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Isolation and molecular characterization of a new vegetative MADS-box gene from *Solanum tuberosum* L.

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Abstract. A cDNA clone, *STMADS11,* encoding a new MADS-box protein was isolated from *Solanum tuberosum* L. (potato). Expression of *STMADS11* was found in all vegetative organs of the plant, but not in floral tissues. The expression was also detected in all developmental stages, from tuber sprouts to mature plants, reaching a maximum in well-developed organs. However, the level of *STMADS11* mRNA was low in tissues such as resting tuber or sprouts developed in the cold, where the metabolic activity is reduced. "In situ" hybridizations performed on leaf and stem sections showed that the *STMADS11* transcript is mainly associated with vascular bundles. Cladistic analysis arising from amino acid sequence comparison revealed that *STMADS11* shows the highest similarity to *STMADS16*, another vegetative MADS-box gene from potato, and to the previously reported "orphan" genes *AGL15* and *AGL17* from *Arabidopsis thaliana*. Possible implications of these data in relation to *STMADS11* function are discussed.

Key words: MADS-box gene – *Solanum* – Vegetative development

Introduction

MADS-box genes have been identified in many plant species and their role as homeotic genes controlling floral organ development has been largely established (Coen and Meyerowitz 1991; Davies and Schwarz-Sommer 1994; Weigel and Meyerowitz 1994; Mena et al. 1995; Riechmann and Meyerowitz 1997). The MADS-box proteins have two conserved regions. One of them is the MADS-box domain, a 56-amino-acid region with DNA-binding properties which was originally recognized for protein MCM1 of yeast (Passmore et al. 1988) and the human SRF transcription factor (Norman et al. 1988). Plant MADS proteins also contain a second conserved domain, the K-box, which possesses regularly spaced hydrophobic residues that have been proposed to allow for the formation of an amphipathic alpha helix. Two additional domains are distinguished in MADS proteins: the I-domain, a weakly conserved intervening region that is located between the MADS-box and the K-box, and the C-domain, at the C terminus, the most variable region both in sequence and length among MADS proteins. The MADS-box proteins bind DNA as dimers. The distribution of molecular determinants controling dimerization specificity varies among different MADS proteins and these determinants are situated in the MADS, K and I domains (Riechmann et al. 1996).

Most of the MADS-box genes reported in plants are expressed exclusively in floral tissues (Ma 1994; Rounsley et al. 1995), although for some of them transcripts are additionally found in various vegetative tissues. Within this group are *AGL3* and *AGL8* from *Arabidopsis thaliana* (Huang et al. 1995; Mandel and Yanofsky 1995), *TM3* from tomato (Pnueli et al. 1991), *TOBMADS1* from tobacco (Mandel et al. 1994), *POTM1* of potato (Kang and Hannapel 1996), *DAL1* and *DAL3* both from *Picea abies* (Tandre et al. 1995) and *SAMADSA* of *Sinapsis alba* (Menzel et al. 1996). Moreover, a few genes (*AGL12, AGL14, AGL15* and *AGL17*) having specific non-floral expression patterns have also been identified in *Arabidopsis* (Heck et al. 1995; Rounsley et al. 1995). The mRNAs for *AGL12, 14,* and *17*, are mainly found in roots, while the *AGL15* transcript accumulates in developing embryos and germinating seedlings.

Nucleotide sequences corresponding to *STMADS11* and *STMADS16* cDNAs have been deposited at the NCBI databank under the accession numbers AF008652 and AF008651, respectively

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Abbreviation: GAPDH = glyceraldehyde-3-phosphate dehydrogenase

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Phylogenetic analyses have been performed for the MADS-box multigene family that revealed a distribution into distinct groups or subfamilies (Purugganan et al. 1995; Theissen et al. 1996; Münster et al. 1997). Members of a subfamily usually share high sequence similarity, similar expression patterns and related functions. This is the case for the major subfamilies involved in flower morphogenesis. In addition, several other genes (among them *AGL12, AGL15* and *AGL17*) are not included in any of these groups, appearing in the phylogenetic tree as solitary sequences (''orphan genes'') which do not fit into any of the subfamilies (Purugganan et al. 1995; Theissen et al. 1996). Interestingly, most of these orphan genes exhibit vegetative expression patterns, providing evidence that a new class/es of MADS-box genes might be involved in the regulatory network directing vegetative organogenesis during plant growth. However, while considerable information is available for the MADS-box genes directing flower development, little is known about vegetative MADS-box genes.

In this paper we report on the isolation and the molecular characterization of a cDNA for *STMADS11*, a MADS-box gene from potato which is specifically transcribed in all vegetative organs, but not in floral tissues. Two other MADS-box genes have been previously reported in potato, the flower-specific *STDEFICIENS*, the ortholog of *DEFICIENS* from *Antirrhinum* (García-Maroto et al. 1993), and *POTM1*, a gene showing both floral and vegetative expression (Kang and Hannapel 1996). *STMADS11* represents a novel MADS-box gene, with an expression pattern not reported so far for other members of this gene family.

