

The stimulation of CAM activity in *Mesembryanthemum crystallinum* in nitrate- and phosphate-deficient conditions

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SUMMARY

Plants of *Mesembryanthemum crystallinum* L. were grown from seed in phosphate-sufficient and phosphate-deficient conditions. Separate plants were subjected to nitrate deficiency by removing nitrate from the rooting medium of previously nitrate-sufficient plants. Subsequently, they were also irrigated with 400 mM NaCl to induce CAM. Plants of *M. crystallinum* with induced CAM that had been grown in complete nutrient medium were deinduced by rehydration.

Nitrate and phosphate deficiencies caused enhanced CAM activity prior to salt treatments. After salinisation, phosphate deficiency also caused higher background levels of malate. Prolonged nitrate deficiency reduced CAM activity.

Endogenous P_i levels in phosphate-sufficient plants correlated with CAM activity. However in phosphate-deficient plants CAM occurred without a significant rise in P_i content. Levels of endogenous P_i appeared more related to nitrate deficiency than to CAM or salt treatment. Nitrate and phosphate deficiencies and salt treatment could all cause a nitrate deficiency within the leaf, causing high endogenous P_i when available in the rooting medium. It is speculated that a change in nitrogen status could play a role in the initiation of CAM induction in *M. crystallinum*. A further possibility is that CAM could be induced by reduced water potential due to dehydration and increased ion content in saline conditions, and by an inhibition of growth and concomitant accumulation of solutes in phosphate- and nitrate-deficient conditions.

Key words: CAM, phosphate, nitrate, *Mesembryanthemum crystallinum*, nutrient deficiency.

INTRODUCTION

Mesembryanthemum crystallinum L., a halophytic member of the Aizoaceae, exhibits inducible CAM. Plants in which CAM can be induced, otherwise known as C_3 -CAM intermediates or facultative CAM plants, attain the CAM mode of photosynthesis in response to an environmental stimulus. In *M. crystallinum* the transition from C_3 to CAM in the field corresponds with the seasonal availability of water (Winter *et al.*, 1978). The flexibility of inducible CAM exhibited by *M. crystallinum* enables the productivity of the C_3 mode in hydrated conditions to be integrated with the endurance of the CAM mode in arid conditions (Paul, 1988).

In the laboratory, CAM may be induced in *M. crystallinum* by treatments that reduce the water potential of the leaves: high light and low relative

humidity (Winter, 1973); exposure of the roots to low temperature or O_2 deficiency (Winter, 1974); irrigation with up to 500 mM NaCl (Winter & von Willert, 1972). CAM activity is also influenced by the complex endogenous factors of plant development and leaf age. Old *M. crystallinum* leaves exhibit greater CAM activity than young ones, a trend associated with phosphoenol pyruvate (PEP) carboxylase activity (von Willert *et al.*, 1976*a,b*). Additionally, *M. crystallinum* plants cannot be induced into CAM until they are 6 weeks old (Ostrem *et al.*, 1987). Endogenous growth regulators that may change during development (Phillips, 1975) do not appear to be involved in CAM induction in *M. crystallinum* (Bohnert *et al.*, 1988).

Von Willert *et al.* (1977) suggest roles for ions in CAM induction in *M. crystallinum*, proposing that the concentration of specific ions at particular sites in response to leaf dehydration may initiate CAM. In addition they speculate that an excess of anions like Cl^- present in young *M. crystallinum* leaves may prevent CAM activity by restricting the accumu-

