RESEARCH PAPER

The AtMYB11 gene from Arabidopsis is expressed in meristematic cells and modulates growth in planta and organogenesis in vitro

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Abstract

In plants, MYB transcription factors play important roles in many developmental processes including cell cycle progression, cell differentiation, and lateral organ polarity. It is shown here that the R2R3-MYB AtMYB11 gene is expressed in root and shoot meristems and also in young still meristematic leaf and flower primordia of Arabidopsis. Knock-out atmbyb11-I mutants and RNAi plants germinate faster, show a faster hypocotyl and primary root elongation, develop more lateral and adventitious roots, show faster development of the inflorescence, and initiate more lateral inflorescences and fruits than wild-type plants. The opposite phenotype was displayed by plants overexpressing AtMYB11. De novo formation of root meristemoids and, consequently, macroscopic roots, from thin cell layers cultured in vitro was enhanced in explants from atmyb11-I and reduced in those from lines overexpressing AtMYB11. These findings indicate that AtMYB11 modulates overall growth in plants by reducing the proliferation activity of meristematic cells and delaying plant development.

Key words: Arabidopsis, AtMYB11, growth rate, meristems, thin cell layers.

Introduction

MYB proteins are transcription factors with a specific DNA-binding domain comprising up to three imperfect tandem repeats (R1, R2, R3), each of about 52 residues, that fold into a helix-turn-helix motif. In vertebrates, the MYB gene family is small and includes c-MYB, A-MYB, and B-MYB; the products of these genes are involved in the control of cell proliferation, differentiation, and apoptosis (Weston, 1998). In plants, the MYB family is much more extensive: at least 198 MYB genes have been identified in Arabidopsis (Yanhui et al., 2006). Plant MYB proteins are classified according to the number of MYB repeats. Those with R1R2R3 are the most similar to their vertebrate counterparts; those with R2R3 constitute the largest group and are plant-specific; those with a single MYB domain, or variants of it, are known as MYB-related proteins (Stracke et al., 2001; Petroni et al., 2002; Yanhui et al., 2006). The plant MYB genes characterized so far are involved in a wide range of processes including cell cycle progression, cell differentiation, lateral organ polarity, flower and seed development, and secondary metabolism; as well as defence and stress responses, light and hormone signal transduction, and the circadian clock (Petroni et al., 2002; Yanhui et al., 2006).

Various R1R2R3-MYB transcription factors have been shown to control cell proliferation in cell cultures. The MYB-related AtCDC5 gene of Arabidopsis is able to complement the growth-defective phenotype of a cdc5 temperature-sensitive Saccharomyces pombe mutant, restoring the G2/M transition (Hirayama and Shinozaki, 1996). The MYB-related NtMYBA1, NtMYBA2, and NtMYBB genes control G2/M transition in tobacco by modulating the expression of B-type mitotic cyclin genes and other co-expressed genes by binding to an MSA-element, required to G2/M phase-specific timing of expression (Ito et al., 2001). A new MYB-related transcription

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factor and a Myc-type protein have been identified as possible regulators of cyclin AtCYCB1;1 in Arabidopsis (Planchais et al., 2002). However, the role of these genes in plant development remains poorly understood.

Local control of cell proliferation has been attributed to R2R3-MYB orthologous genes which control lateral organ polarity, including RS2 from maize, AS1 from Arabidopsis, and PHAN from Antirrhinum majus (Waite et al., 1998; Timmermans et al., 1999; Byrne et al., 2000). PHAN seems to control organ growth by modulating the expression of CycD3a cyclin in leaf and flower primordia (Towers et al., 2003). In addition, some R2R3-MYB genes are involved in the formation of lateral meristems, including the tomato BLIND gene and its Arabidopsis orthologues RAX1-3 (Schmitz et al., 2002; Keller et al., 2006; Muller et al., 2006).

It is shown here that AtMYB11 is expressed in Arabidopsis apical, lateral, and adventitious meristems and in young still meristematic organ primordia. Knock-out atmyb11-I plants are similar to wild-type plants, but show accelerated germination and morphogenesis, increased rates of leaf and lateral root initiation, faster development of the inflorescence, and enhanced rooting response in in vitro culture. Conversely, plants overexpressing AtMYB11 have reduced growth compared to wild-type and atmyb11-I plants, and showed a reduced rooting response in vitro. Our data indicate that AtMYB11 modulates overall growth in plants by reducing proliferation activity in all types of meristematic cells and delaying plant development.

Materials and methods

Plant material and growth conditions

Arabidopsis ecotypes were used Columbia (Col) for transgenic plants (pMYB11::GUS, RNAi::MYB11, and 35S::MYB11) and Landsberg erecta (Ler) for atmyb11-I. Wild-type segregants of mutants and transgenics were used as controls in all experiments. Seed lots to be compared were harvested the same day from plants grown at the same time in the same environmental conditions and stored at 4 °C in plastic tubes. Germination assays were performed in triplicate using seeds obtained from two independent sowings of the seed sets to be compared. Seeds were sterilized and sown on half-strength MS (Sigma) supplemented with 0.5 mM myo-inositol, 0.3 μM thiamine-HCl, 2% (w/v) glucose, and 0.8% agar (w/v, Sigma), in long day conditions (16/8 h light/dark) at a fluence rate of 80 μE m⁻² s⁻¹ and 22±2 °C. Differences in growth parameters were evaluated as mean size increases (±SE) per 12 h intervals and as mean values and percentages (±SE) d⁻¹, and statistically compared using Student t test.