Materials and methods

Isolation and sequencing of cDNA clones. The leaf cDNA library (ca. 1. 10^{10} pfu ml^{-1}) of *S. tuberosum* cv. Desirée (two-month-old plants), was purchased from Clontech (Palo Alto, Calif. USA). The cDNA used was generated from a mixture of random hexanucleotide- and oligodT-primed cDNA and cloned into the *Eco*RI site of λgt11. The library was probed with an *Eco*RI-*NheI* 300-bp cDNA fragment (including the MADS-box plus 5′-untranslated region) of *STMADS16*. This is another MADS-box gene recently isolated in our laboratory upon screening of a tuber cDNA library under non-stringent conditions, using as a probe the *STDEFICIENS* cDNA (García-Maroto et al. 1993). The screening was performed under low-stringency conditions, at 55 °C for 15 h, in Southern hybridization buffer [3 × SSPE (1 × SSPE = 20 mM NaH_2PO_4, 0.3 M NaCl, 2 mM EDTA) 0.02% polyvinyl pyrolidone (PVP), 0.02% Ficoll 400 and 0.1% SDS]. Washes were accomplished twice in buffer containing 2 × SSPE, 0.1% SDS for 15 min at the same temperature. Phage DNA isolation was achieved by using the Wizard lambda Perps DNA Purification System (Promega, Madison, Wis., USA), and the insert was liberated by *Eco*RI digestion, followed by subcloning and sequencing.

3′-Rapid amplification of cDNA ends (3′-RACE) was made on oligodT-primed single-stranded (ss)-cDNA, synthesized from total leaf RNA using a commercial kit (Pharmacia-Biotech), followed by polymerase chain reaction (PCR) amplification with primer NotI-dT18 (5′-AACTGGAAGAATTCGCGGCCGCAGGAAT$_{18}$-3′), the same used for ss-cDNA synthesis, in combination with the gene-specific primer pr#146 (5′-GAATCGGGAGTTAAGGCA-ACTACATG-3′). The 700-bp PCR product obtained was cloned in the pMOSBlue vector-T (Amersham, Bucks, UK) and three independent clones were sequenced with identical results. Sequencing of all clones was performed on both strands of double-stranded DNA using the Sequenase Version 2.0 kit (U.S. Biochemical, Cleveland, Ohio, USA) according to the manufacture's instructions.

Sampling of plant material. To analyse *STMADS11* expression in different organs of the adult plant (Fig. 2B), material was collected during springtime from field-grown plants (*S. tuberosum* L. cv. Jaerla, provided by the Centro de Investigación y Mejora Agrícola, CIMA from Vitoria, Spain). Samples from roots, developing flowers, leaves, stems, developing new tubers (3–4 mm in diameter) and their associated stolons, as well as the mature resting tuber were selected for this experiment. Etiolated sprouts, 3–8 cm in length, which spontaneously germinated at 8 °C from tubers kept in the dark, were also taken.

For the experiment shown in Fig. 4, around 80 pieces excised from mature tubers were placed underground in soil pots, and maintained at 25 °C under a long-day (16 h light) photoperiod, or in the dark. In light conditions, samples of the sprouts emerging from these tubers, the roots which developed at the base of the sprouts, as well as the tuber tissue immediately around the sprout, were collected at the different developmental stages (S1 to S4) depicted in Fig. 4G. The S1 and S2 stages correspond to 3 to 5-mm- and 10 to 15-mm-long sprouts still growing underground, respectively; S3 are 3 to 4-cm-long sprouts with the tip just starting to emerge from the ground; S4 are sprouts with a 3 to 4-cm emerging portion and some leaves beginning to differentiate. Additional samples were taken from S5, which represents young plants (aerial stems, 12–15 cm long, Fig. 4G) and from mature flowering plants. For these stages, samples from roots, stems as well as young (last 2–4 apical leaves) and old, well-developed leaves were taken. In dark conditions, etiolated sprouts were collected until the S4 stage. In both experimental conditions, each sample represents a pool of material from 5–10 single plants or sprouts.

Southern and Northern blot analysis. Potato genomic DNA was isolated from leaves of *S. tuberosum* L. cv. Jaerla using acetyltrimethylammonium bromide (CTAB)-based extraction procedure (Taylor and Powel 1982). Restricted DNA (about 10 μg) was separated on a 0.7% agarose gel, and transferred onto Hybond-N nylon members (Amersham). Filters were hybridized with a ^{32}P-radiolabelled *STMADS11*-specific probe obtained from a 510-bp restriction fragment spanning 67 amino acids of the C-terminus plus the 3′-untranslated region. Hybridization was performed under stringent conditions (65 °C) in the same hybridization buffer used for the screening of the library. A final high-stringency wash in 0.1% SSPE, 0.1% SDS, was applied for 20 min at 65 °C.

