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GENETIC TRANSFORMATION AND HYBRIDIZATION

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The banana actin 1 promoter drives near-constitutive transgene expression in vegetative tissues of banana (*Musa* spp.)

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Abstract We have isolated a banana actin (*ACT1*) gene and characterised its expression levels, tissue-specificity and upstream regulatory sequences in banana. The *ACT1* promoter (2.2 kbp) was isolated by a combination of ligation-mediated polymerase chain reaction and a method for amplifying flanking sequences. A single transcriptional start site was mapped to within 215 bp of the ATG, within which was located a large intron of 840 bp with almost consensus monocot 5' and 3' splice recognition sites. The *ACT1* gene was amplified from genomic DNA, and the deduced amino acid sequence showed the highest levels of similarity to vegetatively expressed plant actins. Northern analysis indicated that the *ACT1* transcript was present in all tissues examined (petals/sepals, stigma/stamens, leaves and roots). Expression studies in transgenic banana plants showed the *ACT1* promoter drove strong reporter gene expression in both leaves and roots. Truncations of the *ACT1* promoter suggested that all of the necessary regulatory elements required for strong (twofold greater than CaMV 35S) near-constitutive expression were located within 1.2 kbp of the *ACT1* ATG.

Keywords Actin · Promoter · GUS · Constitutive expression · Banana

Abbreviations *ACT1*: Banana actin 1 gene · *GUS*: β-Glucuronidase · *uidA*: *Escherichia coli* gene encoding GUS · *Monocot*: Monocotyledonous · *UTL*: Untranslated leader · *UTR*: Untranslated region

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Introduction

Actin is a fundamental component of the cytoskeleton in all eukaryotes and participates in a number of important subcellular processes. In plants, actin is encoded by complex gene families composed of a number of ancient and highly conserved gene classes (McDowell et al. 1996a; An et al. 1999). How these gene families evolved and why they were preserved in angiosperms, however, remains unclear (McDowell et al. 1996a; An et al. 1999; Meagher et al. 1999). Probably the best characterised actin gene family is that from *Arabidopsis*, which consists of ten individual gene members, eight of which are expressed during plant development (An et al. 1996a, b; Huang et al. 1996, 1997; McDowell et al. 1996a, b). The *Arabidopsis* actin gene family can be divided into two classes, vegetative and reproductive, based predominantly on the site of expression (Meagher et al. 1999). The vegetative actin class is strongly expressed in roots, stems and leaves of germinating seedlings, young plants and mature plants as well as in some parts of the floral organ complex. In contrast, reproductive actin genes are characteristically expressed in pollen, ovules and/or seeds with little or no expression in the vegetative organs. Similar differences in actin gene expression have been described in rice (McElroy et al. 1990a), soybean (Hightower and Meagher 1985) and tobacco (Thangavelu et al. 1993). This finding, combined with phylogenetic analyses (An et al. 1999; McDowell et al. 1996a), suggests that these classes may be representative of angiosperm actins.

The different temporal and spatial expression of plant actin genes makes their regulatory sequences potentially useful for transgene expression in plants. Indeed, the rice actin (*ACT1*) promoter has been widely used to direct transgene expression in graminaceous monocotyledonous plants (McElroy et al. 1990b; Chibbar et al. 1993; Takumi et al. 1994; Zhong et al. 1996). However, its expression in certain non-graminaceous monocots is variable (Wilmink et al. 1995), suggesting that the activity of actin promoters may be limited to closely related species. Consequently, the banana actin gene family may

provide a potential source of new promoters for transgene expression in banana and possibly other non-graminaceous monocots. We report here the isolation and characterisation of a banana (*Musa* spp.) actin gene and show that its promoter is capable of directing near-constitutive reporter gene expression in banana.

