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Characterization of Potato Vegetative MADS-Box Gene, *POTM1-1*, in Response to Hormone Applications

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We used petiole/leaf cuttings from potato (*Solanum tuberosum* L.) to study the effect of plant hormones on expression of the vegetative MADS-box gene, *POTM1-1*. Transcript levels were constitutive after treatments with exogenous ABA, GA_3, methyl jasmonate (MeJa), and NAA. However, transcripts amounts were reduced when tissues were treated with BA. High levels of cytokinin disrupted apical dominance and promoted axillary-bud formation. Therefore, based on these results, the *POTM1-1* gene may conceivably be involved in maintaining the proper development of vegetative axillary meristems.

Keywords: MADS-box gene, plant development, plant hormones, *POTM1-1*, *Solanum tuberosum* L.

MADS-box genes encode transcription factors that mediate important functions in plant development. The MADS-box protein contains the MADS-box, a conserved DNA-binding domain, and a conserved protein interaction domain, the K-box (Schwarz-Sommer et al., 1990; Ma et al., 1991). Genetic analyses with *Arabidopsis* and *Antirrhinum* have revealed that the transcription factors encoded by the MADS-box genes play an important regulatory role in flower development (Schwarz-Sommer et al., 1990; Bowman et al., 1991; Coen and Meyerowitz, 1991; An and An, 2000; Lee et al., 2000). Further studies have demonstrated that MADS-box genes function in the formation of various organs, including the endosperm, pollen, fruit, guard cells, trichomes, leaves, tubers, and roots (Colombo et al., 1995; Kang and Hannapel, 1996; Zhang and Forde, 1998; Immink et al., 1999; Sung et al., 1999, 2000; Alvarez-Buylla et al., 2000; An and An, 2000; García-Maroto et al., 2000).

According to phylogenetic analysis, *POTM1-1* is a potato MADS-box cDNA that was isolated from an early-stage tuber library, and which belongs to the SQUAMOSA subfamily (Kang and Hannapel, 1995). In the ABC model of floral-organ development, SQUAMOSA controls perianth identity, the A-function (Coen and Meyerowitz, 1991; Riechmann and Meyerowitz, 1997). However, unlike typical gene-expression patterns in that subfamily, *POTM1-1* is expressed abundantly in vegetative organs, such as roots, leaves, stolons, and immature tubers (Kang and Hannapel, 1995, 1996). It is up-regulated during shoot elongation in the petiole/leaf-cutting system (Kang and Hannapel, 1996). There, axillary buds develop into shoots in potatoes grown under long days (16-h photoperiod), while buds that arise from plants treated under short-days (8-h photoperiods) become microtubers. Kang and Hannapel (1996) have observed that *POTM1-1* transcripts are increased during shoot formation, but are reduced when these buds instead become tubers. These results suggest that *POTM1-1* plays an important role during vegetative-meristem development.

Phytohormones influence various processes in plant growth and differentiation. MADS-box genes, which encode transcription factors, are also involved in intrinsic developmental processes, especially during the transition from the vegetative to the reproductive phase. Therefore, the two conductors, phytohormones and MADS-box genes, may have common, yet unrecognized, features. Although Bonhomme et al. (2000) have suggested that cytokinins and GA activate *SaMADS A*, which is involved in the floral transition of shoot apical meristems (SAM) in *Sinapis alba*, information is still lacking about the relationship between vegetative MADS-box genes and plant growth regulators in vegetative-organ development. Here we investigated the effect of phytohormones on accumulation of the mRNA of a potato MADS-box gene. This is the first report concerning the

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Abbreviations: ABA, abscisic acid; BA, N^6-benzyladenine; GA, gibberellic acid; MeJa, methyl jasmonate; NAA, α-naphthylacetic acid.

expression patterns of the vegetative gene, *POTM1-1*, in response to exogenous hormone treatments.

MATERIALS AND METHODS

Plant Materials and Hormone Treatments

Potato plants (*Solanum tuberosum* L. cv. Superior) were grown at 20°C under short-day conditions (8-h photoperiod). For the hormone treatments, fully expanded leaves with petioles were cut from six-week-old stock, then placed in one of the following: 100 mL water, 100 µM ABA, 100 µM N^6-benzyladenine (BA), 100 µM GA_3, or 50 µM α-naphtalene acetic acid (NAA). Time-course analyses were performed under light for 12, 24, or 48 h under light. For the methyl jasmonate (MeJa) treatment, leaf cuttings were first dipped in water, then placed, either under light or in the dark, in airtight, sealed jars containing cotton soaked with 100 µM MeJa. These tissues were also analyzed at the previously indicated times. After the test periods were completed, all samples were frozen in liquid nitrogen and stored at –80°C.