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lation of other anions like malate. P_i , which accumulates during CAM induction in *M. crystallinum* (von Willert, 1975a) stimulates the activity of PEP carboxylase and reduces its inhibition by malate, and was seen to have a possible controlling role in the induction process (von Willert, 1975b). Ions may have an involvement in other species: Ota, (1988a) found that growth of the inducible CAM plant *Kalanchoe blossfeldiana* in the presence of nitrate as the nitrogen source resulted in greater CAM activity in hydrated and dehydrated conditions than in plants where ammonium was the sole nitrogen source. The acidifying effect of NO_3^- and the alkalinizing effect of NH_4^+ on cellular pH (Raven & Smith, 1974) and the known effects of pH on malic acid synthesis and breakdown (Davies, 1973), may explain the higher levels of malic acid in the presence of NO_3^- , but not the greater diel fluctuations of malic acid. Ota, (1988b) also observed that the removal of nitrogen from the nutrient solution fed to *K. blossfeldiana* plants stimulated CAM. Nutrient and water absorption are closely related processes and there is a possibility that some of the effects of water stress on CAM activity in inducible CAM plants may be mediated through effects on nutrients.

In the present study *M. crystallinum* plants were grown with different concentrations of exogenous P_i , and separate plants were subjected to nitrate deficiency by removing nitrate from the rooting medium of nitrate-sufficient plants. The effect of these treatments on CAM activity, and the relationship between CAM activity and endogenous P_i levels, were assessed.

MATERIALS AND METHODS

Effects of phosphate

Plant material. Plants of *M. crystallinum* were grown from seed in white silvaperl in growth cabinets at temperatures of 25 °C (day) and 16 °C (night) with a 12 h photoperiod (warm-white fluorescent tubes and tungsten lamps giving a photon fluence rate of 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ between 400 and 700 nm). Photon fluence rates were measured throughout with a Li-Cor Li-185 quantum radiometer. Plants were watered with nutrient medium (Edwards & Walker, 1983) containing three different concentrations of KH_2PO_4 : 0, 0.2 and 3 mM. Ten plants were maintained at each level of P_i . When plants were 8 weeks old, 5 at each level of P_i were watered with nutrient medium supplemented with 400 mM NaCl to induce CAM (induced plants). The remaining 5 control plants are termed non-induced plants.

For the analysis of soluble carbohydrate, starch, pinitol, endogenous P_i and malate, leaf discs (0.5 cm diameter, 0.01 g f. wt) were excised from most recently fully expanded leaves and killed and extracted in boiling water. Samples for malate estimation were taken immediately prior to and 6 h

into the light period. There were 3 replicates and each sample consisted of 5 leaf discs. Samples for the estimation of starch, soluble carbohydrate and pinitol were taken 6 h into the photoperiod and consisted of 3 replicates of 5 leaf discs each. Samples for the measurement of endogenous P_i were taken 6 h into the photoperiod and consisted of 5 replicate samples of 5 leaf discs each.

Effects of nitrate

M. crystallinum plants were grown from seed in vermiculite in growth cabinets at temperatures of 25 °C (day) and 18 °C (night) with a 14 h photoperiod (Warm-white fluorescent tubes and tungsten lamps giving a photon fluence rate of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ between 400 and 700 nm). Plants were watered with nutrient medium consisting of: 10 mM $NaNO_3$, 5 mM KH_2PO_4 , 2 mM K_2SO_4 , 2 mM $MgSO_4 \cdot 7H_2O$, 4 mM $CaCl_2$ with micronutrients supplied as in Edwards & Walker, (1983). When the plants were 7 weeks old the rooting medium of 9 of them was flushed thoroughly with nitrate-free medium (-N) and from then on they were watered with medium lacking nitrate. Samples for starch, soluble carbohydrate, pinitol and P_i were taken 17 days later. Eight plants were maintained with complete medium. Thirty days later the medium of 4 plants receiving nitrate was supplemented with 400 mM NaCl (+N+NaCl). Additionally, the medium of three nitrate-deficient plants was supplemented with 400 mM NaCl (-N+NaCl) and three were given 400 mM NaCl and replenished with nitrate (-N+N+NaCl). Further samples were taken 20 days after NaCl treatment.

For the analysis of starch, soluble carbohydrate, pinitol and endogenous P_i leaf disc samples were excised from the most recently fully expanded leaves carried on stolons, as described above. For the estimation of leaf acid content, most recently fully expanded leaves (young) and second most recently fully expanded leaves (old) were excised, killed and extracted in boiling water and titrated to the phenolphthalein end-point with 0.01 M NaOH.