Materials and methods

Isolation and cloning of the *ACT1* promoter

The *ACT1* promoter was isolated using a combination of ligation-mediated polymerase chain reaction (PCR; Mueller and Wold 1989) and a method for amplifying flanking sequences (Hermann et al. 2000). Banana (*Musa* spp. cv. Bluggoe) genomic DNA was isolated from leaves of 2- to 3-month-old in vitro plantlets essentially as described by Stewart and Via (1993). Genomic DNA (100 ng) was digested with *Sac*I at 37°C for 12 h and the restriction enzyme inactivated by incubation at 68°C for 10 min. A linker (50 pmol), created by annealing the LINKsac primer (5'-AGAATTCTGCAGGATCCCGGGGAGCT-3') and KNIL primer (5'-CCCCGGGATCCTGCAGAATTCG-3'; 5' phosphorylated and 3' amino blocked), was ligated to the digested DNA at 14°C for 12 h. The ligation product was used as template for a PCR with 200 µ*M* of each dNTP, 5 µl Buffer 3 (Expand Long Template PCR System, Roche), 20 pmol of primers Actin-B (5'-ACCTTGACCATTCCAGTGCC-3') and LINKsac, 0.5 U Expand polymerase (Expand Long Template PCR System) and ddH_2O to a final volume of 50 µl. The reaction mix was subjected to PCR cycles of: 1 cycle at 92°C for 2 min; 35 cycles of 92°C for 30 s, 50°C for 30 s, 68°C for 3 min; 1 cycle at 68°C for 10 min; a 4°C soak. A final nested PCR was carried out on the resulting products using primers Actin-N (5'-AGTGCCATTGTCACAGAC-3') and LINKsac under similar conditions.

A second amplification reaction was carried out using a method for amplifying flanking sequences, with primers designed to the 5' sequence of the product isolated by ligation-mediated PCR. In brief, 50 ng to 1 µg of genomic DNA was mixed with 20 pmol of a 5' biotinylated primer, BACT1-B (5'-GATACGTGTTGCGGATCCCACAG-3'), and cycled as previously described. The products from this primer extension were purified using Dynabeads (Dynabeads Kilobase BINDER kit) according to the manufacturer's directions. In summary, 5 µl of beads was washed twice in Binding Buffer (provided by the manufacturer) and then resuspended in 20 µl of Binding Buffer. To this, 20 µl of the extension product was added and incubated for 3 h at 25°C with gentle agitation. Template genomic DNA was removed by washing in 0.2 *M* NaOH (1×30 min, and 4×1 min), TE Buffer (5×1 min) and ddH_2O (5×1 min) and the purified beads resuspended in 20 µl of ddH_2O. A PCR was carried out with the BACT1-N primer (5'-ACGGAAGTCGAATATGCC-3') using conditions similar to the BACT1-B extension, except that 1 µl of purified template was added instead of genomic DNA. The putative upstream actin sequence was amplified using an overlapping PCR approach with primers BACT15.0 (5'-ACCTTAGTCTGAGAGCTCTGA-3') and BACT13.0 (5'-GTTATGGATATCTGCAAAACC-3') under similar conditions to BACT1-B extension. The subsequent PCR product was cloned into pGEM-T (Promega) and sequenced using the ABI PRISM BigDye Terminator system (PE Biosystems).

Mapping of the transcriptional start site, leader intron, polyadenylation site and actin gene isolation

The precise 5' end of *ACT1* mRNA was mapped using primer extension. Total RNA from banana embryogenic cells was isolated essentially as described by Chang et al. (1993). Total RNA (20 µg) was annealed to 10 pmol of 6-FAM end-labelled primer (synthesised by Genset, Lismore, Australia) 5'-GTCAGCCATGTTATGGATATCTTACAC-3' for 10 min at 75°C. The reagents for cDNA synthesis were added on ice [10 m*M* DTT, 1 m*M* each dNTP, 20 U RNase inhibitor, 40 U Expand reverse transcriptase in buffer (Roche)] and the reaction incubated for 90 min at 42°C. Following removal of the RNA by incubation with 25 ng DNase-free RNase (Roche) for 30 min at 37°C, cDNA was precipitated in 0.3 *M* sodium acetate with 2.5 volumes of ethanol. The primer extension products were resuspended in 8 µl 95% (v/v) formamide, 10 m*M* EDTA (pH 9.0) and electrophoresed on a 6 *M* urea, 4.5% polyacrylamide TBE (45 m*M* Tris-borate, 1 m*M* EDTA, pH 8.0) gel. The fluorescent signal was detected using a fluorescence spectrophotometer (model ABI 337) with GeneScan analysis software, and the size of the cDNA extension product determined by comparison with GS 500Rox molecular size markers (PE Biosystems).