Semi-quantitative RT-PCR analysis

Total RNA was isolated (van Tunen et al., 1988) from organs harvested at various stages as previously described (Gusmaroli et al., 2001). Approximately 5 μg were reverse-transcribed using RT Superscript II (Invitrogen) and an oligo dT, as previously described (Proccisi et al., 1997). After first strand cDNA synthesis, samples were diluted 50 times and used as templates for semi-quantitative RT-PCR, with atMYB11-specific primers Z17F2 (5'-GGCAAATCCGTGAAATGCCG-3') and Z17R3 (5'-TCGTC-AATATCCAACGGTCTCC-3') and TSBI-specific primers TSBI-F1 (5'-CTCATGGCCGAGGATCTGA-3') and TSBI-R1 (5'-CTTGCTTCTCATATCTTGAGCA-3') as a control of cDNA concentration (Berlyn et al., 1989; Proccisi et al., 1997). The amplifications were carried out within linear ranges (25 cycles). The PCR products were transferred onto Hybond N+ nylon membranes (Amersham) and hybridized with gene-specific probes labelled using the DIG-High Prime kit (Roche). Nested PCR re-amplifications were also performed for an additional 35 cycles on specific samples (Fig. 1A, lower panel), using the AtMYB11-specific primers Z17F3 (5'-AAGAACCAAGAGATCCGCCATGA-3') and Z17R4 (5'-TOGAGCTCCCTTGTGATG-3').

In situ hybridization

Flowers and siliques were collected at various developmental stages from 7-week-old plants grown on soil. Seedlings grown on MS agarose plates supplemented with 1% sucrose were collected at 4 days after germination (DAG). Samples were immediately fixed in freshly prepared 4% (w/v) p-formaldehyde in phosphate buffered saline (130 mM NaCl, 7 mM Na_HPO4, 3 mM NaH2PO4) under vacuum for 3 h. The fixed material was placed in 70% ethanol and stored at 4 °C pending processing. Embedding procedures were performed as previously described (Proccisi et al., 1997), except that each step was reduced to 1 h. The template for the in situ hybridization probe was prepared as described in the Lig'nScribe PCR Promoter Addition Kit (Ambion, USA). A PCR fragment obtained with the primers Z17F2 and Z17R3 (see above) was ligated non-directionally to a T7 promoter adapter as recommended by Ambion. The template for the antisense strand AtMYB11 probe was generated by PCR using an aliquot of the ligation reaction using Z17F2 and the Ambion PCR primer 1 (5'-GCTTCCGGCTCTGATATGTTGGTG-3'). Z17R3 and Ambion PCR primer 1 were used to generate the template for the sense strand AtMYB11 probe. Sense and antisense DIG-11-UTP labelled RNA probes were synthesized with T7 RNA polymerase using components from Boehringer. Eight μm-thick tissue sections were cut and mounted on poly-l-lysine-coated slides and the in situ hybridization carried out as previously described (Coe et al., 1990). Immunological detection of the hybridized probe was carried out as described in the Boehringer digoxigenin-nucleic acid detection kit. For visualizing the red hybridization signal, sections were viewed under dark-field conditions using an Axioscope light microscope (Zeiss) and photographed using a Zeiss camera with a Kodak Ektachrome 100 PLUS film. Digital images were acquired with a scanner CanoScan 2700F.

Isolation of atmyb11-I mutant and Southern analysis

The atmyb11-I mutant allele in the Ler ecotype has previously been isolated by PCR screening of the S3 generation of Wageningen Arabidopsis En-I lines (Speulman et al., 1999), using the AtMYB11-specific primer Z17R3 (see above) in combination with primers It2 and It3, complementary to the 5' and 3' terminal inverted repeats, respectively, of the T transposon as previously described (Meissner et al., 1999). To generate a stable mutant, the progeny of the positive plant were outcrossed with the wild-type Ler ecotype to segregate the T-En5 transposase source (Meissner et al., 1999). F2 segregants free of T-En5 transposase were selected using a PCR screen, as previously described by Speulman et al. (1999).
Genomic DNA was extracted from Ler ecotype and atmyb11-I mutant plants as previously described (Galbiati et al., 2000). DNA was digested with EcoRI, separated by agarose gel electrophoresis, blotted and hybridized as previously described (Dellaporta and Moreno, 1994). As probes, the 1179 bp promoter fragment from AtMYB11 described above and a 267 bp fragment up to a SalI restriction site containing the 5' end of the I element (Aarts et al., 1995) were used to detect I element insertions.

