Plant Physiol. (1992) 99, 759–761
0032-0889/92/99/0759/03/$01.00/0

Received for publication December 2, 1991
Accepted March 2, 1992

Communication

Induction of mRNA for Phospho*enol*pyruvate Carboxylase Is Correlated with a Decrease in Shoot Water Content in Well-Irrigated *Mesembryanthemum crystallinum*[1]

Jürgen M. Schmitt* and Mechtild Piepenbrock

Institut für Pflanzenphysiologie und Mikrobiologie, Freie Universität, 1 Berlin 33, Germany

ABSTRACT

The abundance of mRNA specific for phospho*enol*pyruvate carboxylase (PEPCase) was measured in leaves from well-watered plants of *Mesembryanthemum crystallinum*. Plants grown side by side in pots of four different volumes (0.16, 0.74, 2.6, 6.5 liters) were compared. The time of increase in the steady-state level of PEPCase mRNA in well-watered plants was dependent on soil volume. The larger the pot, the later PEPCase transcripts were increased. PEPCase mRNA induction started when shoot water content decreased to well below 4000% of dry weight. No positive correlation with the developmental status of the plants could be found. The data indicate that PEPCase mRNA induction in well-watered plants up to 10 weeks of age is controlled environmentally rather than developmentally.

Mesembryanthemum crystallinum is a facultative halophyte capable of shifting its carbon metabolism from C_3 to CAM when water stressed (16). In the CAM mode of photosynthesis, several carbon-metabolizing enzymes are increased in extractable activity (7). The induction of one of these enzymes, PEPCase[2], has been studied in some detail (2, 6, 10, 11). Under salt stress, transcription from a stress-specific isogene is increased, leading to accumulation of CAM-specific mRNA (2) and enzyme protein (5). The CAM-specific PEPCase enzyme can be distinguished from its C_3 counterpart by mobility on SDS gels and peptide mapping (5).

Induction of PEPCase mRNA, protein, and the CAM pathway has also been observed in old, well-watered plants (1, 3, 9, 13). Cushman and coworkers (3) have therefore proposed that a developmental program is regulating PEPCase transcription and mRNA stability, and they have drawn the conclusion that C_3 to CAM switching is developmentally programmed (3). Alternatively, mild water deficits caused by a diurnal transient decline in leaf water content have been thought to be sufficient to give rise to PEPCase (9) and CAM induction (14) in well-watered plants.

In this study, we measured the abundance of mRNA coding for PEPCase using well-watered *Mesembryanthemum* plants grown side by side in pots of different sizes. We show that induction in plants up to 10 weeks of age is correlated with neither the age nor the developmental status but rather to shoot water content.

MATERIALS AND METHODS

Growth of Plants

Plants (*Mesembryanthemum crystallinum* L.) were germinated in the greenhouse under supplementary light (14 h at 15 W/m^2) from metal halide lamps and transferred (one plant per pot) to pots of different sizes (0.16, 0.74, 2.6, 6.5 L). Two weeks after germination, plants were placed in a controlled climate chamber (14 h light at 25 W/m^2 from metal halide lamps, 25°C, 50% RH; 10 h dark, 15°C, 60% RH). Smaller pots were elevated to have all the plants the same distance from the lamps. The plants were watered one to two times daily, and the soil was never allowed to dry. Shoots were harvested 2 h before the dark period. Plant age is given in weeks after germination and refers to the time of harvest.

RNA Extraction and Northern-Type Hybridization

Nonsenescent leaves were pooled. RNA was isolated using a guanidine-based protocol modified from ref. 8. Briefly, the tissue was powdered in liquid nitrogen and extracted in 8 M guanidine-HCl, 0.2 M Tris-HCl (pH 8), 2.5% (v/v) 2-mercaptoethanol. After extraction with phenol, RNA was precipitated from the aqueous phase with 0.05 volume of 1 M acetic acid and 0.7 volume of ethanol. The sodium acetate wash of the RNA pellet was omitted. Total RNA (10 μg/lane for PEPCase transcripts and 5 μg/lane for SSU transcripts) was separated on formaldehyde gels. Proper loading was verified by ethidium bromide staining. Transfer to nitrocellulose membranes, hybridization, and washing conditions were as described previously (11).