The intron within the 5' untranslated leader (UTL) sequence was mapped using a PCR strategy described by An et al. (1996a). A sense primer (Ba5utrF; 5'-CACCTCTCACTCTCCATCTCTC-3') and antisense primer (Actex3r; 5'-CACTTCATGATGGAGTTGTA-3') were used to PCR-amplify the RNA leader-intron junction from Actex3r-primed cDNA. Reverse transcriptase (RT)-PCR was carried out essentially as described for primer extension, except that 1 µg of total RNA was used. The leader intron and splice sites were mapped by comparing the cDNA sequence of the PCR product with the previously obtained genomic sequence.

The *ACT1* gene was amplified from genomic DNA using an *ACT1* promoter-specific primer, BaIF (5'-GTTCTTCCTCCTTCGATT-3'), and a degenerate actin primer (5'-TAGAAGCActtcatgtg-3') complementary to the 3' end of exon 4. The 3' untranslated region (UTR) of *ACT1* was isolated using a 3' RACE (random amplification of cDNA end) approach (Frohman et al. 1988). DNA was amplified using primers Actex2f (5'-CCCTGAGGAGCACCCTGT-3') and oligo-dT(5'-NT_{20}) using oligo-dT primed cDNA as template. PCR products were cloned into pGEM-T (Promega) and sequence-verified as described earlier.

Genomic and expression analysis of the *ACT1* gene

Genomic DNA (10 µg) was isolated from banana embryogenic cells as previously described and digested independently with *Bam*HI, *Pst*I, *Eco*RI or *Sac*I. Digests were electrophoresed on a 1.0% agarose gel, capillary-blotted onto a nylon membrane (Roche) and baked for 2 h at 80°C. Prior to hybridisation, the membrane was blocked for 60 min at 42°C with DIG Easy Hyb (Roche). DIG-labelled probes were PCR-amplified using a mixture of DIG-labelled and standard dNTPS (1:9 ratio). Two *ACT1* probes were generated: (1) a 945-bp 5' flanking sequence probe complementary to nucleotides (nt) –945 to +1, using primers Ba5utrF and BACT13.0 and (2) a 210-bp 3' UTR probe complementary to nt +1,387 to +1,596, using primers Ba3utrF (5'-CAGGAAGTGCTTCTGAGTTC-3') and Ba3utrR (5'-ATAAACAGCCTTCATTGCAG-3'). The membrane was hybridised with DIG-labelled probes for 12 h at 42°C followed by two washes at room temperature (10 min) in 2× SSC/0.1% SDS and two washes at 65°C (15 min) in 0.1× SSC/0.1%SDS. Detection of the hybridised probe using CDP-STAR (Roche) was carried out according to the manufacturer's instructions. Localisation of *ACT1* gene expression was assessed by Northern analysis. Total RNA was extracted from the leaves, roots and flowers of mature banana plants as previously described. RNA samples (30 µg) were electrophoresed on an agarose-formaldehyde gel (Sambrook et al. 1989) and capillary-blotted onto a nylon membrane (Roche). The membranes were hybridised in a manner similar to the Southern hybridisation, using the same 3' UTR *ACT1* DIG-labelled probe. To ensure even loading of RNA, the membrane was stripped using two 15 min washes in 0.1% SDS and reprobed with a 900-bp 18S ribosomal DIG-labelled probe, PCR-amplified with primers 18Sf (5'-CAGACTGTGAAACTGCGA-3') and 18Sr (5'-GCTTTCGCAGTGGTTCGT-3') as previously described.