Deinduction of CAM

M. crystallinum plants were grown from seed in wet, peat-based compost in a greenhouse at 25 °C with supplementary mercury vapour lighting to give a 16 h photoperiod. After 5 weeks they were transferred to growth cabinets with the same conditions as the phosphate experiment. Six weeks after germination CAM was induced by irrigating plants with 400 mM NaCl. The soil was allowed to dry out between successive treatments with salt solution. Three weeks later CAM was deinduced by drenching the soil of each plant with tap water and then

maintaining plants in this state. Sampling for malate and P_i was carried as described above.

Analysis of carbohydrate

Water soluble carbohydrate was identified and quantified by gas chromatography of their trimethylsilyl derivatives according to Holligan & Drew (1971). Pinitol was quantified in this way and identified according to Keiller, Paul & Cockburn (1987) and Paul (1988). In the absence of pure pinitol as an internal standard amounts of this substance were expressed as *myo*-inositol equivalents.

Starch was estimated in the boiling water extracted residue of leaf discs by amyloglucosidase digestion followed by enzymic analysis of the released glucose (Macrae, 1971; Jones, Outlaw & Lowry, 1977).

Estimation of malate and inorganic phosphate

Aliquots of aqueous leaf disc extract were analysed enzymically for malate by the method of Mollering (1974) and for inorganic phosphate by the ammonium vanadate-molybdate method of Kitson & Mellon (1944).

RESULTS

Effects of phosphate

Malate. Amounts of malate in non-induced and induced *M. crystallinum* were negatively correlated with exogenous P_i (Fig. 1). The amount of consumption of malate during a $5\frac{1}{2}$ h photoperiod was unaffected by exogenous P_i in induced plants (Table 1). In non-induced *M. crystallinum* consumption of malate was negatively correlated with exogenous P_i . At zero P_i , levels of malate were higher in non-induced than in induced plants until day 21 of the induction period (Fig. 1a), and the consumption of

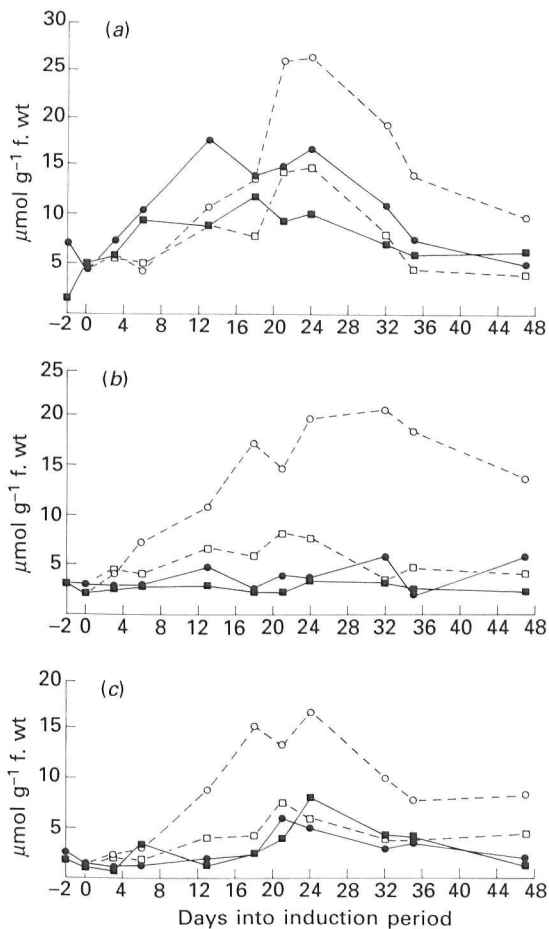


Figure 1. Levels of malate ($\mu\text{mol g}^{-1}$ f. wt) during the induction of CAM in *Mesembryanthemum crystallinum* plants grown at 0 P_i (a), 0.2 mM P_i (b) and 3 mM P_i (c) at the start of the photoperiod [non-induced (\bullet), induced (\circ)] and $5\frac{1}{2}$ h into photoperiod [non-induced (\blacksquare), induced (\square)]. Maximum standard errors of mean, at 0 P_i were non-induced 5.1, induced 3.1; at 0.2 mM P_i non-induced 1.8, induced 4.6; at 3 mM P_i non-induced 1.7, induced 3.5.

