Table 3). *ET304* and *ET304*-like transcripts were readily detected in roots and root-containing tissues such as germinated seeds. In contrast, only weak RT PCR products were detectable in some aerial tissues containing secondary meristems such as shoot apex and lateral buds suggesting that both genes have strong root-predominant expression profile. A search of massively parallel signature sequencing (MPSS) database (http://mpss.udel.edu) suggested that the expression pattern of a ESCAROLA—a putative ET304 homolog from *Arabidopsis* was also root-specific.

Table 3
Detection of transcripts of ET304 and its putative ET304-like homolog in seeds and various tissue types of field-grown poplar trees by RT PCR

Poplar tissue type	RT PCR product	
	ET304 (eugene3.00130441)	ET304-like (gw1.X.5544.1)
Seeds	ND	ND
Germinated seeds (24 h)	++	++
Germinated seeds (43 h)	++	++
Leaf	ND	ND
Shoot apex	+	+
Xylem	ND	ND
Phloem	ND	ND
Root	++	++
Male catkin	ND	ND
Female catkin (post-pollination)	ND	ND
Male flower bud	ND	ND
Female flower bud	ND	ND
Axillary bud	+	+
Flushing axillary bud (including young leaves)	ND	ND

++ and + indicate abundant and weak RT PCR products, respectively; ND, not detectable.