Construction of *uid*A reporter fusions

A 2,217-bp putative promoter region (Ba2.2) was excised from the pGEM-*ACT1* clone as a *Sac*II/*Nco*I fragment and inserted

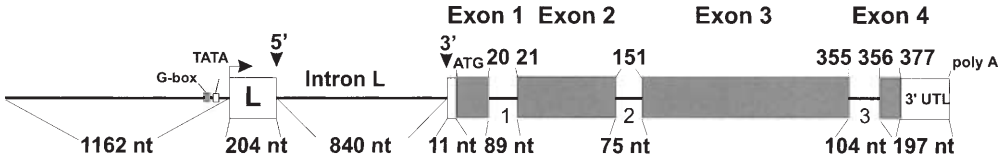

Fig. 1 Structure of the *ACT1* gene. Actin coding regions are denoted by *shaded boxes* and the 5' untranslated leader (*L*) by an *open box*. A *horizontal arrow* depicts the transcription start site, while the intron within the 5' leader is labelled *Intron L*. The sizes of the coding exons (*amino acid*) are indicated *above* the diagram. The sizes of non-coding components (*nt*) are noted *beneath* the diagram

upstream of the *uid*A reporter gene (encoding β-glucuronidase) and the tobacco ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit gene 3' UTR in pUC19. This construct was designated pBa2.2-*uid*A. Three promoter truncations, Ba1.2 (1;253 bp), Ba1.1 (1,100 bp) and Ba0.9 (902 bp), were PCR-amplified as previously described using primers Bact5.1 (5'-CGAGACAGC TCATTGACGAACACAA-3'), Bact5.2 (5'-TTTAAGCTGTCTCG TCGC-3') or Ba5utrF, respectively, and BACT13.0. The PCR products were cloned upstream of *uid*A as described for Ba2.2-*uid*A, and designated pBa1.2-*uid*A, pBa1.1-*uid*A and pBa0.9-*uid*A, respectively. A further truncation which removed the 5' intron splice site was made by digesting Ba2.2 with *Sal*I/*Nco*I. The resulting 847-bp product (Ba0.8) was inserted upstream of the *uid*A reporter gene in pBa0.9-*uid*A, creating pBa0.8-*uid*A. An 800-bp cauliflower mosaic virus (CaMV) 35S promoter and the maize polyubiquitin promoter (*ubi1*), which drives *uid*A, were used to compare promoter expression (Christensen and Quail 1996; Dugdale et al. 1998). Plasmid DNA for microprojectile bombardment was prepared using a Bresapure Plasmid Maxi Kit according to the manufacturer's instructions (Geneworks).

Banana transformation and regeneration

Ba-*uid*A promoter fusions were introduced into banana (*Musa* spp. cv. Bluggoe) embryogenic cell suspensions using a particle inflow gun. Target tissue was prepared essentially as described by Dugdale et al. (1998). Preparation of the microcarrier gold particles and coating of the plasmid DNA were essentially as described by Becker et al. (2000). For the transient expression studies, tissues were assayed for GUS activity 48 h post-bombardment. Transformed banana plants were selected and regenerated essentially as described by Becker et al. (2000).

Analysis of promoter strength

GUS activity was measured both histochemically and fluorometrically essentially as described by Jefferson et al. (1987). For the histochemical GUS assays, tissues were incubated in buffer containing 100 m*M* sodium phosphate (pH 7.0), 50 m*M* ascorbate and 1 m*M* 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-gluc). Samples were incubated for up to 12 h at 37°C and cleared with acetic acid:ethanol (1:3). For the fluorometric GUS assays, total protein was extracted from leaf tissue and GUS activity assayed spectrophotometrically using 4-methylumbelliferone as the substrate.