Preparation of Gene-Specific RNA Probes

Two radioactive (^{32}P)-labeled RNA probes, a MADS-box probe (Probe1) and a *POTM1-1* gene-specific probe (Probe 2), were generated according to the method of Kang and Hannapel (1996). An actin cDNA was used to create an antisense actin RNA probe for our loading control. All RNA probes were synthesized and labeled with [α-^{32}P] UTP, as described in the procedures for the Riboprobe® System (Promega Co., Madison, WI, USA).

Poly $(A)^+$ mRNA Isolation and Gel-Blot Analysis

Total RNA was extracted from frozen samples using the phenol/chloroform extraction method described by Sambrook et al. (1989). Using an oligo d(T) cellulose column for isolation, we separated 5 µg of poly $(A)^+$ mRNA electrophoretically on 1.4% denaturing agarose gels with 5 mM methyl mercury hydroxide (Alfa Products, Danvers, MA, USA) in 1X Tris-borate buffer. RNA was blotted onto nylon membranes and hybridized with [α-^{32}P] UTP-labeled probes for 10 h at 65°C in 50% formamide, 1.0 M NaCl, 10% (w/v) dextran sulfate, 1.0% SDS, and 0.1 mg/mL salmon sperm DNA. The membranes were then washed twice in 2X SSC and 0.1% SDS at 23°C for 5 min; once in 0.2X SSC and 0.1% SDS at 23°C for 10 min; twice in 0.1X SSC and 0.1% SDS at 23°C for 20 min; and once in 0.1X SSC and 0.1% SDS at 65°C for 5 min. Afterward, they were exposed for 24 h on X-ray film (Kodak, Rochester, NY, USA) for autoradiography. The used membranes were stripped in 0.1X SSC and 0.1% SDS at 98°C for 10 min, then exposed to X-ray film for 24 h to confirm removal of the probe. These stripped membranes were then hybridized to the antisense actin RNA probe for the loading control. All the experiments with hormone treatments and northern analysis were duplicated.

RESULTS AND DISCUSSION

Confirmation of a Gene-Specific Probe for *POTM1-1*

The MADS-box gene family consists of several subfamilies with high homology in their MADS-box domains (Theissen et al., 2000). Highly conserved nucleotide sequences that encode the MADS-box domain could lead to cross-hybridization within that family. Therefore, we selected a gene-specific probe for *POTM1-1* in order to perform an accurate analysis of *POTM1-1* gene expression in response to the hormone treatments (Fig. 1). Several related MADS-box genes were used for the cross-hybridization analysis, including *TM4* (83.2% nucleotide homology to *POTM1-1*) from *Lycopersicon esculentum* (Pnueli et al., 1991); *AP1* (58.8% homology) from *Arabidopsis* (Mandel et al., 1992); and *AG* (36.3% homology) from *Arabidopsis* (Yanofsky et al., 1990).

Cross hybridization revealed that Probe 1, which is MADS-box-specific, did not hybridize with *AG* and *AP1*, although a trace amount of signal remained in *TM4* (Fig. 1). However, Probe 1 failed to differentiate between *POTM1-1* and *POTM1-2*, which is its homologue (Kang and Hannapel, 1995). No signal was detected with the gene-specific Probe 2 used in cross-hybridization with *TM4*, *AG*, and *AP1* (Fig. 1); that probe significantly reduced the signal in the *POTM1-2* blot. This demonstrates that the signals in the northern blots represent transcripts of only the *POTM1-1* gene.

Northern-Blot Analysis of *POTM1-1* Gene Expression after Hormone Treatment

Little information is available about the relationship between MADS-box-mediated development and the actions of phytohormones. Therefore, we investigated whether *POTM1-1* gene expression was affected by hormone treatments. First, we determined the basal levels of *POTM1-1* transcripts by placing the leaf cuttings