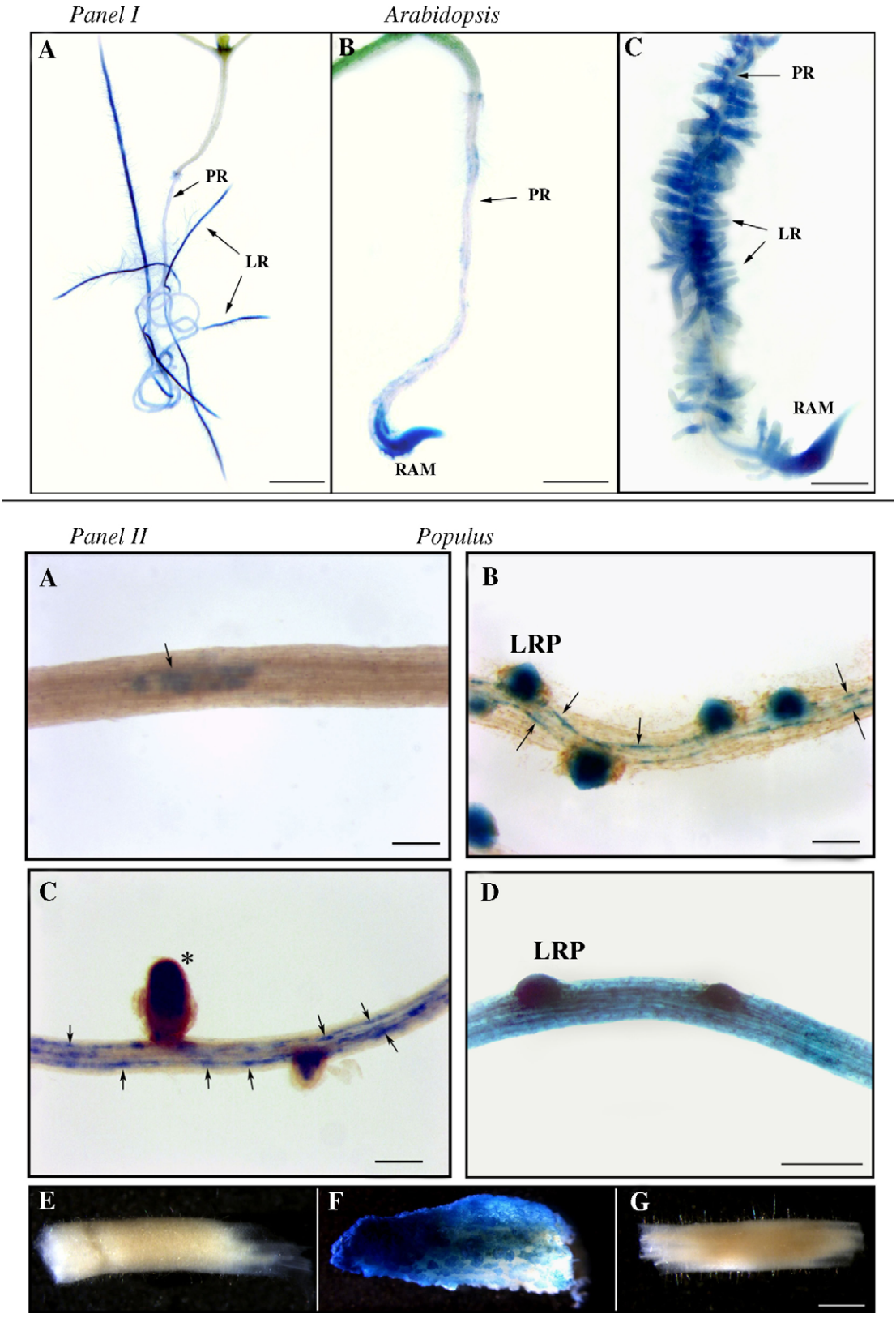

Fig. 4. Synchronized lateral roots auxin induction system: initiation of LRP and GUS staining in *pET304::GUS Arabidopsis* and *Populus* transgenics.Panel I: *Arabidopsis*. Seeds were germinated and grown vertically on control hormone-free medium (A), or medium supplied with 10 μM NPA for 7 days (B), followed by transfer on medium supplied with 10 μM NAA for 4 days (C). LR, lateral root; PR, primary root; RAM, root apical meristem. Scale bars = 1.6, 0.8 and 0.6 mm for A–C, respectively.Panel II: *Populus*. Transgenic *pET304::GUS* plantlets were grown hydroponically under the sterile conditions on media supplied with 20 μM NPA (A) or 20 μM NAA (B). Cultivation on medium containing NPA for 20 days arrested formation of new LRP and reduced GUS accumulation in roots to the trace amounts (residual GUS staining is shown by an arrow in A). Auxin treatment for 7 days induced multiple LRP and "strings" of GUS-stained cells clusters in root cortex apparently linked with LRP (indicated by arrows in B). Transfer from NPA to NAA-containing medium for 72 h reactivated GUS activity in cortex cell clusters (C)

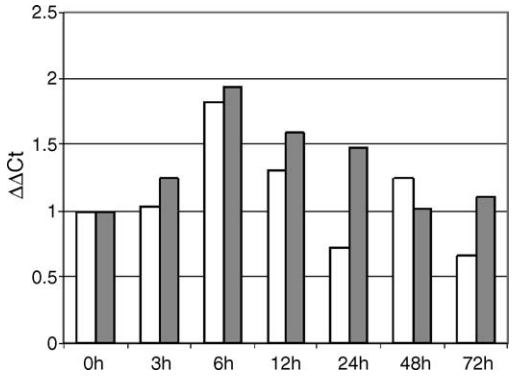

Fig. 5. Time course of ET304 mRNA accumulation in poplar roots after synchronized auxin induction. Relative levels of ET304 mRNA were estimated as $\Delta\Delta C_T$ ratios to the zero time point (i.e. NPA-treated roots prior NAA induction). Each time point (in hours post-NAA induction) is represented by two biological replicates.

4. Discussion

We have identified and characterized a novel gene from *Populus* with root-predominant expression pattern.

Three independent lines of evidence strongly supported conclusion that *ET304* promoter directs highly specific gene expression in LRP and RAM of adventitious and lateral roots. First, examination of poplar enhancer trap line ET304 showed that GUS activity was localized in RAM, LRP and LR elongation zone. Second, many transgenic poplar *pET304::GUS* lines produced a consistent pattern of histochemical GUS staining that was nearly identical to that of enhancer trap line ET304. Finally, the expression of *pET304::GUS* in *Arabidopsis* roots showed a LR-specific patterning resembling GUS distribution in tissues of both enhancer trap line ET304 and *pET304::GUS* transgenic poplar lines. The LR-predominant expression pattern of *pET304* was confirmed by the analysis of T_1, T_2 and T_3 generations of transgenic *Arabidopsis*.

