

Electronic supplementary materials

Title:

Physiological roles of Arabidopsis MCA1 and MCA2 based on their dynamic expression patterns

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Plant materials and growth conditions

The Columbia-0 (Col-0) ecotype of *Arabidopsis thaliana* (Arabidopsis) and its isogenic, transgenic lines were used in this study. The *MCA1* and *MCA2* promoters fused to the reporter gene β -glucuronidase (GUS), *MCA1p::GUS* and *MCA2p::GUS*, respectively, have been described previously (Yamanaka et al., 2010) and were used to localize the expression of MCA1 and MCA2. The *MCA1p::GUS* lines 1, 3, and 5 and the *MCA2p::GUS* lines 3, 4, and 5 were obtained from our laboratory's stock.

Seeds were treated for 10 min with 1 mL of 5% sodium hypochlorite/10% Triton X-100 solution, washed five times with autoclaved Milli-Q water (1 mL each), and stored for 3 days at 4 °C in the dark. Then, the seeds were sown on Murashige and Skoog (MS) media (Murashige and Skoog, 1962; FUJIFILM Wako Pure Chemical Corp., Osaka, Japan) supplemented with 0.8% (w/v) agar (Difco Laboratories, Detroit, Michigan, USA), and the germinated seedlings were grown under a 16 h light/8 h dark cycle with white light from fluorescent lamps at approximately 90-110 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity at 22 °C.

Histochemical analysis

The plants were placed in 1.5 mL Eppendorf tubes containing 1 mL of 90% acetone and placed on ice for 1 h for fixation. The plants were then washed twice with 1 mL of 0.1 M sodium phosphate buffer (5x dilution of 0.5 M sodium phosphate buffer, pH 7.0). Then, 1 mL of X-Gluc buffer (0.1 M sodium phosphate buffer (pH 7.0), 0.5 mg/ml X-Gluc [5-bromo-4-chloro-3-indolyl glucuronidase; Bio Medical Science, Ltd., Tokyo], 0.5 mM potassium ferrocyanide, and 0.5 mM potassium ferricyanide) was added, and the mixture was incubated at 37 °C in an incubator (Sanyo, MLR-153) for 12-18 h. The plants were then decolorized (50% $\rightarrow$ 70% $\rightarrow$ 90% $\rightarrow$ 100%) by immersion in ethanol (09-0770-3; Sigma-Aldrich Japan) at different concentrations in stages. The samples were soaked in 50% ethanol for 10 min and sterile Milli-Q water for 10 min. Then, the

samples were soaked in sterilized Milli-Q water for preparation and observed and photographed using a binocular stereomicroscope (Leica, DFC300FX, Germany) or phase contrast microscope (Olympus, 3G05308, Tokyo, Japan).

Semiquantitative RT–PCR

Total RNA was obtained from the whole organs of interest within *Arabidopsis* plants at 10, 15, and 20 days after sowing (DAS) with a NucleoSpin RNA Plant Kit (Macherey-Nagel, Rev. 06.1012/001; Allentown, PA, USA) according to the manufacturer's protocol. cDNA was synthesized for 30 min at 37 °C with oligo (dT) primers and a PrimeScript RT reagent kit (RR037A; Takara, Shiga, Japan) on a T00 Thermal Cycler (186-1096J1; Bio-Rad, Hercules, CA, USA). RT–PCR was performed using the primer pairs listed in Table S1 on a T00 Thermal Cycler. Each reaction was carried out in a mixture (9 µl) containing 1 µl of 40 ng/µl cDNA, 5 µl of 2x Quick Taq HS DyeMix (Toyobo, Osaka, Japan), 0.2 µl of 10 µM forward primer, 0.2 µl of 10 µM reverse primer, and 3.6 µl of Milli-Q water. The thermal profile consisted of 30 s of hot-start enzyme activation at 94 °C, followed by 32 cycles of PCR at 94 °C for 2 min (denaturation), 55 °C for 30 s (annealing), and 68 °C for 1 min (elongation). After the reaction, 5 µl of the reaction mixture and 1 µl of 6x GR Red loading buffer (Bio Craft, Tokyo, Japan) were mixed, and 5 µl of the mixture was electrophoresed for 20 min at 100 V on a 2% agarose gel. Following electrophoresis, the gels were photographed with a ChemiDoc XRS+ (Bio-Rad), and the fluorescence intensity of each PCR product in the gel was quantified with the image processing software ImageJ. The intensity of the *MCA1* and *MCA2* products is indicated by the relative value when that of β -tubulin was set to 1.

References

Murashige T, Skoog S (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15, 473–497.

Table S1 List of primers used for RT–PCR

Name of primer	Nucleotide sequence (5' to 3')
FAM1AG0007 (<i>MCA1</i> -f ¹)	TGCTCCCTCTTTTAGTCAATTCTC
RAM1Ao981 (<i>MCA1</i> -r ²)	CCTGTTGGATGCTGCAGTGGCAATC
FAM1B565S1 (<i>MCA2</i> -f)	CATCCGTAATTTGCTAGAGCA
FAM1BG0129 (<i>MCA2</i> -r)	ACAAGTACCATCTCTGTAATTCTTGAC
FAM1B630S2 (<i>MCA2</i> -f)	CGCTTCAAGGACTTGATGATGATGC
RAM1Bo888 (<i>MCA2</i> -r)	CAAGGTTCTGAACAACAATCCAGC
Tubulin-f	ACCACTCCTAGCTTTGGTGATCTG
Tubulin-r	AGGTTCACTGCGAGCTTCCTCA

¹f, forward; ²r, reverse.