**Constructs and generation of transgenic lines**

The pAtMYB11::GUS fusion was constructed from a 1179 bp genomic fragment amplified by PCR with a forward primer containing a HindIII restriction site at the 5’ end (5’-AAGCTTACACCAATCGGATTAAAG-3’) and a reverse primer containing a SalI restriction site at the 3’ end (5’-CCCCGGGAAATCACTCAGCTTCACT-3’). The PCR fragment was cloned in the pCR4-TOPO (Invitrogen), excised with HindIII-SalI and subcloned between the HindIII-SalI sites preceding the GUS gene in the binary vector pGPTV-Kan (Becker et al., 1992). The construct for 35S::MYB11 lines was obtained as follows: AtMYB11 cDNA was amplified by PCR (Z17F8 5’-TGCGCGCCGAGAAATGGGA-3’ and Z17R15 5’-AATCTTTCAAGACAAAAGCCAAG-3’) and cloned in pCRII-TOPO (Invitrogen) and sequenced. A BamH1-XbaI fragment containing full-length AtMYB11 cDNA was excised and subcloned in pRT2Nl/Asc (Oberlacker and Werr, 1996). The overexpression cassette was then excised with AscI and subcloned in the pGPTV-Kan-Acs vector. The construct for RNAi::MYB11 lines was obtained as follows: an AtMYB11-specific PCR fragment was amplified with primers Z17F2 and Z17R3 modified with attB sequences at the ends to create an entry vector in pDONR™207 (Invitrogen) and then recombined into pFGC5941 (www.ChromDB.org).

Constructs were introduced into A. tumefaciens GV3101 (Labeke et al., 1974) and transferred to Col by the floral dip method (Clough and Bent, 1998). Homozygous T2-independent lines with a single T-DNA insertion were selected by segregation analysis on MS agar plates supplemented with 1% (w/v) sucrose containing 50 mg ml−1 kanamycin (pAtMYB11::GUS and 35S::MYB11) or 50 mM BASTA® AgrEvo (RNAi::MYB11).

**GUS staining and histology**

The pAtMYB11::GUS transgenic plants were harvested daily from sowing up to the mature silique stage, exposed to the GUS staining procedure for 16 h, cleared and examined as previously described by Cominelli et al. (2005) or cleared as described by Malamy and Benfey (1997), observed using Nomarski optics on a Zeiss Axioscope D1 microscope with a video camera Axiocam MRc5. Alternatively, GUS-stained material was fixed in 70% (v/v) ethanol, embedded, and sectioned as described for the in situ analysis. For the other histological analyses, seedlings and thin cell layers (TCLs) were fixed in 70% (v/v) ethanol, embedded in Technovit 7100 (Heraeus Kulzer), sectioned at 4 μm intervals and stained with 0.05% (w/v) toluidine blue. Histological sections were examined under a DAS Leica DMRB microscope with a DC500 video camera. Measures were carried out with a personal computer (Optiplex GX 240MT) using the Leica IM1000 IMAGE ANALYSIS-SIS software and the means (±SE) compared using the Student t test.

of AtMYB11 transcripts in Ler (wt) and atmyb11-I seedlings, indicating the absence of the AtMYB11 transcript in the atmyb11-I mutant. PCR products were blotted and hybridized with random primed probes. The tryptophan synthase β subunit (TSB1) gene was used as a control.
In vitro culture of thin cell layers

Superficial thin cell layers (TCLs, 5×3 mm, six cell layers including the epidermis) were excised from the inflorescence stem internodes of 35-d-old plants of *atmyb11-1*, 35S::MYB11, and the corresponding wild-type ecotype (Ler for *atmyb11-1* and Col for 35S::MYB11). One hundred TCLs per genotype were cultured for 30 d on medium consisting of Murashige and Skoog (1962) salts supplemented with 0.55 mM myo-inositol, 0.1 μM thiamine-HCl, 1% (w/v) sucrose, 0.8% agar (w/v, Sigma) (pH 5.8), and with 10 μM indole-3-butyric acid (IBA) and 0.1 μM kinetin (kin), under continuous darkness, at 24±2 °C, according to Falasca et al. (2004). The analysis of the rooting response was conducted under a dissecting microscope (MZ8 LEICA stereomicroscope) at the end of the culture period. The experiments were repeated three times (100 explants per replicate) without significant differences in the percentage of rooting explants (χ² test). The response was quantified as the mean percentage (±SE) of TCLs that had rooted in the three replicates. The differences between the means were evaluated using Student t test. The images of TCLs were acquired in digital form with a colour video camera (LEICA DC500) applied to the stereomicroscope. The experiments were repeated three times with similar results.

Results

**AtMYB11 is expressed in meristematic cells**

In order to determine the function of *AtMYB11* in *Arabidopsis*, its expression pattern was first analysed in different organs and developmental stages. RT-PCR showed that *AtMYB11* is mainly expressed in 4-d-old seedlings and throughout flower development, but increasing the number of cycles the *AtMYB11* transcript was detected at very low levels in all vegetative organs (Fig. 1A).

The cellular and tissue distribution of *AtMYB11* was further investigated by *in situ* hybridization experiments on seedlings, flowers, and seeds (Fig. 2). In 4-d-old seedlings, the *AtMYB11* transcript was mainly present in shoot apical meristem (SAM) and meristematic leaf primordia (Fig. 2E and inset). During flower development, *AtMYB11* was expressed in inflorescence meristem and in the still meristematic floral organ primordia (Fig. 2A, bud at stage 6 according to Smyth et al., 1990), but as the latter developed into complete flowers, the transcript became essentially confined to ovule primordia (Fig. 2B). After anthesis, *AtMYB11* was found in fertilized ovaules developing into seeds and in the ovary wall developing into silique wall (Fig. 2C). In the mature embryo, the transcript was mainly located close to the protoderm and the root pole (Fig. 2D). Similar hybridization experiments performed with the sense probe gave no signal (not shown).