Extraction of RNA was performed following essentially the method of Lagrimini et al. (1987). In Northern blot analysis, about 20 μg per lane of total RNA was loaded onto an agarose/formaldehyde gel, electrophoretically separated, and transferred to Hybond-N membranes. Filters were hybridized at 65 °C in Church buffer (Church and Gilbert 1984) with a radiolabelled *STMADS11*-specific probe, obtained from a 480-bp *Bam*HI-*Xho*I restriction fragment comprising the last 116 amino acids of the coding region plus 130 bp of 3′-untranslated sequence. Washes were performed at 65 °C, once in buffer containing 40 mM sodium phosphate, pH 8.0, and 5% SDS, for 15 min, followed by a 10-min stringent wash in the same buffer containing 1% SDS. As a control, the filters were rehybridized with a 900-bp cDNA probe from tobacco, which encodes part of the cytosolic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene (Shih et al. 1986). Hybridization was done in the same conditions, but the final wash was also performed in 5% SDS buffer.

Radiolabelling with ^{32}P of DNA fragments used as hybridization probes was done as described by Feimberg and Vogelstein (1984).

In-situ hybridization. In-situ RNA hybridization was carried out essentially following procedures previously described (Huijser et al. 1992) with some minor modifications. Pieces of leaves and stems

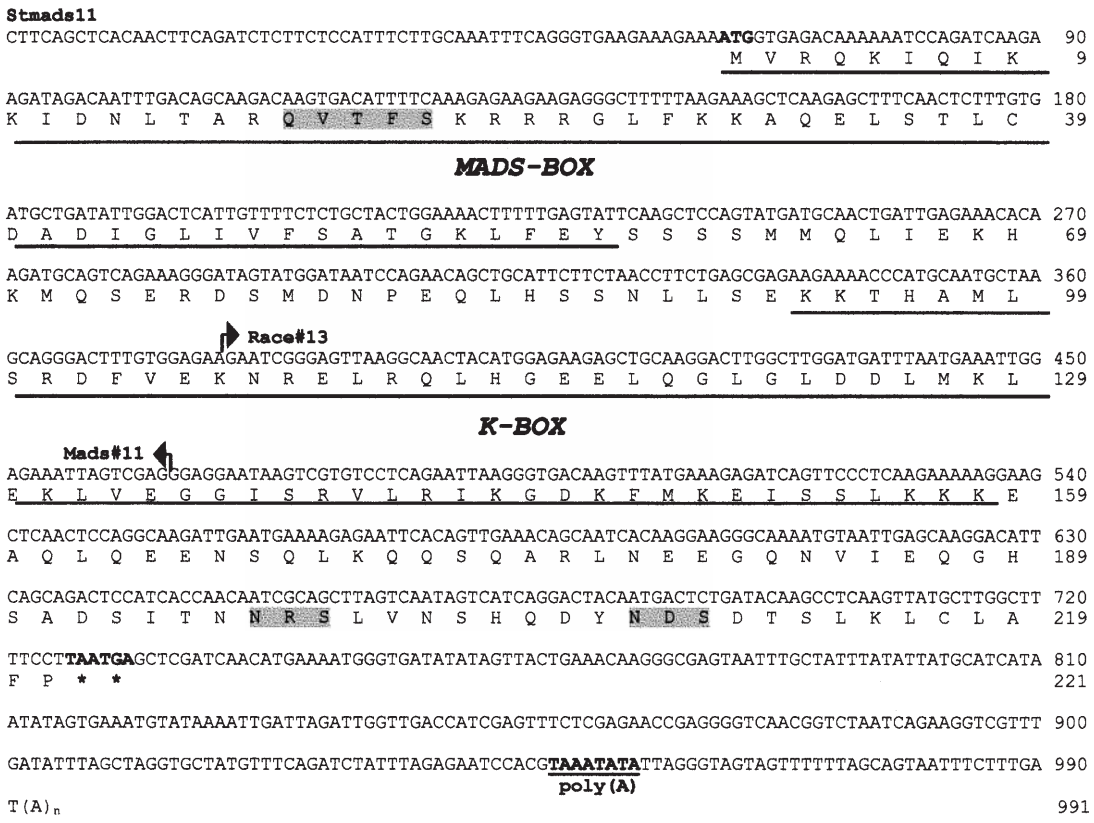

Fig. 1. Nucleotide and deduced amino acid sequences of *STMADS11* cDNA. The MADS-box and K-box domains are *underlined*. The length of Mads#11 and Race#13 clones used for the reconstruction of the whole cDNA are indicated by *arrows*. Both clones overlap in an 86-bp region within the K-box. Stop codons are marked by *asterisks* and the putative polyadenylation signal is *underlined*. Potential phosphorylation and glycosylation sites are *shaded*

were fixed in FAE (2% formaldehyde, 5% acetic acid, 60% ethanol) for 48 h at 4 °C. Tissue slices were hybridized with a mixture containing 1×10^7 cpm ml^{-1} of ^{35}S-labelled antisense or sense RNA probes. Probes were generated by in-vitro transcription, using a commercial kit (Ambion, Austin, Texas, USA) and following instructions from the manufacturer. [^{35}S]Uridine 5′-triphosphate was employed as a radioactive precursor and synthesis was performed from a linearized pBluescript vector (Stratagene, La Jolla, Calif., USA) carrying the *Bam*HI-*Xho*I (480 bp) fragment used as a probe in Northern blots. For autoradiography, hybridized slides were coated with photographic emulsion (NTB2; Kodak, Rochester, N.Y., USA) and exposed for four weeks at 4 °C. Tissue sections were stained with 0.01% Calcofluor White and the underlying tissue was examined using UV epifluorescence. Silver-grain distribution was observed under dark-field illumination.