malate was greater in non-induced plants until day 18 (Table 1). CAM activity in plants grown at 0.2 and 3 mM P_i was greater in induced than in non-

Table 1. Consumption (–) or accumulation (+) of malate ($\mu\text{mol g}^{-1}$ f. wt) during the first $5\frac{1}{2}$ h of the photoperiod in *Mesembryanthemum crystallinum* grown at 3 concentrations of P_i during CAM induction

Days into induction period	0		0.2 mM		3 mM	
	Induced	Non-induced	Induced	Non-induced	Induced	Non-induced
–2	–0.76	–0.76	0	0	–0.10	–0.10
0	+0.08	+0.08	–0.14	–0.14	–0.05	–0.05
3	–0.07	–0.22	+0.06	–0.03	–0.03	–0.06
6	+0.11	–0.14	–0.41	+0.02	–0.18	+0.30
13	–0.24	–1.17	–0.56	–0.23	–0.64	–0.10
18	–0.77	–0.33	–1.54	–0.02	–1.47	+0.03
21	–1.51	–0.74	–0.86	–0.21	–0.78	–0.27
24	–1.50	–0.87	–1.61	–0.03	–1.44	+0.42
32	–1.48	–0.50	–2.28	–0.35	–0.82	+0.19
35	–1.28	–0.19	–1.84	+0.10	–0.52	+0.08
47	–0.52	–0.17	–1.31	–0.45	–0.52	–0.09

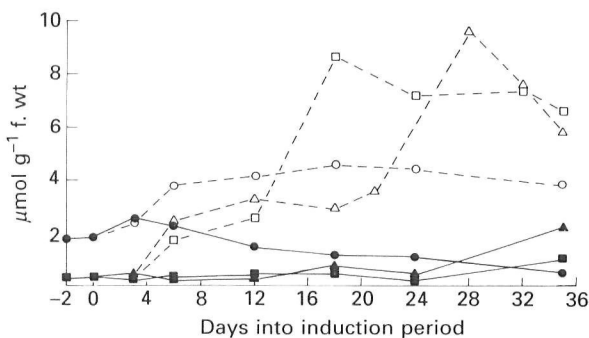


Figure 2. Levels of pinitol (inositol equivalents, $\mu\text{mol g}^{-1}$ f. wt) during the induction of CAM in *Mesembryanthemum crystallinum* plants grown at 0 P_i [non-induced (\bullet), induced (\circ)], 0.2 mM P_i [non-induced (\blacksquare), induced (\square)] and 3 mM P_i [non-induced (\blacktriangle), induced (\triangle)]. Maximum standard errors of mean at 0 P_i were non-induced 0.8, induced 1.3; at 0.2 mM P_i non-induced 0.6, induced 1.5; at 3 mM P_i non-induced 0.1, induced 1.4.

induced *M. crystallinum* by day 6 of the induction period [Fig. 1(b, c)]. Levels of malate and malate consumption until day 24 were similar in non-induced plants at 0 P_i and induced plants at 3 mM P_i .

Pinitol. Amounts of pinitol were higher in induced than in non-induced plants after day 6 of the induction period (Fig. 2). In non-induced plants the amounts of pinitol were highest at 0 P_i until day 24. Conversely, in induced *M. crystallinum*, after day 12 levels of pinitol were half as much at 0 P_i as at 0.2 and 3 mM P_i .

Carbohydrate and P_i . Amounts of starch were higher at zero P_i in both non-induced and induced forms (Table 2). Differences in levels of starch between phosphate treatments were most marked in non-induced plants due to higher levels of starch than in the induced form at zero P_i . There was no difference in levels of starch between the non-induced and induced forms at 0.2 and 3 mM P_i .