Additional information was obtained from transgenic plants carrying a 1179 bp promoter fragment of *AtMYB11* (–1220 to –21 bp from the ATG start codon) fused to the β-glucuronidase reporter gene (pAtMYB11::GUS, Fig. 3). GUS staining was detected from the torpedo (not shown) to the mature embryo stage (Fig. 3A). In 4-d-old seedlings...
GUS staining was present in the SAM and at the tip of cotyledons (Fig. 3B). It was also present in the root tip (RAM and columella; Fig. 3D) and in adventitious root meristems (Fig. 3C). In 10-d-old plants, strong GUS staining was present in axillary vegetative meristems (Fig. 3E, F). Leaf primordia were also intensely stained, but as leaf development proceeded, GUS staining declined, becoming progressively confined towards the base until it was visible only in still-developing hydathodes and in the procambium of the petiole (i.e. where meristematic and dividing cells are present) (Fig. 3E, F).

After floral transition, GUS staining was present in the axillary meristems of rosette leaves and stems, and in procambium at their bases (Fig. 3G, H). The pattern of GUS expression in axillary meristems during the development of secondary inflorescences and individual flowers was the same as in the SAM (Fig. 3I).

During flower development, GUS staining was found in all floral organ primordia (sepals, petals, stamens, and carpels) until they showed meristematic features and division activity (Fig. 3J). At stage 10 of flower development, GUS expression was present in developing ovules, ovary walls, and petals (Fig. 3K) and also in developing nectaries at the base of the flower (Fig. 3L). After anthesis, GUS staining was strong in the abscission zone and in the valves of developing siliques (Fig. 3M).

Plants in all developmental stages had GUS staining at the sites of lateral root formation (i.e. in dividing pericycle cells) (Fig. 3N). Strong activity was also present in protruding root domes (Fig. 3O). Root primordia also remained strongly stained after protrusion (Fig. 3P), but later GUS expression became restricted to their procambium, forking region, and root tip (arrow in Fig. 3Q). In all, the GUS expression results corroborate the results.
of the in situ analyses and show that AtMYB11 is expressed almost exclusively in meristems and other actively dividing meristematic cells.

**The atmyb11-I mutant grows faster than wild type**

To determine the function of AtMYB11, a knock-out mutant, atmyb11-I, previously isolated in the Landsberg erecta (Ler) ecotype was analysed by PCR screening of the Wageningen En-I lines, in which the I defective transposon is inserted into the R3 repeat of the MYB domain (Meissner et al., 1999) (Fig. 1B).

Southern blot analyses revealed the presence of at least four additional I insertions in atmyb11-I (Fig. 1C). RT-PCR analysis performed on 4-d-old seedlings showed that the AtMYB11 transcript was not detectable in homozygous atmyb11-I individuals (Fig. 1D).

The atmyb11-I mutant was morphologically similar to Ler plants except that it germinated and grew faster than Ler plants. Thus, 2 d after sowing about 50% of atmyb11-I seeds had germinated compared to 20% of Ler seeds (Fig. 4A). Considering plants germinated at the same time, i.e. on the same day after germination (DAG), a first peak in hypocotyl elongation already occurred at 1.5 d in atmyb11-I seedlings and was followed by another one, of similar value, at 2.5 d (Fig. 4B). By contrast, in Ler seedlings the first peak occurred at 2 d, and a second similar peak at 3 d and they were both significantly ($P < 0.01$) lower than the two peaks occurring in atmyb11-I seedlings (Fig. 4B). The growth of the hypocotyl continued to be significantly ($P < 0.01$) faster in atmyb11-I than in Ler up to 3.5 d (Fig. 4B). Subsequently, hypocotyl elongation was completed in the atmyb11-I mutant, whereas it slowly continued in Ler seedlings (Fig. 4B). As a consequence, the final length of the organ was reached in atmyb11-I three days earlier than in Ler seedlings, and it was not significantly different in the two genotypes (i.e., $2.7 \pm 0.02$ and $2.2 \pm 0.02$ mm, respectively).

Also the elongation of the primary root was accelerated in atmyb11-I plants (Fig. 5A). In fact, seedlings of both genotypes showed peaks in elongation rate at the same times (i.e. at 1 d and 2.5 d), but those of atmyb11-I were significantly ($P < 0.01$) higher (Fig. 5A). Interestingly at 5 d, the root apex (measured including the division zone and excluding the root cap) was significantly longer ($P < 0.01$) in the atmyb11-I seedlings than in Ler (i.e. $405 \pm 10.5$ μm and $320.1 \pm 10.1$ μm, respectively).

Initiation of lateral and adventitious roots was also increased in atmyb11-I plants compared to Ler (Figs 5C, 6E, F). Two lateral root primordia were formed in 5-d-old atmyb11-I seedlings, whereas no root primordia were visible in Ler seedlings (Fig. 5C). Seven days after germination atmyb11-I plants had more and longer lateral and adventitious roots than in Ler plants (Fig. 6E, F). Moreover, the difference in lateral root formation per day...
remained constant up to day 9 (Fig. 5C) and this resulted on day 10, when atmyb11-I primary roots were not significantly longer than Ler roots (i.e. 16.0 ± 0.1 mm and 14.4 ± 0.5 mm, respectively), into an enhanced number of lateral roots in atmyb11-I compared to Ler (Table 1), indicating a higher induction of root meristems per root length unit.