Cladistic analysis. Alignment of amino acid sequences for MADS-box proteins was done by using the program Clustal 4 (Higgins and Sharp 1989) and the resulting alignment was improved by manual adjusting. This output was fed to the program clustal W v.1.5 (Thompson et al. 1994) which generated a phylogenetic tree based on the neighbor-joining algorithm of Saitou and Nei (1987) with the following parameters: the whole amino acid sequence of the protein was considered, positions with gaps were excluded, and distances were corrected for multiple substitutions. Bootstrap values were also calculated using the same program. The resulting phenogram was drawn with "drawgram" from the Phylogeny Inference Package, Phylip v.3.5c, (J. Felsenstein, University of Washington, USA).

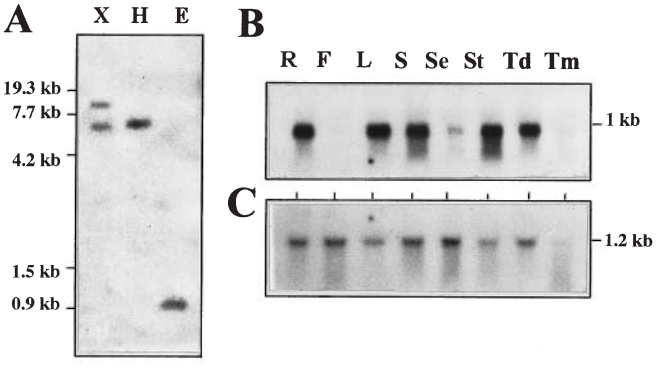

Fig. 2. A Southern blot analysis of *STMADS11* in *S.tuberosum*. The DNA was digested with *Xba*I (*X*), *Hind*III (*H*) or *Eco*RI (*E*) restriction endonucleases and hybridization was performed under stringent conditions as described in *Materials and methods*, using an *STMADS11* 3′-specific probe. Size markers (*kb*) positions are indicated. **B** Expression pattern of *STMADS11* in organs from mature flowering plants. Equivalent amounts of total RNA from roots (*R*), developing flowers (*F*), leaves (*L*), stems (*S*), etiolated sprouts developed in the cold (*Se*), developing stolons and tubers (*St*, *Td*), and mature resting tubers *Tm* were hybridized with an *STMADS11* 3′-specific porbe under high-stringency conditions as indicated in *Materials and methods*. **C** Expression pattern of cytosolic GAPDH, a constitutive gene used as a control. The membrane from the experiment shown in **B** was re-hybridized using as a specific probe a 900-bp GAPDH cDNA clone from tobacco

Results

Isolation of a new MADS-box gene from Solanum tuberosum. A leaf cDNA library from *S. tuberosum* was screened under non-stringent conditions (55 °C)