In contrast to poplar, *pET304* in *Arabidopsis* was highly active in stipules and to a lesser extent in primary shoot apex and in secondary meristems located in leaf axils. Interestingly, *ET304* promoter activity in *Arabidopsis* had a striking resemblance to the GUS expression pattern of *DR5::GUS* promoter marker line [2]. *DR5* is a synthetic IAA-inducible promoter containing AuxRE TGTCTC [39,40,41]. *DR5* is widely used as a marker to monitor distribution of free endogenous IAA [3,7,32]. The presence of the canonic AuxRE in *pET304* could explain coincidental accumulation of GUS activity in *Arabidopsis* tissues rich in free endogenous IAA such as stipules, as well as in primary and secondary shoot and

```
ET304           1 MANRWWTGQVG------LPGMDTSTSSSSPMKKPDLGISMSNNNREATESGAGKEDEQED
ET304-like      1 LANPWWTGQVG------LPGLDSSSNSPS-LGKINRELSIN-----ETSNRSGGRDEDDD
ESCAROLA        1 MEGGYEQGGGASRYFHNLFRPEIHHQQLQPQGGINLIDQHHHQHQQHQQQQQPSDDSRES
consensus       1 man wwtGqvg      Lpgmdtstns spmgkinl ism  n    t n  g eDe ed

ET304          55 ERENSDEPREG--------AIDIASRRPRGRPPGSKNKPKPPIFVTRDSPNALKSHVMEI
ET304-like     49 DRDTGDEAKEG--------AVEVGNRRPRGRPPGSKNKPKPPIFVTRDSPNALRSHVMEI
ESCAROLA       61 DHSNKDHHQQGRPDSDPNTSSSAPGKRPRGRPPGSKNKAKPPIIVTRDSPNALRSHVLEV
consensus      61 dren De reG         aidia rRPRGRPPGSKNKpKPPIfVTRDSPNALrSHVmEi

ET304         107 ASGSDIAENLACFARKRQRGVCVLSGSGMVTNVTLKQPSASG---------AVMALHGRF
ET304-like    101 AGGADVAESVAQFARRRQRGVCVLSGSGSVANVTLRQPAAPG---------AVVALHGRF
ESCAROLA      121 SPGADIVESVSTYARRRGRGVSVLGGNGTVSNVTLRQPVTPGNGGGVSGGGGVVTLHGRF
consensus     121 a GaDiaEsva fARrRqRGVcVLsGsGsVtNVTLrQP apG          aVvaLHGRF

ET304         158 EILSLTGAFLPGPAPPGATGLTIYLAGGQGQVVGGSVVGSLVASGPVMVIAATFSNATYE
ET304-like    152 EILSLTGAFLPGPAPPGSTGLTVYLAGGQGQVVGGSVVGSLIAACPVMVIAATFANATYE
ESCAROLA      181 EILSLTGTVLPPPAPPGAGGLSIFLAGGQGQVVGGSVVAPLIASAPVILMAASFSNAVFE
consensus     181 EILSLTGafLPgPAPPGatGLtiyLAGGQGQVVGGSVVgsLiAsgPVmviAAtFsNAtyE

ET304         218 RLPLEDEEEGSGGAQGQLG-GGNGS-----GEGNGGGMGDPATSMPVYQLPNMVPNG--Q
ET304-like    212 RLPLEDDEEAGSGGQGHIQSGANNSPPAIGSSGQQAGLPDPS-SMPVYLPPNLMQSGAQQ
ESCAROLA      241 RLPIEEEEEGGGGGGGGG-GGPPQ------MQQAPSASPPSGVTGQGQLGGNVGGYGFS
consensus     241 RLPlEdeEEgggGgqG lg Ggn s        gqgggm dPs smpvyqlpnmv  ga q

ET304         270 LNHEGYGWAHG---RPPY
ET304-like    271 LGHDAYAWAHAA--RPPY
ESCAROLA      294 GDPHLLGWGAGTPSRPPF
consensus     301 l hegygWahg    RPPy
```

Fig. 6. Multiple sequence alignment of *P. trichcarpa* ET304 (eugene3.00130441, chromosome 13), its putative ET304-like homolog (gw1.X.5544.1, chromosome 10) and *Arabidopsis* ESCAROLA (At1g20900). Globally conserved amino acid residues are shown in white on black background, identical highlighted in grey, and similar, in light grey. AT hook motif residues centered around GRP tripeptide are boxed. Predicted DNA binding domain DUF296 of protein family pfam02178 encompasses residues 100-161of ET304. The consensus sequence of the predicted nuclear localization signal ARrRqRG is underlined.