Probes

Inserts from pMcCAM7 (12) or pPPC1 (10) were used to probe for transcripts of PEPCase (*ppc*). These clones are specific for the stress-inducible isoform 1 of the enzyme (2).

[1] This work was supported by Deutsche Forschungsgemeinschaft.

[2] Abbreviations: PEPCase, phospho*enol*pyruvate carboxylase; PEP, phospho*enol*pyruvate; *ppc*, gene for PEPCase; *rbcS*, gene for the small subunit of Rubisco; SSU small subunit of Rubisco.

An insert from pMcss5 (4) was used to probe for *rbcS* transcripts coding for SSU.

RESULTS

Mesembryanthemum plants grown in different sized pots had very different fresh weights. The weight of the plants (Fig. 1a) was dependent on soil volume.

Young *M. crystallinum* are rosette-type plants. Leaves are inserted at the main axis. At the plant age of 4 to 6 weeks, branches start to arise from axillary buds, on which smaller leaves develop (Fig. 1, b and d). When the side shoots start growing vigorously at week 7 (Fig. 1d), cotyledons and primary leaves show symptoms of senescence, and the lowest foliar leaf pair (leaf pair 2) already loses turgor. Eventually, most of the organic matter ends up in stems of side shoots and reproductive organs. Flower buds start to develop at approximately week 10 (9). The ratio of leaves to stems has been used previously as a quantitative indicator of the developmental status of a plant (15). Regardless of the widely differing sizes, plants grown in various volumes of soil were similar in their development as measured by leaf to stem fresh weight ratios (Fig. 1b), onset of side shoot growth (Fig. 1d), and leaf emergence (Fig. 1e) at different times after germination.

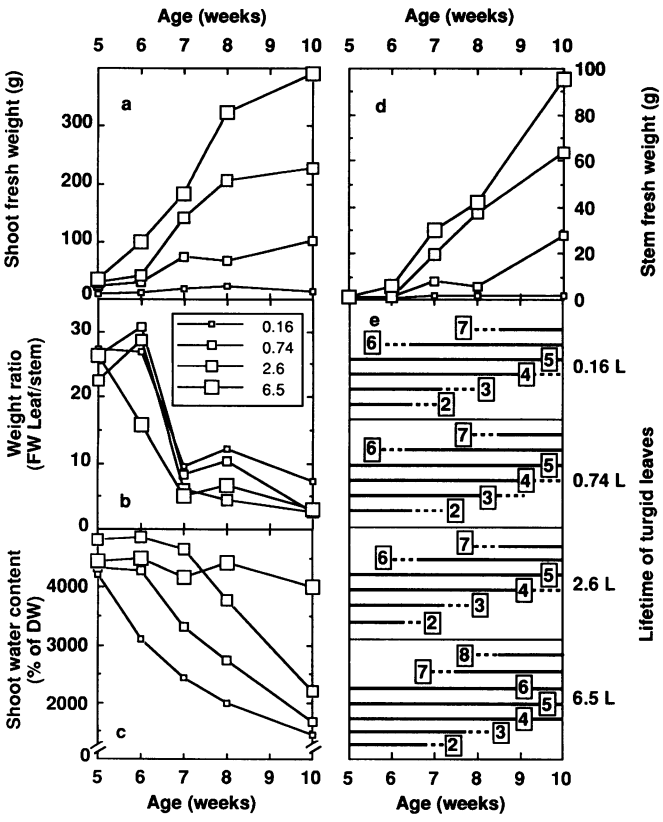

Figure 1. Growth, development, and water content of shoots from well-watered plants grown side by side in pots of different sizes. The sizes of the symbols (see inset) reflect the sizes of the pots (0.16 L [smallest symbol], 0.74 L, 2.6 L, and 6.5 L). Developmental status was quantified as the fresh weight (FW) ratio of leaves to stems (b), fresh weight of stems (d), and the lifetime of the leaf pairs that are attached to the main shoot (e). In e, leaf pairs 2 to 8 have been numbered in the order of emergence (cotyledons = leaf pair 0). A schematic drawing of a plant together with the leaf numbering scheme is shown in ref. 9. Water content of the shoots (c) is given as the percentage of dry weight (DW).