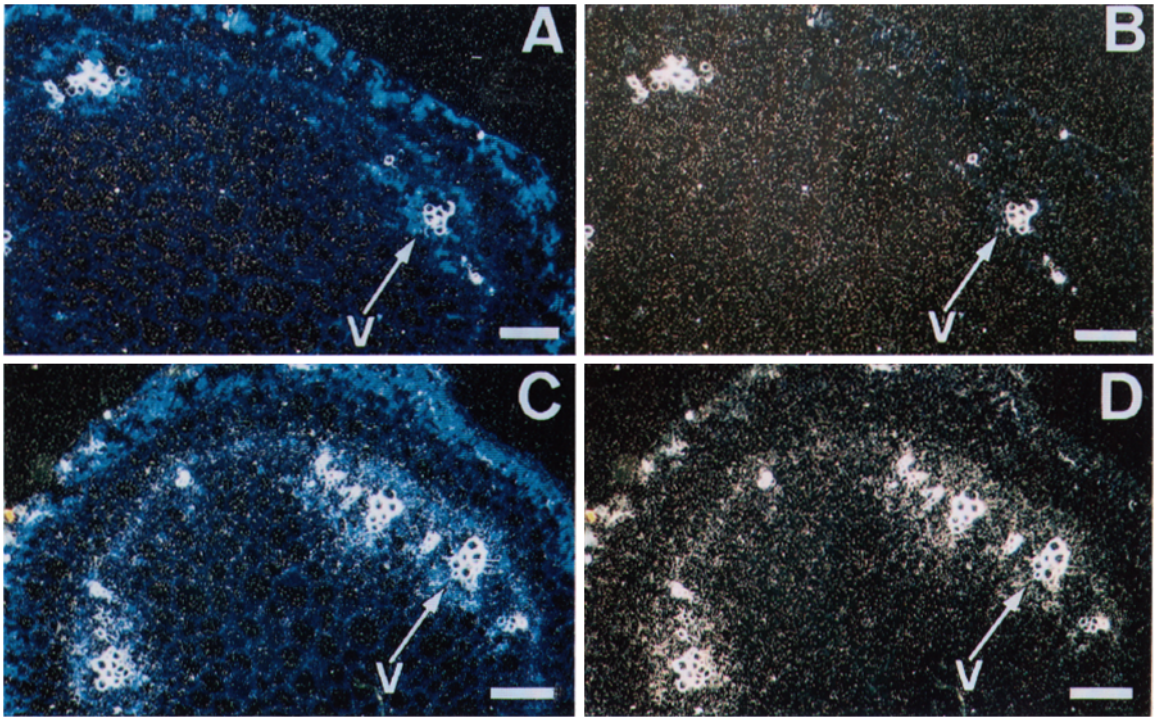

Fig. 3A–D. In-situ localization of the *STMADS11* transcript in potato stems. Stem sections were hybridized with the sense (**A**, **B**) or antisense (**C**, **D**) RNA *STMADS11*-specific probes. **A**, **C** UV epifluorescence images of transverse sections. **B**, **D** Dark-field views of the same sections. Bar 80 μm; vascular bundles (*v*) are marked by an *arrow*

with an *STMADS16* cDNA fragment coding the MADS-box plus the 5′-untranslated region. *STMADS16* is a MADS-box gene recently isolated in our laboratory by sequence homology to *STDEFICIENS* cDNA (data not shown). Around 850,000 lambda clones were screened and five positive clones were finally selected for further studies. Clone Mads#11, which after sequencing was shown to be truncated at the 3′ end (Fig. 1), was initially used in this study. To complete the nucleotide sequence, a 3′-rapid amplification of cDNA ends (3′-RACE) was performed as indicated in *Materials and methods*. The 700-bp product obtained was cloned and three independent clones were sequenced with identical results (clone Race#13, Fig. 1). The overlapping nucleotide stretch (86 bp) of Race#13 and Mads#11 clones was shown to be identical, thus allowing the reconstruction of the whole cDNA for *STMADS11* (Fig. 1). It should be noted that the overlapping sequence corresponds to the K-box, a domain which is only moderately conserved and highly variable at the nucleotide level among MADS-box genes even from the same plant species.

The *STMADS11* cDNA is 991 nucleotides (nts) long, with the longest open reading frame (ORF) spanning 663 nts (positions 63 to 725) and flanking 5′- and 3′-untranslated regions of 62 and 266 nts, respectively. Sequence context around the first ATG codon of the cDNA [AAGAAA AUG GT] fits fairly well to the reported consensus for dicots, [ATAA(A/C)A AUG *G*C], where the underlined −3 and +4 positions play a critical role (Cavener and Ray 1991). Two contiguous stop codons are located at the end of this ORF, thus encoding a 221-amino-acid protein. The conserved MADS-box (57 amino acids) and K-box (66 amino acids) domains, as well as the I and C-terminal domains are present in this protein (Fig. 1). The potential phosphorylation site (QVTFS) for calmodulin-dependent protein kinases, highly conserved among MADS-box genes is also present within the *STMADS11* MADS-box domain (Fig. 1). Moreover, two putative glycosylation sites (NRS and NDS) were identified at the C-terminal region (Fig. 1).

Genomic analysis and expression pattern of STMADS11. Southern blot analysis under stringent conditions of restricted potato genomic DNA revealed single hybridization bands when digested with *Hind*III or *Eco*RI, and two hybridizing bands with *Xbo*I (Fig. 2A). These results are compatible with a single copy or a low copy number for the *STMADS11* gene in the potato genome. Generation of two *Xba*I hybridizing fragments is probably due to the presence of such a site in some of the introns probably covered by the probe.

The RNA blot analysis was performed for different organs collected from mature plants at the flowering time. Similar high levels of the *STMADS11* transcript were found in roots, leaves, and stems, as well as in developing tubers and their associated stolons, while in the mature resting tuber it was only slightly detectable. The *STMADS11* transcript was undetectable in developing flowers (Fig. 2B). The *STMADS11* mRNA levels were similar in vegetative tissues collected from plants before flowering (data not shown). In etiolated sprouts, which developed slowly from tubers stored in darkness