(shown by arrows). Developmentally arrested LRP formed prior NPA treatment (indicated by asterisk) showed GUS staining inside of characteristic outgrowth layer surrounding the bulge of primordium. A continuous auxin treatment for 7 days after NPA block induced multiple LRP and background GUS staining through the whole root (D). The whole root mounts in B and D were clarified by sodium hydroxide treatment. E through G. *ET304* promoter was activated in callus cells in the presence of auxin. The sterile explants were excised from the stems of *pET304::GUS* transgenic plantlets and placed on CIM media without NAA (E), or containing 10 μM NAA (F), or CIM supplied with both NAA and NPA (10 μM of each) (G). The cells of callus formed in the presence of 10 μM NAA showed intense GUS staining. Scale bars = 0.4 mm for A–D, and 1 mm for E–G, respectively.

root meristems. GUS expression was not visible in poplar stipules, suggesting that in *Populus* these tissues may not represent true sites of *ET304* expression. Instead, weak GUS staining consistent with the detection of ET304 transcripts by RT PCR, was observed in poplar axillary vegetative buds. Altogether, these data suggested a possibility that in addition to strong expression of *ET304* gene in root meristems its low promoter activity could be associated with the secondary meristems of aerial organs. This conclusion is consistent with the similar expression pattern of a putative poplar ET304-like homolog.

The insertion of enhancer trap T-DNA in close proximity to the putative *ET304* initiation codon suggested a likelihood of functional transcriptional fusion of the *GUS* reporter gene to upstream *cis*-acting regulatory elements of the *pET304* promoter including the AuxRE.

In addition to common eukaryotic promoter elements, the upstream sequence of *pET304* contained two conserved motifs implicated in development of RAM and in initiation of LRP. AAACCCTAA ''telo-boxes'' participate in control of gene expression during cell cycle progression in *Arabidopsis* root meristems [37]. This conserved motif was found in the 5′-region of genes encoding components of the translational apparatus, and particularly in all known plant *eEF1A* gene promoters. The AAACCCTAA sequence has been found to be absolutely required for the activation of GUS expression in *Arabidopsis* root primordia [38]. The functional significance of the ''telo-box'' in the *pET304* promoter remains to be established.

The presence of TGTCTC AuxRE and coincidental GUS accumulation in IAA-rich tissues suggests the possibility that the expression of *ET304* may be auxin-regulated. To test this hypothesis we used synchronized cell cycle activation system, which is widely used to study developmental regulation of LR initiation [20,21]. The formation of lateral roots was blocked by the application of auxin transport inhibitor NPA or induced by NAA in a synchronized manner. Deprivation of a polar root auxin transport by NPA arrested growth of new LR in both poplar and *Arabidopsis pET304::GUS* transgenic lines. GUS activity in NPA-treated roots of both *Populus* and *Arabidopsis pET304::GUS* transgenic plants was restricted predominantly to the existing RAM. In contrast, NAA-treated roots developed multiple LRP with intensive GUS staining suggesting that GUS expression correlated with auxin-induced formation of LRP.

A spike in ET304 mRNA synthesis after synchronized induction by NAA reinforced the conclusion that ET304 expression is auxin-regulated. The residual activity of ET304 promoter in the presence of the auxin transport inhibitor could be explained by the accumulation of free endogeneous IAA in the apical meristems formed prior to treatment by NPA auxin transport inhibitor. Altogether, our results strongly suggest that the activation of *ET304* promoter in LRP occurs in an auxin dependent manner. It appears possible that *ET304* expression may have similarities to the regulation of expression of a number of cyclins that are activated by auxin in pericycle cells during lateral root initiation [20].