Results and discussion

As an initial investigation of the actin gene family of banana, we isolated a banana actin (*ACT1*) gene and characterised its promoter region. The *ACT1* promoter was isolated using a combination of ligation-mediated PCR (Mueller and Wold 1989) and a method for amplifying flanking sequences (Hermann et al. 2000). Ligation-mediated PCR involved restriction enzyme digestion of genomic DNA, ligation of a dsDNA linker and subsequent amplification with a linker-specific primer and a degenerate actin primer. Using this strategy, we amplified a 1.2-kbp fragment from banana genomic DNA. Sequence analysis of this product revealed strong homology to the rice actin exon 1 sequence (McElroy et al. 1990c). To obtain further upstream sequences we designed primers to the 5' end of this fragment and amplified the flanking sequences. This resulted in a single 1-kbp product, and subsequent Southern hybridisation with an *ACT1*-specific oligonucleotide probe indicated that the product was specific. Cloning and sequencing confirmed this, and the *ACT1* promoter sequence (2.2 kbp) was assembled from these two fragments using overlapping PCR.

Alignment of the 3' end of the *ACT1* promoter with other actin promoters revealed the presence of a putative 3' intron splice site (TTTTGCAG/AT) that was almost identical to the monocot consensus (TTTTGCAG/GT) (Simpson and Filipowicz 1996). The precise location of the intron was mapped using an RT-PCR approach targeted to the 5' UTL. Comparison of the cDNA and genomic sequences revealed that the *ACT1* 5' UTL contained a relatively large intron of 840 bp that was spliced 11 nt upstream from the translation start codon (Fig. 1). Analysis of the 5' intron splice site (AG/GTCAGT) showed that, like the 3' splice site, it was similar to the monocot consensus (AG/GTAAGT) (Simpson and Filipowicz 1996). The *ACT1* transcriptional start site was mapped by primer extension using a fluorescently labelled oligonucleotide. A single product of 224 bp was generated (Fig. 2a) and mapped to position –1,055 from the translational start site, indicating the *ACT1* gene has a 215 nt 5' UTL sequence. Like other plant actin 5' UTLs, this sequence contained a number of C+T-rich stretches and had a high overall pyrimidine content (approximately 65%). Analysis of the sequence upstream from the transcriptional start site using published regulatory element databases (Wingender et al. 1996; Rombauts et al. 1999) identified a G-box-like motif (CACGTA) and a TATA-box-like motif (TTAATA) located 98 nt and 60 nt upstream from the transcription start site, respectively. As eukaryotic genes transcribed by RNA polymerase II generally contain a TATA box located approximately 30 nt upstream from the transcription start site, the significance of this TATA-box-like motif, with respect to location and function, remains unclear.

The *ACT1* gene was amplified from both genomic DNA and cDNA using *ACT1*-specific primers designed to the promoter region in combination with degenerate