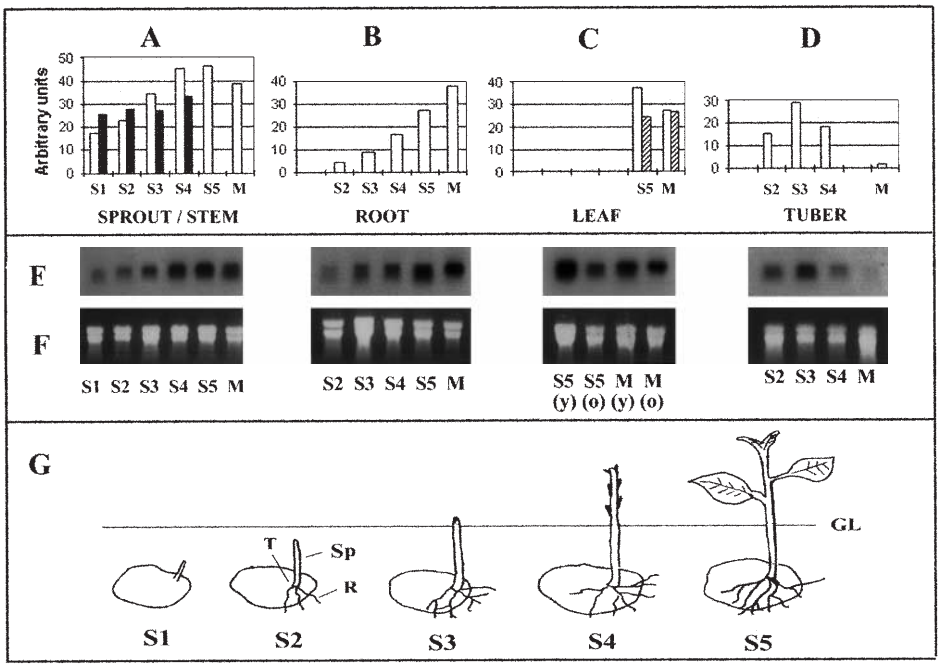

Fig. 4A–G. Expression of *STMADS11* at different developmental stages during tuber sprouting. Total RNA samples from various plant tissues were analyzed by Northern blot as described in Fig. 2. Densitometry was performed on the autoradiogram (**E**) and values expressed as arbitrary units. Data were corrected for loading differences, based on densitometry of ribosomal RNA bands stained with ethidium bromide (**F**). Samples were collected at the tuber sprouting stages (*S1* to *S4*) depicted in **G** as well as from young developing plants (*S5*) and mature flowering plants (*M*). Experiments **B-D** were performed under the illumination conditions described in **A.** **A** Expression in sprout/stem tissues. Sprout samples (*Sp*) were taken from stages *S1* to *S4*, and fully developed stems from *S5* stage and from mature plants (*M*). The experiment was performed either under long photoperiod (16 h light) illumination (*white bars*), sampling all stages, or in the dark (*black bars*) along stages *S1* to *S4*. **B** Expression in roots (*R*) associated to developing sprouts. **C** Expression in leaves. Young (*y*) developing leaves (2–4 apical leaves, white bars) and old (*o*) leaves (*stripped bars*) were analyzed in S5 plants and in mature flowering plants (*M*). **D** Expression in tuber tissues. Tuber tissues (*T*) around the sprouts was analyzed for stages S2 to S4. Transcript was almost absent in mature resting tubers (*M*) . **E** Autoradiogram of the Northern blot. Only the experiment under illumination is shown for sprout/stem samples. **F** Ribosomal RNA stained with ethidium bromide. **G** Depicted developmental stages (*S1* to *S5*) during tuber sprouting. See *Materials and methods* for a further description

at 8 °C, and mainly consisting of stem tissue, the mRNA level was much lower than that of normal stems (Fig. 2B). The size of the detected transcript was approx. 1kb, in agreement with the *STMADS11* cDNA length. Control hybridization (Fig. 2C) with a probe for the cytosolic GAPDH, a constitutively expressed gene of the glycolytic pathway (Shih et al. 1986), reveals a similar transcript level in all tissues examined except for the mature resting tuber where the GAPDH mRNA was present, as expected, at a very low level. This indicates that the reduced *STMADS11* expression observed in flowers and etiolated cold-developed sprouts is certainly due to a down-regulation of the gene expression in these tissues.

In-situ hybridization experiments in stem tissues revealed a preferential accumulation of *STMADS11* mRNA along the ring delimited by the vascular bundles (Fig. 3). A similar distribution was also found in leaves, where the *STMADS11* transcript was mainly associated with the vascular tissues present at the leaf veins (data not shown). Hybridization with an *STMADS16* probe reveals a uniform distribution of the transcript along those tissue sections (data not shown), thus suggesting that this particular expression pattern for *STMADS11* is not simply due to a higher cellular activity at the vascular ring.

We further investigated the temporal and spatial expression of *STMADS11* during plant development. Since clonal propagation is the usual way to cultivate potato, we decided to study the mRNA accumulation in developing sprouts and associated tissues during tuber sprouting (S1 to S4 stages, Fig. 4G). To test for a possible effect of light on the regulation of that process, we analyzed sprout development both under darkness or illumination (long-day photoperiod) conditions. The transcript level was also determined in different organs of developing young plants (S5) and mature flowering plants. The onset of *STMADS11* expression occurred as soon as the sprouts started to emerge from the tuber (S1 stage) and increased throughout their development (S2 to S4 stages), reaching a similar level in the stems of young developing S5 plants and the mature flowering plants (Fig. 4A, white bars). The accumulation of the *STMADS11* transcript does not seem to depend on the illumination sensed by the emerging plant, because similar mRNA levels were also found in tuber sprouts developed in the dark (Fig. 4A, black bars). In the roots, the *STMADS11* transcript was hardly detectable at the S2 stage (roots are still not developed at the S1 stage) and its level progressively increased until the plant was mature, reaching at this stage a level similar to that