Poplar and *Arabidopsis* genomes encode approximately 39 and 34 predicted proteins with sequence similarity to ET304, respectively. Nineteen putative proteins encoded by intronless genes in poplar genome share a high degree of global and local homology with ET304. The *ET304* gene encodes a putative protein containing predicted AT-hook—a conserved motif generally centered around GRE tripeptide which has been shown to bind to the DNA minor groove [1,31]. ET304 as well as two homologous proteins from poplar and *Arabidopsis* share conserved AT-hook motifs, putative DNA binding domains and nuclear localization signals (Fig. 6). Presence of these domains strongly suggests that ET304 protein and its putative homologs may function as transcriptional factors. Interestingly, all three genes have had root-predominant expression patterns suggesting similar biological functions. The activation-tagged *esc-1D* dominant mutant overexpressing *ESCAROLA* gene had larger wavy leaves, short stem internodes and late flowering in long days [42]. The overexpression/knock down phenotypes and the exact functions of ET304-like proteins in plants remain yet to be established.

In mammals, nuclear non-histone proteins and putative transcription factors that contain a single or multiple AT-hook domains have been implicated in control of gene transcription, chromatin remodeling, DNA replication, DNA repair, and regulation of mRNA processing (reviewed in [30]).

Further investigation of root-predominant *ET304*-like genes including functional dissection of their promoters will help to define their functions in root meristems establishment, and in initiation and development of lateral roots. *ET304* could be a valuable promoter in production tissue-specific marker lines and in applications requiring LR-, LRP- and/or RAM-specific gene expression or suppression.

Acknowledgements

This work was supported in part by grants from the US Department of Energy (award no. 4000023558 ''Genome-enabled discovery of carbon sequestration genes in poplar''), the National Science Foundation Industry/University Cooperative Research Centers (award no. 9980423), and by industrial members of the Tree Biosafety and Genomics Research Cooperative at Oregon State University (http://wwwdata.forestry.oregonstate.edu/tgbb/). The authors are grateful to Elizabeth Etherington for poplar transformation and tissue culture maintenance. The authors also thank Dr. Renee Arias for critical discussions during manuscript preparation.

References

[1] L. Aravind, D. Landsman, AT-hook motifs identified in a wide variety of DNA-binding proteins, Nucl. Acids Res. 26 (1998) 4413–4421.
[2] O. Avsian-Kretchmer, J.-C. Cheng, L. Chen, E. Moctezuma, Z.R. Sung, Indole acetic acid distribution coincides with vascular differentiation pattern during *Arabidopsis* leaf ontogeny, Plant Physiol. 130 (2002) 199–209.
[3] I. Blilou, J. Xu, M. Wildwater, V. Willemsen, I. Paponov, J. Friml, R. Heidstra, M. Aida, K. Palme, B. Scheres, The PIN auxin efflux facilitator network controls growth and patterning in *Arabidopsis* roots, Nature 433 (2005) 39–44.
[4] V.B. Busov, E. Johannes, R.W. Whetten, R.R. Sederoff, S.L. Spiker, C. Lanz-Garcia, B.G. Goldfarb, Auxin-inducible, nodulin-like gene from