In contrast, shoot water content was strongly dependent on the size of the pots (Fig. 1c). The plants grown in the smallest pots showed a decrease in water content to about half of the initial value between the ages of 5 and 10 weeks. With increasing pot size, the rate of reduction in leaf water content was smaller, the decrease in water content occurred later, and the absolute loss was less severe. Plants grown in large 6.5-L pots showed a negligible decline in water content during the 5-week observation period.

The time of increase in the steady-state level of PEPCase mRNA in well-watered plants was correlated with soil volume (Fig. 2). Five-week-old plants had extremely low and comparable levels of PEPCase mRNA regardless of the volume of their pots. Well-watered plants grown in the 0.16-L pots showed a sharp increase in mRNA abundance at the age of 6 weeks. Plants grown in 0.74-L pots induced when they were 7 weeks of age, plants grown in 2.6-L pots induced at week 8, and plants grown in the large 6.5-L pots did not induce at all up to 10 weeks of age. Transcripts coding for the SSU of Rubisco were used as controls. The abundance of *rbcS* transcripts declined with the age of the plants (Fig. 2). This has been observed previously for plants grown in 0.7-L pots (9). In comparison with the transcripts coding for PEPCase, *rbcS* transcripts showed, however, only minor changes, indicating that the observed changes in PEPCase mRNA abundance were specific.

DISCUSSION

It is obvious that the accumulation of PEPCase mRNA in well-watered plants is not correlated with plant age (Fig. 2). It is also not correlated with the age of the individual leaf pairs, as we have shown previously (9). It could be argued that chronological age is a poor indicator of developmental status in plants. The data in Figure 1, b, d, and e, show, however, that the plants in different pot sizes develop closely in parallel.

PEPCase mRNA induction (Fig. 2) is best correlated with the water content of the shoots (Fig. 1c). Under our conditions, transcripts for PEPCase increase in abundance when shoot water starts to decline to well below 4000% of dry weight (*cf.* Figs. 1c and 2). Plants grown in 6.5-L pots did not reach this threshold during the 5-week observation period (Fig. 1c) and did not accumulate significant levels of PEPCase mRNA (Fig. 2). Growth conditions favoring water conservation, such as low-intensity light and high humidity, are known to retard the expression of CAM in well-watered plants (13). In salt-stressed plants, transient reductions in leaf water content (*cf.* Fig. 1c) precede the phenotypic expression of CAM (14).

The cause of the increased water loss in shoots of plants grown in small pots is not clear. It is obvious that smaller pots

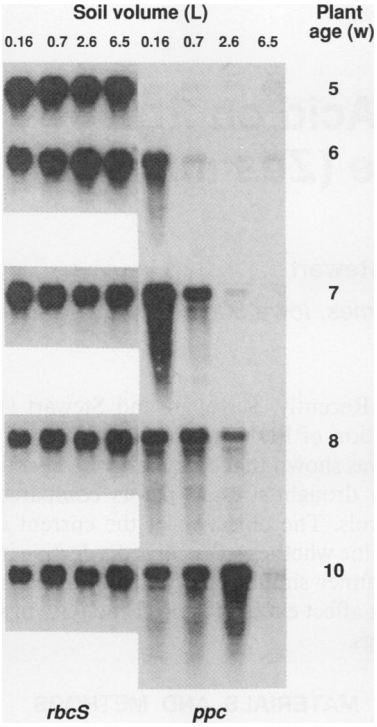

Figure 2. Abundance of mRNAs in leaves of well-watered plants grown in different soil volumes. Soil volume is indicated at the top of the figure. At the age indicated, in weeks (w), plants were harvested, and RNA was separated on agarose gels. The loading was verified by ethidium bromide staining of the gels. RNA was transferred to nitrocellulose and probed with DNA specific for SSU of Rubisco (*rbcS*) and PEPCase (*ppc*). Blots for *rbcS* and *ppc*, respectively, were hybridized, washed in the same solution, and exposed side by side on the same autoradiographic film.

have a smaller water capacitance. Visual inspections indicate that larger plants have a larger root system that would favor water uptake. Quantitative determinations of root mass have, however, not been made.

It was pointed out (14) that the occurrence of CAM in well-watered *Mesembryanthemum* plants would only be truly developmental if it could be demonstrated that CAM appears in the absence of any leaf water deficit. Our results show that PEPCase transcript induction in well-watered plants up to 10 weeks of age is correlated with a decline in leaf water content. The results, therefore, corroborate previous conclusions that PEPCase mRNA accumulation (9) and CAM induction (14) in well-watered, vegetative plants are caused by water deficit rather than by a developmental program.