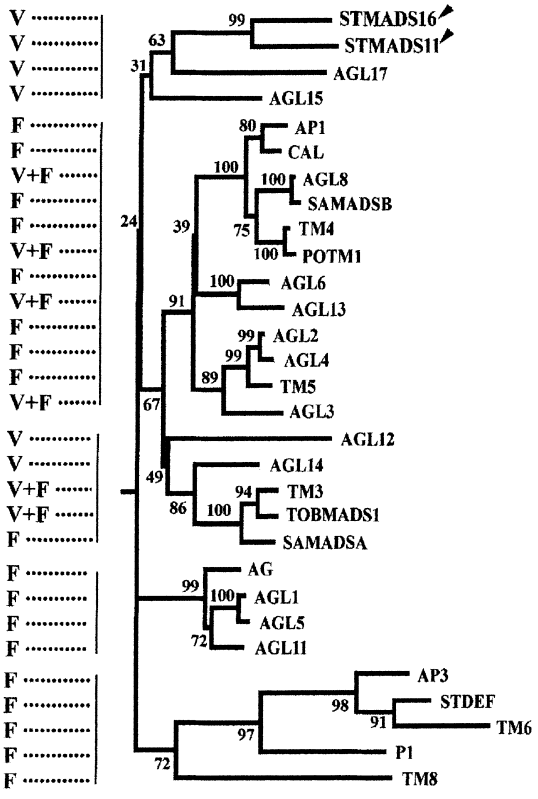

Fig. 5. Neighbor-joining tree illustrating relationships among MADS-box proteins from dicot species. Orthologs in other species different from *Arabidopsis* were excluded except for potato. STMADS11 and STMADS16 proteins reported in this paper are indicated by *arrowheads*. The expression pattern for each gene is represented on the left side by *V* (vegetative) or *F* (floral). Bootstrap values are expressed as a percentage (over 500 replicates) at the corresponding nodes

found in mature stems (Fig. 4B). Nevertheless, a delay in the induction of *STMADS11* expression was observed (see S2–S4 stages) in the roots as compared to the stems. At the S4 stage, there was a similar expression level in roots from dark-growing sprouts and those grown under illumination (data not shown), thus suggesting that *STMADS11* expression is not regulated by light in the roots. The *STMADS11* transcript was detected at a similar high level in young (2–4 last apical leaves) and old leaves from developing (S5 stage) or mature flowering plants (Fig. 4C). Although the *STMADS11* mRNA was nearly absent in the mature resting tubers, a substantial increase in transcript level through S2 to S4 stages was found during sprouting in the tuber tissue surrounding the sprout (Fig. 4D).

Sequence comparison. The deduced amino acid sequence of the STMADS11 protein was aligned with those from other dicot MADS proteins already reported. Orthologs of the *A. thaliana* MADS proteins were excluded from this analysis, except those from potato. The output from the alignment was used to perform a neighbor-joining cladistic analysis and stadistical significance of nodes was checked by computing the corresponding bootstrap values. The resulting phenogram (Fig. 5) showed that the STMADS11 protein is grouped with STMADS16 from potato, AGL17 and AGL15, both from *A. thaliana*, thus defining a cluster separated from the rest of the tree. Conversely, other vegetative-specific members like *AGL12* and *AGL14* from *A. thaliana*, as well as genes like *AGL3* from *A. thaliana*, *TM3* from tomato or *POTM1* from potato, which are additionally expressed in flower tissues, appeared interspersed along other clades (Fig. 5).

The alignment of the deduced amino-acid sequences for *STMADS11*, *STMADS16*, *AGL15* and *AGL17* cDNAs (Fig. 6) revealed that STMADS11 shared the highest overall homology with STMADS16 (69%