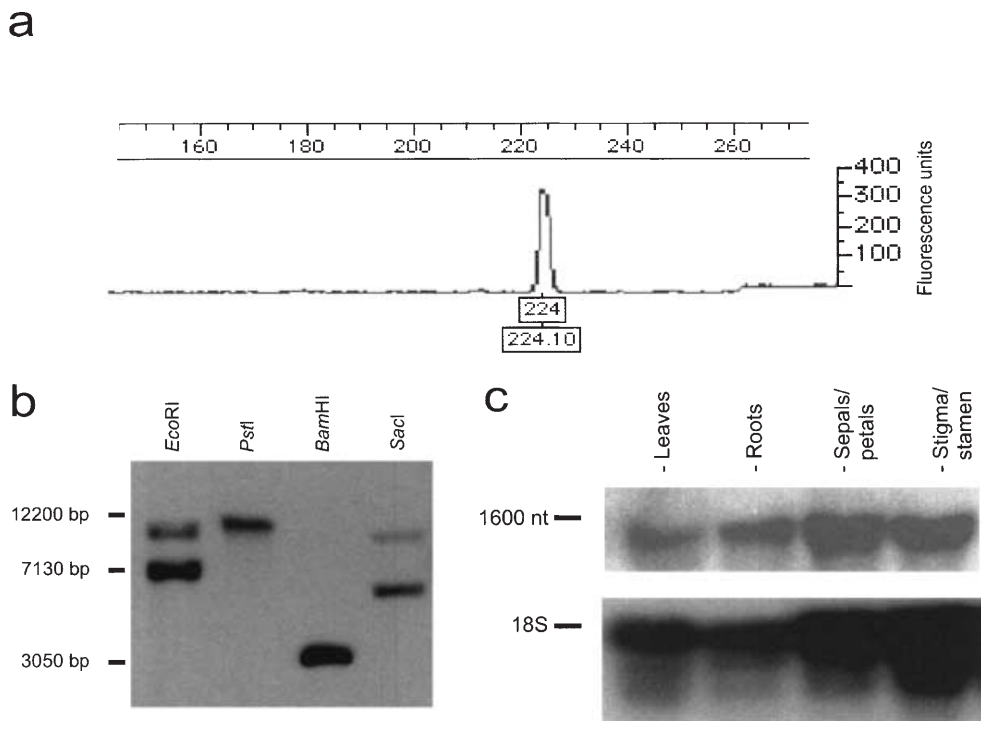

Fig. 2a–c Characterisation of the *ACT1* gene. **a** Primer extension analysis was carried out on total banana RNA using a 6-FAM-labelled primer specific to the 5' UTR of *ACT1*. The primer sequence is given in the text, and the size of primer extension products greater than 40 fluorescence units are shown *under* the trace. **b** Southern analysis of banana (*Musa* spp. cv. Bluggoe). Genomic DNA (10 µg) was digested with either *Eco*RI, *Pst*I, *Bam*HI or *Sac*I and resolved on a 1.0% agarose gel, blotted to a nylon membrane and hybridised with a 945-bp actin gene-specific probe from the 5' flanking sequence. **c** Northern analysis of the *ACT1* transcript in major banana organs. Thirty micrograms of total RNA from leaves, roots, sepals/petals and stigma/stamen was resolved on a 1% agarose-formaldehyde gel, blotted onto nylon, and hybridised with a *ACT1*-specific probe from the 3' untranslated region (*upper panel*). To observe relative loading of RNA, the membrane was stripped and reprobed with a 900-bp 18S rRNA-specific probe (*lower panel*)

actin primers and 3' RACE to amplify the 3' UTR. The *ACT1* gene potentially encoded a protein of 377 amino acids and contained three small introns at identical locations to most other plant actins. Intron 1 (89 bp) separated codons 20 and 21; intron 2 (75 bp) split codon 151; intron 3 (104 bp) separated codons 356 and 357. One *ACT1* polyadenylation site was mapped 197 bp downstream of the stop codon; however, the possible existence of additional poly(A) sites was not determined. Similarity searches on the potential gene product (Altschul et al. 1997) revealed that the amino acid sequence was highly similar to that of the vegetatively expressed *Arabidopsis thaliana ACT7* protein (8 amino acid changes, 4 non-conservative). Furthermore, the putative *ACT1* gene product contained residues common to vegetatively expressed actins (An et al. 1999), including valine-219, serine-232 and serine-358, and lacked serine-79, which is conserved in *Arabidopsis* actins expressed in mature pollen (Kandasamy et al. 1999).

To ascertain the presence of genes closely related to *ACT1*, we extracted banana genomic DNA and used it in Southern hybridisation with an *ACT1* promoter-specific probe. Either one or two bands hybridised with the probe, depending on the restriction enzyme used (Fig. 2b). Similar results were also obtained using an *ACT1*-3' UTR-specific probe (results not shown). As none of the restriction enzyme sites were present within the probe sequence, the hybridisation pattern observed is probably due to allelic differences in either the *ACT1* gene and/or adjacent sequences. Northern hybridisation was subsequently used to investigate the distribution and level of *ACT1* mRNA in various banana tissues. The *ACT1* 3' UTR-specific probe was used to detect the *ACT1* transcript in total RNA isolated from leaves, roots and petals/sepals, and from stigma/stamens from flowers (Fig. 2c). To determine the relative amounts of RNA loaded, we stripped the blot and reprobed it with an 18 S ribosome-specific probe. Northern analysis revealed the presence of a 1,600-nt *ACT1*-specific transcript which was similar in size to those from *Arabidopsis* (An et al. 1996a, 1996b; McDowell et al. 1996b). The *ACT1*-specific transcript was present in all tissues tested and, although equal amounts of RNA were not loaded, it appeared that *ACT1* transcript levels were approximately equal between tissues.