Amounts of total soluble carbohydrate before

CAM induction were much greater at zero P_i (Table 2). By day 18 there were no differences in amounts of total soluble carbohydrate between the non-induced and induced forms and between levels of exogenous P_i .

Levels of endogenous P_i rose with increasing exogenous P_i (Table 2). At 3 mM P_i levels of endogenous P_i were appreciably greater in induced plants.

Nitrate experiment

Levels of acid and P_i . The withdrawal of nitrate from the nutrient medium for 17 days ($-N$) substantially increased levels of acid at the start of the photoperiod and the amount of deacidification during the day (Fig. 3). Levels of acid and deacidification during the photoperiod were greater in old leaves. Endogenous P_i levels in young leaves were higher in nitrate-free plants ($-N$) (Table 3) and hence positively correlated with the amount of deacidification and negatively correlated with nitrate in the nutrient medium.

Twenty days after the addition of NaCl to the nutrient media and the replenishment of nitrate to the nutrient medium of plants also receiving NaCl ($-N+N+NaCl$), nitrate-free ($-N$) plants no longer exhibited greater levels of acid at the start of the photoperiod and deacidification during the photoperiod (Table 4). The application of NaCl resulted in higher levels of malate at the start of the photoperiod. Plants irrigated with NaCl and nitrate exhibited the greatest amount of deacidification. Levels of endogenous P_i were significantly higher in nitrate deficient plants ($-N$, $-N+NaCl$) (Table 5). Thus endogenous P_i levels were again negatively correlated with nitrate, but were now negatively correlated with the amount of deacidification.

Carbohydrate content. The removal of nitrate from the rooting medium for 17 days resulted in higher amounts of pinitol and starch (Table 3). The

Table 2. Amounts of total soluble carbohydrate (TSC) ($\mu\text{mol g}^{-1}$ f. wt) before (0) and 18 days CAM induction and amounts of inorganic phosphate (P_i) ($\mu\text{mol g}^{-1}$ f. wt) 22 days after CAM induction in *Mesembryanthemum crystallinum* grown at 0, 0.2 and 3 mM phosphate

	0		0.2 mM		3 mM	
	Non-induced	Induced	Non-induced	Induced	Non-induced	Induced
TSC						
0	5.3 ± 1.2	—	0.2 ± 0.1	—	0.2 ± 0.02	—
18	1.3 ± 0.7	1.5 ± 0.5	1.0 ± 0.4	1.4 ± 0.3	0.7 ± 0.1	0.8 ± 0.2
P_i						
22	1.4 ± 0.4	1.8 ± 0.5	6.1 ± 0.5	7.5 ± 0.1	9.9 ± 0.7	20.1 ± 4.2
Starch						
0	13.8 ± 0.8	13.8 ± 0.8	2.7 ± 0.7	2.7 ± 0.7	1.8 ± 0.1	1.8 ± 0.1
18	9.6 ± 0.7	7.1 ± 1.4	4.5 ± 0.2	4.2 ± 0.5	3.2 ± 0.5	3.2 ± 0.8