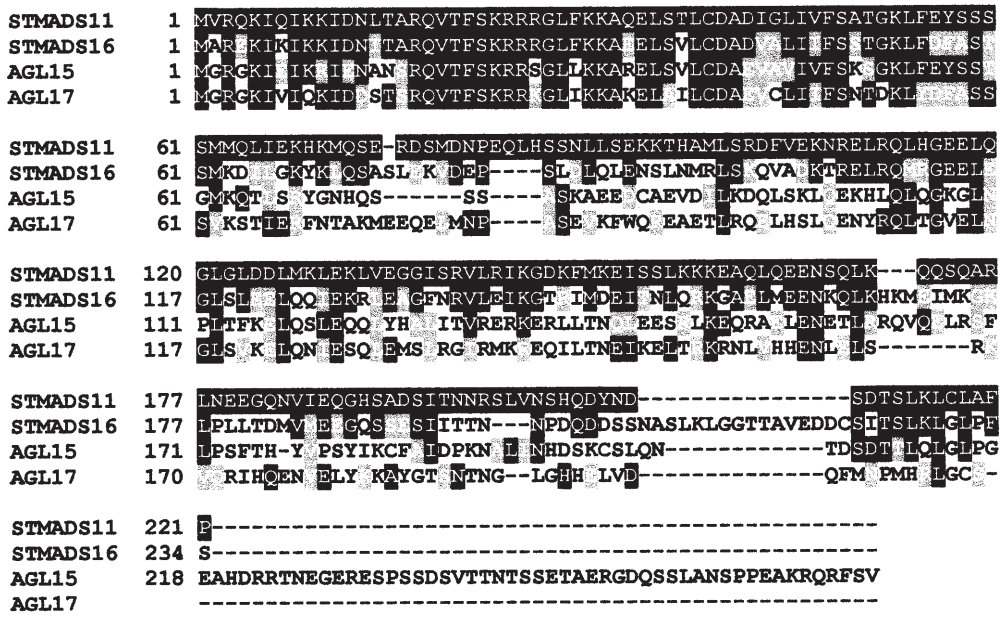

Fig. 6. Alignment of the deduced amino acid sequences for proteins STMADS11, STMADS16, AGL17 and AGL15. Amino acids identical to STMADS11 are indicated by the *black boxes* and similar residues by *grey boxes*. Gaps introduced to optimize the alignment are indicated by *dashes*

similarity, 52% for identical residues) followed by AGL17 (52% similarity, 33% identity). When the highly conserved MADS-box domains were compared, STMADS11 similarity to STMADS16 and AGL17 rose to 95% and 81% respectively, while for the moderately conserved K-box domain of STMADS11 these was a 67% similarity to STMADS16 and 41% to AGL17. Within the C-terminal region, the less conserved region, the homologies dropped to 56% and 39%, respectively.

Discussion

In this paper we report on the isolation of the cDNA for *STMADS11*, a new MADS-box gene from *Solanum tuberosum*. Its deduced amino acid sequence contains the typical domain structure, common to most plant MADS proteins, with the MADS, I, and K boxes as well as a C-terminal variable domain. However, the STMADS11 amino acid sequence and its specific vegetative expression make it different from previously reported MADS-box genes. As we have shown, *STMADS11* transcript is found at a similar level in all vegetative organs of the mature plant, but not in floral tissues. Moreover, *STMADS11* expression is not dependent on the light presence, and is detected in all developmental stages. These data suggest that *STMADS11* may participate in some common and basic process of vegetative growth. This particular expression pattern has not been reported for other genes of the MADS-box family. In this context, most of the vegetative MADS-box genes so far described, like *AGL3*, *TM3* and *POTM1*, are also active in flowers, so it is likely that their primary function is rather related to reproductive development. In addition, other vegetative-tissue specific genes like *AGL15* and *AGL17* are only transcribed in a few tissues or at some particular stages. On the other hand, since the *STMADS11* transcript is mainly found in vascular bundles of stems and leaves, it is tempting to speculate about a possible role for *STMADS11* as a vegetative regulator involved in vascular morphogenesis. It remains intriguing that *STMADS11* is induced during the sprouting process in the tuber tissue surrounding the sprouts, a place where growth or differentiation do not seem to occur. An appealing explanation is that signal(s) responsible for sprout initiation at the dormant meristem might originate within the tuber tissue, and that *STMADS11* would be activated during this process. Nevertheless, this possibility deserves further investigation.

Light has an important influence on seedling morphogenesis, mainly during the switch from heterotrophic to autotrophic growth (Chory 1997). As a similar situation is likely to occur during tuber sprouting, we have studied the effect of illumination conditions on *STMADS11* expression. However, transcript accumulation does not seem to be affected in stages covering transition from under-ground (S1–S2 stages) to above-ground (S3–S4 stages) growth, indicating that *STMADS11* function is not dependent on photoreceptor networks.

Cladistic analysis based on protein sequence comparison reveals that *STMADS11* shows the highest similarity (69% overall) to *STMADS16*. This is another potato MADS-box gene recently cloned in our lab, with an expression pattern similar to that of *STMADS11*, although with different relative levels among tissues (data not shown). Within the same cluster are also found *AGL17* and *AGL15* from *Arabidopsis thaliana*, two genes initially designated as "orphan genes" (Purugganan et al. 1995) because no other related genes were known at that time. Recent comprehensive studies (Theissen et al. 1996; Münster et al. 1997) also grouped *AGL15* and *AGL17* in the same cluster although they were considered as belonging to different gene subfamilies, on the basis of particular amino acid deviations from the MADS-box consensus sequence. Following this criterion, only *STMADS11* and *STMADS16* would be considered within the same subfamily. Moreover, the expression patterns of *AGL15* (whose transcript is present in seed embryos and seedlings) and *AGL17* (mRNA mainly found in roots) are different from those of *STMADS11* and *STMADS16*. All these data suggest that although they might have related functions in vegetative development, *STMADS11* and *STMADS16* are not functional homologous of *AGL17* or *AGL15*. Finally, the high similarity between *STMADS11* and *STMADS16* as well as the overlaping expression patterns make them candidates for involvement in the same developmental pathway, as in the case of the *GLOBOSA*/*DEFICIENS* tandem (Tröbner et al. 1992).

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