Furthermore, after 2 DAG atmyb11-I seedlings exhibited two leaf primordia, whereas no primordia were visible in Ler seedlings (Fig. 7A). The initiation of leaf primordia was highly accelerated in atmyb11-I compared to Ler plants up to day 9 (Fig. 7A, P < 0.01) and in particular between days 2 and 4 (Figs 7A, 6A, B). There was no significant difference between atmyb11-I and Ler in terms of number of rosette leaves that developed prior to floral transition (i.e. eight leaves on average were produced by each genotype before floral transition). However, as a consequence of the faster leaf production rate per day, the mutant plants reached the leaf number necessary to floral transition at day 10 (Table 1), i.e. about two days before Ler.

In addition, bolting and floral dome production occurred earlier in the mutant compared with Ler plants of the same age, as shown by the histological analysis on 12-d-old plants (Fig. 6I, J). On day 17, 11 ± 1.0% of atmyb11-I plants showed macroscopic bolting, whereas only 4 ± 0.5% of Ler plants had bolted. The difference in the percentage of bolted plants per day remained quite constant up to day 19, but at 21 DAG 74.3 ± 1.6% of atmyb11-I plants had bolted inflorescences macroscopically visible compared with 55.6 ± 1.4% of Ler plants (Fig. 7B, P < 0.05).

At 32 DAG the difference in the percentage of bolted atmyb11-I and Ler plants was not significant, but atmyb11-I stems were longer and had more internodes than Ler stems (Table 2) and more atmyb11-I plants were fruiting (90.7% versus 63.3%). Siliques were shorter and contained fewer seeds than in Ler (Table 3).

**AtMYB11 overexpression reduces growth rate**

To confirm that faster growth was due to inactivation of the AtMYB11 gene, two independent transgenic lines (RNAi::MYB11), showing a complete absence of the AtMYB11 transcript and two lines (35S::MYB11) showing elevated expression levels of the AtMYB11 gene under the control of the CaMV35S promoter were selected (data not shown). Both types of transgensics were similar in appearance to Columbia (Col) wild-type plants, but the constructs had opposing effects on germination and growth rate.

As was the case with the atmyb11-I mutant in comparison with Ler, significantly more RNAi::MYB11 seeds than Col seeds had germinated 2 d and 4 d after sowing (Fig. 4A). Shoot and root development in RNAi::MYB11 was faster than in Col. In fact, a main peak in hypocotyl elongation of RNAi::MYB11 seedlings has already been observed at 1.5 d, followed by a second lower peak at 2.5 d, whereas the two peaks occurred later in Col (i.e. at 2 d and 3 d) and were both significantly (P < 0.01) lower than the first one of RNAi::MYB11 seedlings (Fig. 4C). However, the final length of the organ was not significantly different (i.e. 2.3 ± 0.5 mm and 2.0 ± 0.5 mm, respectively). Primary root elongation showed three peaks of growth in RNAi::MYB11 seedlings which were contemporary to those in Col, but the first and the third were significantly (P < 0.01) higher (Fig. 5B).
Similarly to atmyb11-I seedlings, the root apex in 5-d-old RNAi::MYB11 seedlings was significantly longer (P < 0.01) than in Col (i.e. 421.4 ± 13.7 µm and 295.1 ± 9.6 µm, respectively). Initiation of lateral and adventitious roots in RNAi::MYB11 was also accelerated as in atmyb11-I plants (Figs 5C, 6F, G). Considering that the length of RNAi::MYB11 primary roots at day 10 was the same as in Col plants (i.e. 10.1 ± 0.2 mm and 10.8 ± 0.3 mm, respectively), lateral root formation was also significantly enhanced in RNAi::MYB11 with respect to Col (Table 1). The initiation of rosette leaves per day was accelerated as in atmyb11-I mutant (Fig. 6B, C), i.e. at day 4, there were 3-fold more leaf primordia in RNAi::MYB11 seedlings than in Col (Fig. 7A).

By contrast, 35S::MYB11 seeds germinated more slowly than Col (Fig. 4A) and seedlings had significantly (P < 0.01) shorter hypocotyls (1.4 ± 0.2 mm), roots (4.9 ± 0.4 mm), and root apex (i.e. 190.5 ± 17.7 µm at day 5) compared to Col. Furthermore, the hypocotyl and primary root elongation rates showed only one peak of growth (i.e. at 2 d and 2.5 d, respectively), and a reduced growth period, i.e. about four days (Figs 4C, 5B).