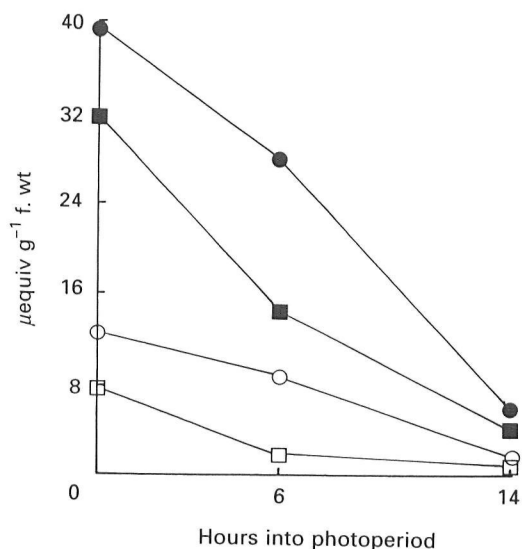


Figure 3. Amounts of acid ($\mu\text{equiv g}^{-1}$ f. wt) during the photoperiod in *Mesembryanthemum crystallinum* grown in complete nutrient medium [old (○), young (□)] and in plants with nitrate withdrawn for 17 days [old (●), young (■)]. Least significant difference at 5% is $9.3 \mu\text{equiv g}^{-1}$ f. wt at the start of the photoperiod, $5.3 \mu\text{equiv g}^{-1}$ f. wt 6 h into the photoperiod and $1.5 \mu\text{equiv g}^{-1}$ f. wt 14 h into the photoperiod.

Table 3. Amounts of pinitol, total soluble carbohydrate (TSC), starch and P_i ($\mu\text{mol g}^{-1}$ f. wt) prior to the removal of nitrates from the rooting medium (day 0) and 17 days afterwards (day 17-N) in *Mesembryanthemum crystallinum*

	Pinitol	TSC	Starch	P_i
Day 0	1.8	6.3	5.2	7.7
+N Day 17	3.9	4.8	4.9	9.1
-N Day 17	7.1	5.4	8.3	29.6
L.S.D. (5%)	2.1	1.2	0.7	4.3

Table 4. Amounts of acid ($\mu\text{equiv g}^{-1}$ f. wt) during the photoperiod in older *Mesembryanthemum crystallinum* grown with nitrate (+N), nitrate and NaCl (+N+NaCl), nitrate free (-N), nitrate free and NaCl (-N+NaCl) and previously nitrate-free plants supplemented with nitrate and NaCl (-N+N+NaCl)

	0 h into photoperiod	14 h into photoperiod	Change in acid during photoperiod
Nitrate present			
+N	29.2	2.9	26.3
+N+NaCl	45.4	7.1	38.3
-N+N+NaCl	39.3	2.2	37.1
Nitrate absent			
-N	26.4	6.2	20.2
-N+NaCl	41.7	12.7	29.0
L.S.D. (5%)	9.3	2.8	

Table 5. Amounts of pinitol, total soluble carbohydrate (TSC), starch and P_i ($\mu\text{mol g}^{-1}$ f. wt) in older *Mesembryanthemum crystallinum* grown with nitrate (+N), nitrate and NaCl (+N+NaCl), nitrate free (-N), nitrate free and NaCl (-N+NaCl) and previously nitrate free plants supplemented with nitrate and NaCl (-N+N+NaCl)

	Pinitol	TSC	Starch	P_i
Nitrate present				
+N	20.4	11.6	1.9	16.0
+N+NaCl	20.7	4.9	3.4	15.5
-N+N+NaCl	19.4	41.5	2.9	14.9
Nitrate absent				
-N	34.4	103.6	4.1	21.0
-N+NaCl	21.2	65.1	0.7	19.2
L.S.D. (5%)	4.4	20.6	1.6	3.1

extended period of nitrate deficiency resulted in higher levels of pinitol, total soluble carbohydrate and starch (Table 5). Nitrate rather than NaCl was the overriding influence on these components.

Appearance of plants

The withdrawal of nitrate from the nutrient medium for 17 days resulted in smaller plants, with thicker more succulent leaves, duller green than the nitrate-sufficient plants, and they resembled plants induced to exhibit CAM by salt treatment.

Deinduction of CAM

The consumption of malate during the first $5\frac{1}{2}$ h of the photoperiod declined rapidly during deinduction from day 0 to day 7 (Fig. 4). Subsequently, on days 27 and 34 malate accumulated during the first $5\frac{1}{2}$ h of the photoperiod. In *M. crystallinum* with CAM consumption of malate remained high until day 27 when there was a sharp reduction in consumption.

Deinduction of CAM caused a sharp decline in levels of P_i during the first 5 days of the deinduction period (Fig. 5), but during the remainder of this period levels of P_i remained stable. In plants with CAM the levels of P_i were relatively higher than those undergoing deinduction of CAM.