loblolly pine is differentially expressed in mature and juvenile phases and encodes a putative transmembrane protein, Planta 218 (2004) 916–927.
[5] A.M. Brunner, V.B. Busov, S.H. Strauss, Poplar genome sequence: functional genomics in an ecologically dominant plant species, Trends Plant Sci. 9 (2004) 49–56.
[6] V.B. Busov, A.M. Brunner, R. Meilan, S. Filichkin, L. Ganio, S. Gandhi, S.H. Strauss, Genetic transformation: a powerful tool for dissection of adaptive traits in trees, New Phytol. 167 (2005) 9–18.
[7] I. Casimiro, A. Marchant, R.P. Bhalerao, T. Beeckman, S. Dhooge, R. Swarup, N. Graham, D. Inze, G. Sandberg, P.J. Casero, M. Bennett, Auxin transport promotes *Arabidopsis* lateral root initiation, Plant Cell 13 (2001) 843–852.
[8] S.J. Clough, A.F. Bent, Floral dip: a simplified method for *Agrobacterium* mediated transformation of *Arabidopsis thaliana*, Plant J. 16 (1998) 735–743.
[9] D.I. Dickmann, J.G. Isebrands, J.E. Eckenwalder, J. Richardson (Eds.), Poplar Culture in North America, NRC Research Press, National Research Council of Canada, Ottawa, ON, 2001.
[10] J.F. Farrar, D.L. Jones, The control of carbon acquisition by roots, New Phytol. 147 (2000) 43–53.
[11] S.A. Filichkin, R. Meilan, V.B. Busov, C. Ma, A.M. Brunner, S.H. Strauss, Alcohol-inducible gene expression in transgenic Populus, Plant Cell Rep. (2006), doi:10.1007/s00299-005-1112-3, PMID: 16496153.
[12] M. Geisler, B. Jablonska, P.S. Springer, Enhancer trap expression patterns provide a novel teaching resource, Plant Physiol. 130 (2002) 1747–1753.
[13] A.P. Gleave, A versatile binary vector system with a T-DNA organizational structure conducive to efficient integration of cloned DNA into the plant genome, Plant Mol. Biol. 12 (1992) 1203–1207.
[14] V. Grbic, A.B. Bleecker, Axillary meristem development in *Arabidopsis thaliana*, Plant J. 21 (2000) 215–223.
[15] A. Groover, J.R. Fontana, G. Dupper, C. Ma, R. Martienssen, S. Strauss, R. Meilan, Gene and enhancer trap tagging of vascular-expressed genes in poplar trees, Plant Physiol. 134 (2004) 1742–1751.
[16] K.-H. Han, R. Meilan, C. Ma, S.H. Strauss, An *Agrobacterium tumefaciens* transformation protocol effective on a variety of cottonwood hybrids (genus *Populus*), Plant Cell Rep. 19 (2000) 315–320.
[17] F.D. Hempel, L.J. Feldman, Bi-directional inflorescence development in *Arabidopsis thaliana*. Acropetal initiation of flowers and basipetal initiation of paraclades, Planta 192 (1994) 276–286.
[18] K. Higo, Y. Ugawa, M. Iwamoto, H. Higo, PLACE: a database of plant *cis*-acting regulatory DNA elements, Nucl. Acids Res. 26 (1998) 358–359.
[19] K. Higo, Y. Ugawa, M. Iwamoto, T. Korenaga, Plant *cis*-acting regulatory DNA elements (PLACE) database, Nucl. Acids Res. 27 (1999) 297–300.
[20] K. Himanen, E. Boucheron, S. Vanneste, J. de Almeida Engler, D. Inze, T. Beeckman, Auxin-mediated cell cycle activation during early lateral root initiation, Plant Cell 14 (2002) 2339–2351.
[21] K. Himanen, M. Vuylsteke, S. Vanneste, S. Vercruysse, E. Boucheron, P. Alard, D. Chriqui, M. Van Montagu, D. Inze, T. Beeckman, Transcript profiling of early lateral root initiation, PNAS 101 (2004) 5146–5151.
[22] M. Holstein, D. De Wacek, A. Depicker, E. Messers, M. van Montagu, J. Schell, Transfection and transformation of *Agrobacterium tumefaciens*, Mol. Gen. Genet. 163 (1978) 181–187.
[23] C. Koncz, J. Schell, The promoter of TL-DNA gene 5 controls the tissue-specific expression of chimaeric genes carried by a novel type of *Agrobacterium* binary vector, Mol. Gen. Genet. 204 (1986) 383–396.