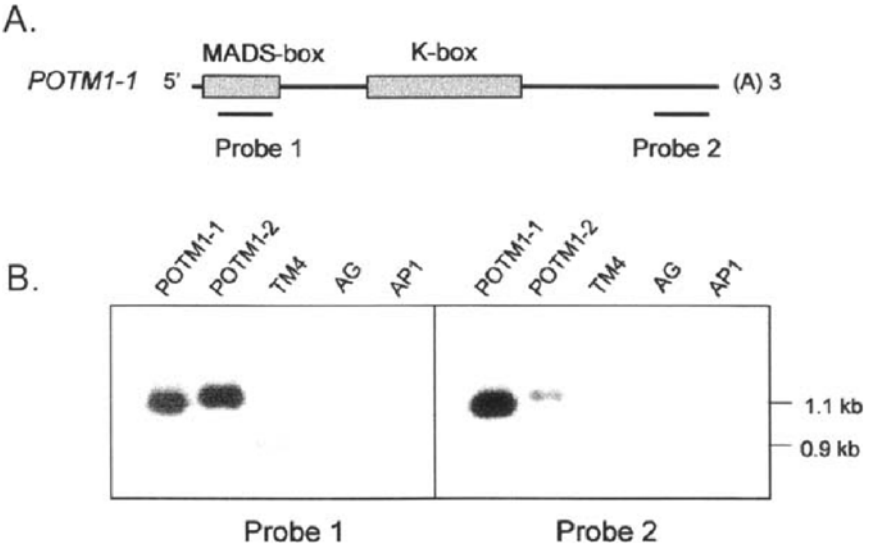

Figure 1. Schematic diagrams of *POTM1-1* cDNA (A), and cross-hybridization analysis of a MADS-box probe (Probe 1) and a gene-specific probe (Probe 2) (B). A, translated region of the *POTM1-1* cDNA is shown as an open box. Solid box indicates the MADS-box domain; striped box, the K-box domain. The 130 bp of the MADS-box domain and 170 bp of the 3'-UTR ir *POTM1-1* cDNA were used for RNA probes, and were designated Probe 1 and Probe 2, respectively. B, Ten nanogarms of full-length cDNAs of *POTM1-1*, *POTM1-2*, *TM4*, *AG*, and *AP1* were loaded in each lane in 1.0% agarose gels. ^{32}P-UTP-labeled RNA probes were used.

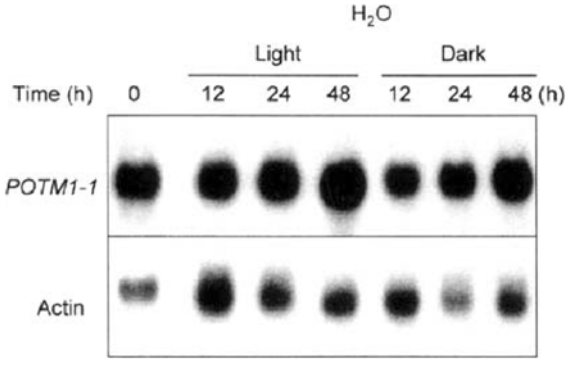

Figure 2. Northern-blot analysis for the accumulation of *POTM1-1* transcripts in potato petiole/leaf cuttings that were treated with water. Time-course analyses were performed for 0, 12, 24, or 48 h, either under light or in darkness. Poly (A^+) mRNA (5 μg) was loaded in each lane. The *POTM1-1* gene-specific RNA probe (Probe 2) was used. An actin RNA probe (actin) served as the control for loading.

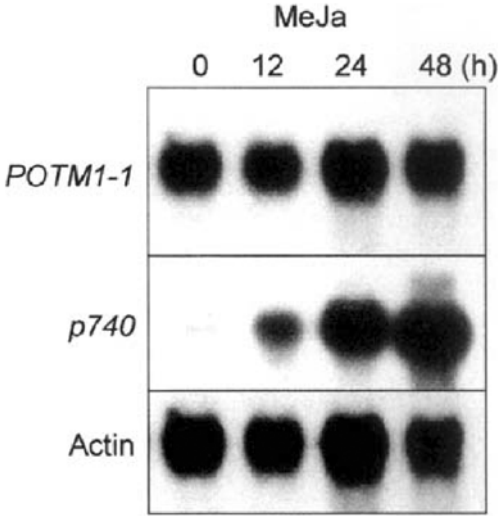

Figure 3. Northern-blot analysis of *POTM1-1* transcripts treated with methyl jasmonate (MeJa). Time-course analyses were performed for 0, 12, 24, or 48 h, as treated with 100 μM MeJa. Poly (A^+) mRNA (5 μg) was loaded in each lane. The *POTM1-1* gene-specific RNA Probe 2 was used. A probe for the proteinase inhibitor gene (p740) served as a MeJa response control, and an actin probe (Actin) was used as a control for loading.

in water, either under light or in the dark. Although transcript levels did not change significantly in the former (Fig. 2), they did gradually increase under continuous dark. Because of these results, we decided to conduct our hormone treatments under light to accurately measure any changes in *POTM1-1* transcript levels.