Table 1. Number of rosette leaves and of lateral and adventitious roots

The number of rosette leaves and of lateral and adventitious roots per primary root was determined in 10-d-old seedlings of atmyb11-I, RNAi::MYB11, and 35S::MYB11 plants compared to wild type (Ler for atmyb11-I, Col for RNAi::MYB11 and 35S::MYB11) and expressed as mean values ± SE. At day 10, atmyb11-I and RNAi::MYB11 plants were at floral transition, whereas 35S::MYB11, Ler and Col were still vegetative. Means were compared using Student t test.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Rosette leaves</th>
<th>Lateral roots</th>
<th>Adventitious roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ler</td>
<td>6.5 ± 0.3</td>
<td>3.0 ± 0.3</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>atmyb11-I</td>
<td>7.9 ± 0.2a</td>
<td>6.2 ± 0.3a</td>
<td>2.0 ± 0.1b</td>
</tr>
<tr>
<td>Col</td>
<td>6.6 ± 0.1</td>
<td>3.9 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>RNAi::MYB11</td>
<td>7.5 ± 0.4b</td>
<td>4.9 ± 0.2b</td>
<td>1.8 ± 0.2b</td>
</tr>
<tr>
<td>35S::MYB11</td>
<td>6.0 ± 0.2c</td>
<td>2.6 ± 0.6c</td>
<td>1.0 ± 0.2</td>
</tr>
</tbody>
</table>

a Significantly different to Ler at P < 0.01.
b Significantly different to Col and 35S::MYB11 at P < 0.01.
c Significantly different to Col at P < 0.05; (n=30).

Four days after germination the number of leaf primordia of 35S::MYB11 (i.e. 2 ± 1.0; Fig. 6D) was the same as in Col (Figs 6D, 7A), but at later times there were
fewer rosette leaves and fewer lateral roots in the 35S::MYB11 plants (Fig. 6H; Table 1).

At 12 DAG, the inflorescence apex of RNAi::MYB11 and No. of siliques were evaluated as length of primary stem. Measurements were done on RNAi::MYB11 and No. of siliques atmyb11-I mutant, and its wild type Columbia (Col), Landsberg erecta (Ler), and in 35S::MYB11 and 35S::MYB11 genotypes, and their wild type Columbia (Col). (A) Mean number of rosette leaf primordia at 0–9 DAG; n=90. (B) Mean percentage of plants showing macroscopic bolting after floral transition evaluated daily from 17 DAG to 21 DAG; n=90. Bars represent SE.

![Graph](https://academic.oup.com/jxb/article-abstract/59/6/1201/481559)

**Fig. 7.** Analysis of the formation of rosette leaves, and floral bolting in atmyb11-I mutant, and its wild type Landsberg erecta (Ler), and in RNAi::MYB11 and 35S::MYB11 genotypes, and their wild type Columbia (Col). (A) Mean number of rosette leaf primordia at 0–9 DAG; n=90. (B) Mean percentage of plants showing macroscopic bolting after floral transition evaluated daily from 17 DAG to 21 DAG; n=90. Bars represent SE.

production of floral domes, showing an acceleration in development similar to atmyb11-I plants (Fig. 6K, J). By contrast, 35S::MYB11 (Fig. 6L) and Col, similarly to Ler plants (Fig. 6I), exhibited only the inception of floral meristems.

As for atmyb11-I plants, macroscopic bolting initiated at day 17 in RNAi::MYB11 plants, whereas it was occasional in Col (Fig. 7B). Again as for atmyb11-I plants, the difference in the percentage of RNAi::MYB11 and Col bolted plants was constant up to day 21, when 65±2% of RNAi::MYB11 plants had bolted inflorescences macroscopically visible compared to 58±2.6% of Col plants (Fig. 7B; P <0.05). At day 32, the percentage of RNAi::MYB11 bolted plants was not significantly different than in Col, similarly to atmyb11-I and Ler plants (Table 2). Bolting in 35S::MYB11 plants was rather scarce (Fig. 7B), resulting at day 32 in 30±1.2% of 35S::MYB11 bolted plants, i.e. less than half compared to Col and RNAi::MYB11 (Table 2). In addition, 35S::MYB11 plants exhibited significantly shorter stems, and fewer internodes and flowers than Col and RNAi::MYB11 plants (Table 2). The number of siliques was reduced in 35S::MYB11 and enhanced in RNAi::MYB11 plants compared to Col (Table 2). However, siliques of both genotypes were similarly shorter and contained fewer seeds than Col, but the number of seeds was much more reduced in 35S::MYB11 than in RNAi::MYB11 (Table 3).

**Root formation in vitro is enhanced in atmyb11-I and reduced in 35S::MYB11**

To evaluate the effects of AtMYB11 on the morphogenic potential of tissues, thin cell layer (TCL) explants were obtained from stems of atmyb11-I, Ler, 35S::MYB11, and Col plants and cultured on a root-inducing medium.

The formation of adventitious roots occurred in thin cell layers (TCLs) of 35-d-old plants of all genotypes. However, Col and Ler explants showed a similar rooting response, as shown in Fig. 8A for Ler, whereas differences in the production of roots were observed between the wild type and the two RNAi::MYB11 genotypes (Fig. 8B). At 32 DAG, the mean number of roots was significantly higher in Col (80±2.0) than in RNAi::MYB11 (12±0.4; P <0.05) and 35S::MYB11 (16±0.5; P <0.05).