LITERATURE CITED

1. **Chu C, Dai ZY, Ku MSB, Edwards GE** (1990) Induction of Crassulacean acid metabolism in the facultative halophyte *Mesembryanthemum crystallinum* by abscisic acid. Plant Physiol **93:** 1253–1260
2. **Cushman JC, Meyer G, Michalowski CB, Schmitt JM, Bohnert HJ** (1989) Salt-stress leads to differential expression of two isogenes of phosphoenolpyruvate carboxylase during CAM induction in the common ice plant. Plant Cell **1:** 715–725
3. **Cushman JC, Michalowski CB, Bohnert HJ** (1990) Developmental control of CAM inducibility by salt-stress in the common ice plant. Plant Physiol **94:** 1137–1142
4. **DeRocher EJ, Ramage RT, Michalowski CB, Bohnert HJ** (1987) Nucleotide sequence of a cDNA encoding rbcS from the desert plant *Mesembryanthemum crystallinum*. Nucleic Acids Res **15:** 6301
5. **Höfner R, Vazquez-Moreno L, Abou-Mandour AA, Bohnert HJ, Schmitt JM** (1989) Two isoforms of phosphoenolpyruvate carboxylase in the facultative CAM plant *Mesembryanthemum crystallinum*. Plant Physiol Biochem **27:** 803–810
6. **Höfner R, Vazquez-Moreno L, Winter K, Bohnert HJ, Schmitt JM** (1987) Induction of Crassulacean acid metabolism in *Mesembryanthemum crystallinum* by high salinity. Mass increase and *de novo* synthesis of PEP-carboxylase. Plant Physiol **83:** 915–919
7. **Holtum JAM, Winter K** (1982) Activity of enzymes of carbon metabolism during the induction of Crassulacean acid metabolism in *Mesembryanthemum crystallinum* L. Planta **155:** 8–16
8. **Logemann J, Schell J, Willmitzer L** (1987) Improved method for the isolation of RNA from plant tissue. Anal Biochem **163:** 16–20
9. **Piepenbrock M, Schmitt JM** (1991) Environmental control of phospho*enol*pyruvate carboxylase induction in mature *Mesembryanthemum crystallinum* L. Plant Physiol **97:** 998–1003
10. **Rickers J, Cushman JC, Michalowski CB, Schmitt JM, Bohnert HJ** (1989) Expression of the CAM-form of phosphoenolpyruvate carboxylase and nucleotide sequence of a full-length cDNA from *Mesembryanthemum crystallinum*. Mol Gen Genet **215:** 447–454
11. **Schmitt JM** (1990) Rapid concentration changes of phosphoenolpyruvate carboxylase mRNA in detached leaves of *Mesembryanthemum crystallinum* L. in response to wilting and rehydration. Plant Cell Environ **13:** 845–850
12. **Schmitt JM, Michalowski CB, Bohnert HJ** (1988) Gene expression during CAM induction under salt stress in *Mesembryanthemum*: cDNA library and increased levels of mRNA for phosphoenol pyruvate carboxylase and pyruvate orthophosphate dikinase. Photosynth Res **17:** 159–171
13. **Winter K** (1973) CO_2-Fixierungsreaktionen bei der Salzpflanze *Mesembryanthemum crystallinum* unter variierten Außenbedingungen. Planta **114:** 75–85
14. **Winter K, Gademann R** (1991) Daily Changes in CO_2 and water vapor exchange, chlorophyll fluorescence, and leaf water relations in the halophyte *Mesembryanthemum crystallinum* during the induction of Crassulacean acid metabolism in response to high NaCl salinity. Plant Physiol **95:** 768–776
15. **Winter K, Lüttge U, Winter E, Troughton JH** (1978) Seasonal shift from C_3 photosynthesis to Crassulacean acid metabolism in *Mesembryanthemum crystallinum* growing in its natural environment. Oecologia **34:** 225–237
16. **Winter K, von Willert DJ** (1972) NaCl induzierter Crassulaceen-Säurestoffwechsel bei *Mesembryanthemum crystallinum*. Z Pflanzenphysiol **67:** 166–170