[24] S. Kurup, J. Runions, U. Kohler, L. Laplaze, S. Hodge, J. Haseloff, Marking cell lineages in living tissues, Plant J. 42 (2005) 444–453.
[25] M. Lescot, P. Dehais, G. Thijs, K. Marchal, Y. Moreau, Y. Van de Peer, P. Rouze, S. Rombauts, PlantCARE, a database of plant *cis*-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences, Nucl. Acids Res. 30 (2002) 325–327.
[26] T.D. Livak, Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method, Methods 25 (2001) 402–408.
[27] J. Malamy, P. Benfey, Organization and cell differentiation in lateral roots of *Arabidopsis thaliana*, Development 124 (1997) 33–44.
[28] J.E. Malamy, K.S. Ryan, Environmental regulation of lateral root initiation in *Arabidopsis*, Plant Physiol. 127 (2001) 899–909.
[29] T. Murashige, F. Skoog, A revised medium for rapid growth and bioassays with tobacco tissue culture, Physiol. Plant. 15 (1962) 473–497.
[30] R. Reeves, HMGA proteins: flexibility finds a nuclear niche? Biochem. Cell Biol. 81 (2003) 185–195.
[31] R. Reeves, M. Nissen, The A.T-DNA-binding domain of mammalian high mobility group I chromosomal proteins. A novel peptide motif for recognizing DNA structure, J. Biol. Chem. 265 (1990) 8573–8582.
[32] S. Sabatini, D. Beis, H. Wolkenfelt, J. Murfett, T. Guilfoyle, J. Malamy, P. Benfey, O. Leyser, N. Bechtold, P. Weisbeek, B. Scheres, An auxin-dependent distal organizer of pattern and polarity in the *Arabidopsis* root, Cell 99 (1999) 463–472.
[33] P.S. Springer, Gene traps: tools for plant development and genomics, Plant Cell 12 (2000) 1007–1020.
[34] S.H. Strauss, F.M. Martin, Poplar genomics comes of age, New Phytol. 164 (2004) 1–4.
[35] V. Sundaresan, P. Springer, T. Volpe, S. Haward, J.D.G. Jones, C. Dean, H. Ma, R. Martienssen, Patterns of gene action in plant development revealed by enhancer trap and gene trap transposable elements, Genes Dev. 9 (1995) 1797–1810.
[36] G. Thijs, K. Marchal, M. Lescot, S. Rombauts, B. De Moor, P. Rouze, Y. Moreau, A Gibbs sampling method to detect overrepresented motifs in the upstream regions of coexpressed genes, J. Comput. Biol. 9 (2002) 447–464.
[37] D. Tremousaygue, L. Garnier, C. Bardet, P. Dabos, C. Herve, B. Lescure, Internal telomeric repeats and 'TCP domain' protein-binding sites co-operate to regulate gene expression in *Arabidopsis thaliana* cycling cells, Plant J. 33 (2003) 957–966.
[38] D. Tremousaygue, A. Manevski, C. Bardet, N. Lescure, B. Lescure, Plant interstitial telomere motifs participate in the control of gene expression in root meristems, Plant J. 20 (1999) 553–561.
[39] T. Ulmasov, G. Hagen, T.J. Guilfoyle, ARF1, a transcription factor that binds to auxin response elements, Science 276 (1997) 1865–1868.
[40] T. Ulmasov, G. Hagen, T.J. Guilfoyle, Dimerization, DNA binding of auxin response factors, Plant J. 19 (1999) 309–319.
[41] T. Ulmasov, Z.B. Liu, G. Hagen, T.J. Guilfoyle, Composite structure of auxin response elements, Plant Cell 7 (1995) 1611–1623.
[42] D. Weigel, J.H. Ahn, M.A. Blázquez, J.O. Borevitz, S.K. Christensen, C. Fankhauser, C. Ferrándiz, I. Kardailsky, E.J. Malancharuvil, M.M. Neff, J.T. Nguyen, S. Sato, Z.-Y. Wang, Y. Xia, R.A. Dixon, M.J. Harrison, C.J. Lamb, M.F. Yanofsky, J. Chory, Activation tagging in *Arabidopsis*, Plant Physiol. 122 (2000) 1003–1014.
[43] D. Wiegel, J. Glazenbrook, *Arabidopsis*: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2002.
[44] Q. Wu, Isolation of genes associated with adventitious root development in *Populus*. MS Thesis, Department of Forestry, North Carolina State University, 2004.