In order to determine regions of the *ACT1* promoter responsible for activity, we fused various truncations of the promoter to a *uid*A reporter gene (Fig. 3a). The promoter fragments included the full-length 2,217-bp fragment (Ba2.2), a 1,253-bp fragment containing the putative TATA and G-boxes (Ba1.2), a 1,100-bp fragment that lacked these putative elements but still contained the complete 5' UTL (Ba1.1), a 902-bp fragment including

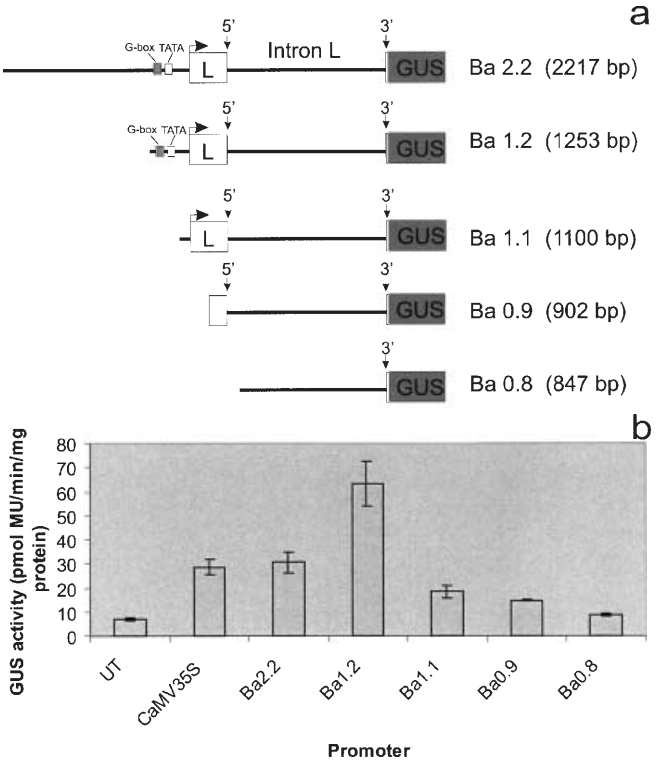

Fig. 3a, b Transient activity of *ACT1* promoter truncations in banana embryogenic cells. **a** *ACT1* promoter truncations fused to the *uid*A reporter gene were used in subsequent transient GUS assays. **b** GUS expression was assessed 48 h post-bombardment. The CaMV 35S promoter was included as a promoter comparison, and untransformed (*UT*) banana tissue was included as a background control. Values are shown as mean GUS activity from four replicates ± the standard error

the 5' intron splice site and part of the 5' UTL (Ba0.9) and an 847-bp fragment lacking the 5' intron splice site (Ba0.8). Varying levels of transient GUS activity were detected from all promoter fusions following microprojectile bombardment into banana embryogenic cell lines. The Ba1.2 promoter showed the greatest activity and directed approximately a twofold greater expression than the Ba2.2 and CaMV 35S promoters (Fig. 3b) but was fourfold less active than the maize *ubi*1 promoter (results not shown). Removal of both the putative TATA and G-boxes (Ba1.1) resulted in a fivefold decrease in expression relative to the Ba1.2 fragment, suggesting that these, or other, upstream elements are indeed important to promoter activity. Interestingly, removal of the transcriptional start site (Ba0.9) did not result in any significant decrease in expression (relative to Ba1.1), whilst a truncation removing the 5' intron splice site (Ba0.8) reduced expression to background levels. Similar results have been reported by Dugdale et al. (2001), who demonstrated that a small part of the 5' UTL and intron of both the maize *ubi1* and rice *ACT1* promoters were capable of directing significant GUS expression in banana. However, the origin and mechanism of transcription initiation from these minimal "promoter" fragments remains undetermined.