**Table 2. Inflorescence height and number of internodes, flowers, and siliques**

<table>
<thead>
<tr>
<th></th>
<th>Ler</th>
<th>atmyb11-I</th>
<th>Col</th>
<th>RNAi::MYB11</th>
<th>35S::MYB11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bolting plants (%)</td>
<td>83.3±2.8</td>
<td>93.3±2.9</td>
<td>70.0±1.9</td>
<td>80.0±2.0</td>
<td>30.0±1.2^a</td>
</tr>
<tr>
<td>Inflorescence height (cm)</td>
<td>7.4±0.4</td>
<td>9.7±0.3^b</td>
<td>15.5±0.5</td>
<td>16.7±0.5</td>
<td>10.0±0.4^a</td>
</tr>
<tr>
<td>No. of internodes</td>
<td>3.2±0.2</td>
<td>3.9±0.1^b</td>
<td>8.0±0.2</td>
<td>8.7±0.2</td>
<td>3.4±0.5^a</td>
</tr>
<tr>
<td>No. of flowers</td>
<td>nd^c</td>
<td>nd^c</td>
<td>3.0±0.2</td>
<td>3.0±0.2</td>
<td>2.0±0.3^c</td>
</tr>
<tr>
<td>No. of siliques</td>
<td>nd^c</td>
<td>nd^c</td>
<td>6.1±0.4</td>
<td>8.2±0.5^d</td>
<td>3.3±0.6^a</td>
</tr>
</tbody>
</table>

^a Significantly different to Col and RNAi::MYB11 at P <0.01.
^b Significantly different to Ler at P <0.01.
^c Significantly different to Col and RNAi::MYB11 at P <0.05.
^d Significantly different to Col at P <0.05.
^e nd, Not determined.
in the response of *atmyb11-I* (Fig. 8C) and *35S::MYB11* (Fig. 8E) TCLs were observed compared to the corresponding wild type (Fig. 8A). In fact, the mean percentage of root-forming TCLs of *atmyb11-I* plants was significantly (*P* <0.01) higher than that of *Ler* (i.e. 90.6±3.5 and 62.9±4.8, respectively), whereas that of *35S::MYB11* TCLs was significantly (*P* <0.01) lower than that of *Col* (i.e. 50.6±2.8 and 74.5±3.8, respectively). Furthermore, *atmyb11-I* TCLs were the only explants that appeared totally covered by roots at the end of culture (compare Fig. 8C with Fig. 8A and E). Furthermore, the histological analysis showed that *atmyb11-I* TCLs exhibited less callus and a higher density of root meristemoids and primordia compared to wild-type TCLs (Fig. 8D, B), whereas *35S::MYB11* TCLs showed a very low formation of root meristemoids and primordia (Fig. 8F), thus indicating that the silencing of the *AtMYB11* gene increases de novo formation of meristemoids in the explants, whereas the overexpression of *AtMYB11* has an opposite effect.

**Table 3. Size of siliques and number of seeds per siliqua**

<table>
<thead>
<tr>
<th></th>
<th>Ler</th>
<th><em>atmyb11-I</em></th>
<th>Col</th>
<th>RNAi::MYB11</th>
<th><em>35S::MYB11</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (µm)</td>
<td>8.3±0.4</td>
<td>6.6±0.2</td>
<td>12.3±0.4</td>
<td>10±0.4</td>
<td>10.5±0.3</td>
</tr>
<tr>
<td>Width (µm)</td>
<td>0.97±0.12</td>
<td>0.95±0.11</td>
<td>0.97±0.2</td>
<td>0.97±0.3</td>
<td>0.95±0.3</td>
</tr>
<tr>
<td>No. of seeds</td>
<td>27.4±1.5</td>
<td>23.2±1.3</td>
<td>30±0.3</td>
<td>25±0.4</td>
<td>19.6±0.3</td>
</tr>
</tbody>
</table>

*Significantly different to Ler at *P* <0.01.*
*Significantly different to Ler at *P* <0.05.*
*Significantly different to Col at *P* <0.01.*
*Significantly different to Col and *35S::MYB11* at *P* <0.01.*

**Fig. 8.** Adventitious root formation from TCLs excised from the inflorescence stem of 35-d-old *Ler* (A, B), *atmyb11-I* (C, D), and *35S::MYB11* (E, F) plants and cultured for 30 d on a rooting medium in darkness. (A, C, E) macroscopic response, (B, D, F) histological longitudinal sections (bars=100 µm). Arrows indicate root primordia.