When the petiole/leaf cuttings were held for up to 48 h after exogenous application with ABA, GA_3, NAA, or MeJa, the amount of *POTM1-1* RNA did not change significantly (Figs. 3 and 4). However, transcript levels gradually decreased for BA treatments of similar length (Fig. 4). Jasmonates and their derivatives modulate the

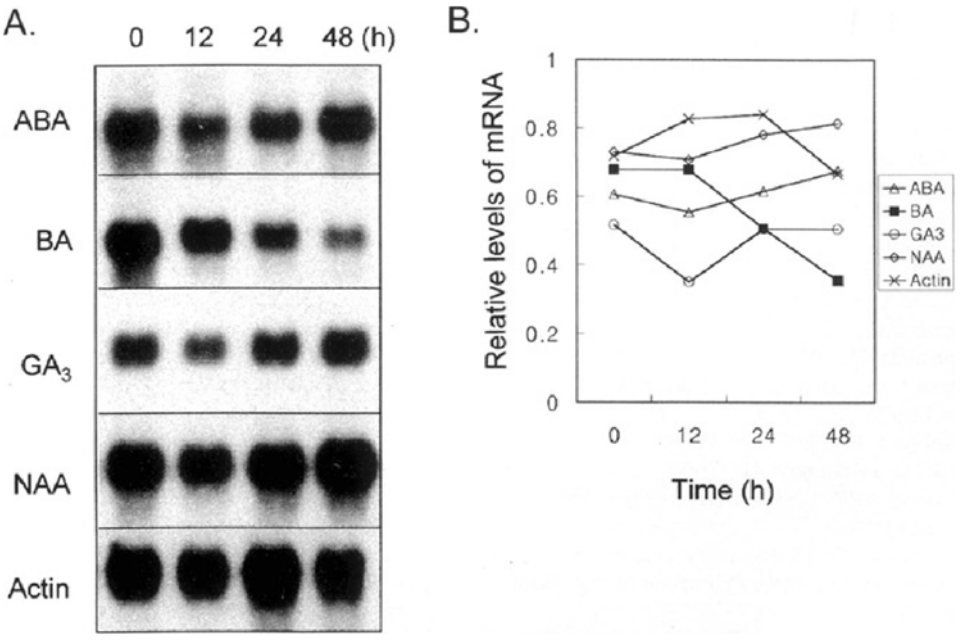

Figure 4. Northern-blot analysis of *POTM1-1* transcripts in potato petiole/leaf cuttings treated with various phytohormones. Time-course analyses were performed for 0, 12, 24, or 48 h, as treated with 100 μM ABA, 100 μM N^6 BA, 100 μM GA_3, or 50 μM NAA. The *POTM1-1* gene-specific RNA Probe 2 was used. Poly (A^+) mRNA (5 μg) was loaded in each lane. An actin RNA probe served as a control for loading.

expression of numerous genes in response to environmental stress (Creelman and Mullet, 1997). We found that treatment with MeJa induced a cathepsin D proteinase inhibitor gene, p740 (Fig. 3), which indicates that the exogenous hormone treatments had affected the gene-expression systems in the petiole/leaf cuttings. In contrast, levels of *POTM1-1* transcripts were unchanged by the exogenous MeJa (Fig. 3), which implies that this particular gene is not involved in MeJa-mediated plant growth and development.

GA-mediated signal-transduction pathways lead to the flowering of *Arabidopsis* (Koornneef et al., 1998; Levy and Dean, 1998). In the floral transition of *Sinapis alba*, *SaMADS A* (Menzel et al., 1996) is activated by cytokinins and GA (Bonhomme et al., 2000). Although *POTM1-1* belongs to the *SQUAMOSA* subfamily (Kang and Hannapel, 1995; Theissen et al., 2000), exogenous GA did not affect its transcription here (Fig. 4), which suggests that *POTM1-1* is independent of GA-mediated floral transition in shoot apical meristems.

Cytokinins can mediate various physiological processes, such as shoot development, cell proliferation, and apical dominance (Skoog and Miller, 1957; Medford et al., 1989). This class of plant growth regulator is synthesized in roots, then transported through the xylem to target tissues and organs. With this information, we presumed that the amount of endogenous cytokinins would be decreased in the leaf/petiole cuttings. Furthermore, exogenous BA was expected to affect gene expression in the cuttings. Northern-blot analysis showed that levels of *POTM1-1* transcripts were noticeably reduced 48 h after BA treatment (Fig. 4.). These results suggest that the functions of *POTM1-1* might differ from those of floral meristem-identity genes, e.g., the *SQUA* subfamily genes and *SaMADS A*.

In conclusion, we propose that gene expression of *POTM1-1*, a potato vegetative MADS-box gene, may be independent of any exogenous applications of ABA, GA, NAA, and MeJa, but that it can be suppressed by BA treatment.

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