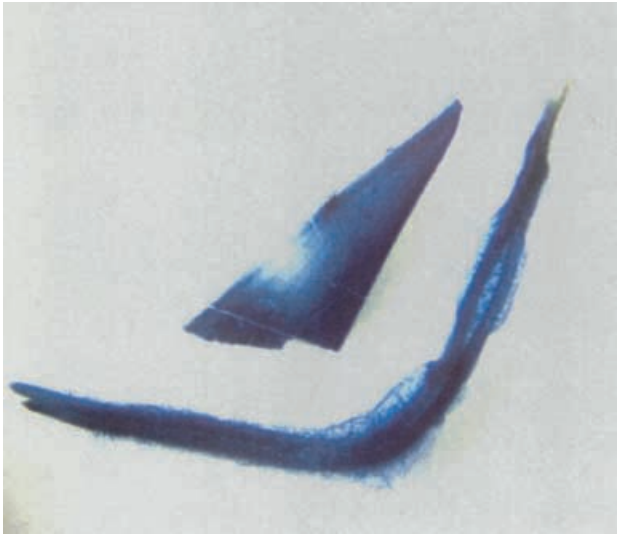

Fig. 4 Histochemical localisation of GUS expression in leaf and root sections of banana transformed with the Ba2.2-*uid*A gene fusion. GUS activity is indicated by an indigo dye precipitate after the addition of X-gluc substrate and clearing in ethanol:acetic acid (1:5)

Table 1 Mean fluorometric GUS activities (pmol MU/min per mg protein) from leaf tissue of transgenic banana plants ± the standard error of the mean (SEM). The number of lines of each plant is shown. The CaMV 35S and maize polyubiquitin (*ubi1*) promoters were included for promoter comparisons, and untransformed (UT) banana was included as a background control

Promoter	Plant lines	Activity (pmol MU/min per mg protein) ± SEM
UT	3	2.5±0.2
CaMV 35S	2	1,670±1,181
ubi1	3	5,338±1,771
Ba2.2	3	1,263±510
Ba1.2	2	3,869±871

To study spatial and temporal regulation of the *ACT1* promoter in whole plants, we regenerated and analysed several independent banana lines transformed with either the Ba.2.2-*uid*A (three lines) or Ba1.2-*uid*A fusions (two lines). Histochemical GUS staining of both the Ba2.2-*uid*A and Ba1.2-*uid*A transformed plants revealed strong reporter expression throughout the pseudo-stem, leaves and roots (Fig. 4), suggesting a near-constitutive pattern of expression. Similar to the transient assays, fluorometric assays from leaf extracts of transgenic plants indicated that the Ba1.2 promoter directed approximately a two-fold greater expression than the Ba2.2 and CaMV 35S promoters, and a twofold lower expression than the maize *ubi1* promoter (Table 1). These results supported the Northern hybridisation and *ACT1* gene sequence analysis and suggested that the banana *ACT1* gene belongs to the vegetative class of plant actins.

This study has indicated that the banana *ACT1* promoter appears to drive strong near-constitutive expression in banana. Of the promoters assessed for

activity in transgenic banana, only a few have demonstrated strong constitutive expression, namely maize *ubi1*, CaMV 35S (Dugdale et al. 1998), the genomic promoter from sugarcane bacilliform badnavirus (Schenk et al. 1999) and at least one promoter from the banana bunchy top nanovirus (Dugdale et al. 1998). Thus, the *ACT1* promoter, which represents the first actin promoter characterised from a non-graminaceous monocot, may be useful for driving transgene expression in banana and possibly in other related monocots.

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