**Discussion**

It has been shown that the *AtMYB11* gene is expressed at all developmental stages and in all organs of *Arabidopsis*, and that it is confined to actively dividing meristematic cells. Specifically, the *AtMYB11* gene was expressed during embryogenesis, in primary, lateral, and adventitious meristems, and in the still meristematic leaf, root, and floral organ primordia. Interestingly, in young leaf primordia, the expression pattern of *AtMYB11* showed a basipetal gradient similar to that of cell division activity. In fact, the outgrowth of leaf primordia and formation of the leaf blade are driven by cell proliferation until the size of a primordium of a few millimetres is reached, after which a progressive cessation of cell division proceeds from the distal tip towards the base (Donnelly *et al.*, 1999; Fleming, 2002). Later on in mature organs, the *AtMYB11* transcript was abundant in the cells which had reprogrammed to generate meristemoids giving rise to hydathodes and nectaries.
Mutated plants (\textit{atmyb11-I} and RNAi::\textit{MYB11}) not expressing the \textit{AtMYB11} gene were characterized by faster germination and faster vegetative growth. This acceleration of growth continued after floral transition, resulting in plants with taller stems, and more internodes and siliques. The opposite phenotype was displayed by 35S::\textit{AtMYB11} plants that overexpress \textit{AtMYB11}. Plant development is generally due to a balance between cell division in meristematic tissues to produce tissues/organs and cell growth and differentiation to allow organ development and maturation. Since initiation and early growth of leaf primordia involves both cell proliferation within the primordium and recruitment of cells from the meristem, a faster initiation of leaf primordia would result in a shoot meristem depleted of stem cells, if its cells were not replenished at the same rate of leaf initiation. As a consequence, a reduction in leaf number may also be observed. Since the number of rosette leaves produced prior to bolting remained unaltered, the faster initiation of leaf primordia observed in \textit{atmyb11-I} and RNAi::\textit{MYB11} plants suggests that the inactivation of \textit{AtMYB11} may cause an accelerated cell production rate in the SAM and in the early primordium. Similar conclusions can be drawn for the root, where the accelerated cell production rate in RAM had already resulted into a more extended root apex in \textit{atmyb11-I} and RNAi::\textit{MYB11} primary roots at day 5. In addition, a faster initiation rate of lateral and adventitious roots, but no alterations in overall root morphology and length, or in the pattern of initiation was observed. These findings suggest that \textit{AtMYB11} may control growth along the longitudinal axis of the plant possibly by reducing the cell production rate in SAM, RAM, and axillary lateral and adventitious meristems, without discriminating between initial and derivative cells and being active in lateral/adventitious organ primordia until proliferation activity is present.

In \textit{Arabidopsis}, floral transition in the SAM is followed by bolting of the stem and the phenotype of the early flowering mutants is characterized by a reduction in rosette leaf number in comparison with the wild type. It is shown that the lines with inactivated \textit{AtMYB11} reached the bolting stage faster than both wild type and \textit{AtMYB11} overexpressing lines, but this was a consequence of an overall faster growth and not of an earlier floral transition, since no difference in the number of rosette leaves was found. Furthermore, the differences in flower and silique number after 32 d of growth between RNAi::\textit{MYB11} and 35S::\textit{AtMYB11} lines compared to wild type may also be due to a different rate of cell division in the inflorescence and floral meristems. It is not clear how both loss of \textit{AtMYB11} expression and its overexpression may lead to shorter siliques with less seeds than wild-type, since many factors affect reproduction and fertility. However, some examples of mutants are known in which a delayed growth is associated to reduced silique size and seed set, as observed for 35S::\textit{MYB11} (Huang et al., 2001; Himanen et al., 2003; Yang et al., 2007). On the other hand, in mutated plants (\textit{atmyb11-I} and RNAi::\textit{MYB11}) an energy imbalance might cause a reduced silique size and seed production, since the additional lateral outgrowths (flowers/fruits) are additional sinks for nutrients.

The role of \textit{AtMYB11} in reducing the production rate of meristematic cells is further sustained by the results of TCL experiments. The cells of the TCLs are excised from the epidermal and cortical layers of an adult organ, i.e. stem, and are totally reprogrammed by the hormones added to the culture medium for proliferating into a callus and producing the root meristems \textit{de novo} (Falasca et al., 2004). The enhancement of root meristemoid formation observed in the knock-out \textit{atmyb11-I} explants (not associated with an enhanced callus proliferation) and the reduced number of neoformed meristemoids observed in the explants from 35S::\textit{MYB11} plants compared to wild type demonstrate that \textit{AtMYB11} plays a meristem-inhibiting action in TCL explants. Since it has been demonstrated that the development of neoformed meristemoids and organs on TCLs is comparable with that observed \textit{in planta} (Altamura et al., 1991; Falasca and Altamura, 2003; Falasca et al., 2004), these results probably reflect the role played by \textit{AtMYB11} \textit{in planta}.

Accelerated growth is typically associated with alterations in cell cycle genes in meristems. Indeed, preliminary results suggest that the silencing of \textit{AtMYB11} determines a change in the timing of expression of cell cycle genes, for example, \textit{AtCYCD2;1}, \textit{AtCYCB1;1}, and \textit{AtCDKA;1} (Doerner et al., 1996; Cockcroft et al., 2000; Beemster et al., 2002). However, an in-depth analysis of germinating seedlings and developing plants is necessary to unravel how the expression of these and other cell cycle genes is locally affected in different meristems and primordia, when the \textit{AtMYB11} expression is altered.

Recently, the analysis of the closely related \textit{MYB} genes, \textit{AtMYB11}, \textit{AtMYB12}, and \textit{AtMYB111}, has been reported showing that, in developing seedlings \textit{AtMYB12} and \textit{AtMYB111} are mainly responsible for the control of flavonol biosynthesis in roots and cotyledons, respectively, but no significant differences in flavonol accumulation were detected in their \textit{atmyb11} mutant compared with wild type (Stracke et al., 2007). Based on the results here presented, we propose that \textit{AtMYB11} controls the rate of germination and modulates growth in \textit{Arabidopsis} by reducing proliferation activity in the meristematic cells. Further studies are required to identify the direct targets of \textit{AtMYB11} and to determine how the timing and extent of expression of these targets are controlled during germination and throughout plant development.

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References


AtMYB11 modulates growth rate