DISCUSSION

Phosphate and nitrate deficiencies, like leaf age and plant development, stimulate CAM in *M. crystallinum* without an apparent water deficit. It is unlikely that such parameters predispose the plants to water stress since nitrate and phosphate deficiencies reduce stomatal conductances (Radin & Ackerson, 1981; Wong, Cowan & Farquhar, 1985). The possibility of CAM being induced as a result of stomatal closure,

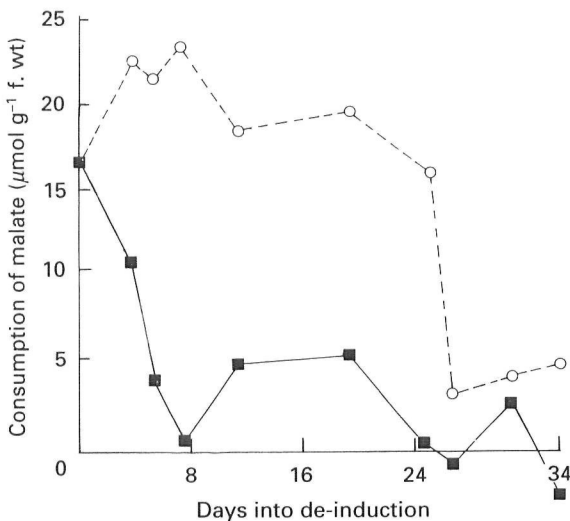


Figure 4. The consumption or accumulation of malate in *Mesembryanthemum crystallinum* ($\mu\text{mol g}^{-1}$ f. wt) during the first $5\frac{1}{2}$ h of the photoperiod during CAM deinduction [deinducing (■), CAM (○)].

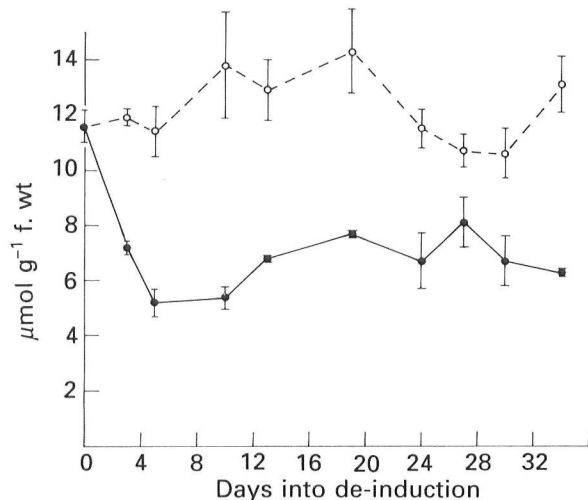


Figure 5. Levels of inorganic phosphate ($\mu\text{mol g}^{-1}$ f. wt) [deinducing (●), CAM (○)] during the deinduction of CAM in *Mesembryanthemum crystallinum*. Vertical bars = standard error of mean.

which would be facilitated with these deficiencies, has been dismissed (Winter, 1979).

The induction of CAM both by salinity (Keiller *et al.*, 1987), and withdrawal of nitrate (Table 3), resulted in marked rises in levels of endogenous P_i . Correspondingly, the deinduction of CAM produced a rapid fall in levels of P_i (Fig. 5). This initial correlation of P_i with CAM activity supports the view of von Willert (1975b) that P_i may have a role in the induction process. However, the stimulation of CAM in response to phosphate deficiency did not correspond to a high endogenous level of P_i (Table 2). Furthermore, the CAM activity of plants after the application of NaCl in the nitrate experiment was negatively correlated with endogenous P_i content (Tables 4, 5). Thus, the correlation of high en-

dogenous P_i with CAM activity breaks down, and high endogenous P_i cannot be an absolute requirement for, or necessary result of, CAM activity.

Phosphate uptake in response to salinity (Grattan & Maas, 1985; Treeby & Steveninck, 1988) and nitrate deficiency (Barneix & Arnois, 1980; Breeze & Hopper, 1987) is well-established. In *M. crystallinum* P_i uptake was more related to nitrate deficiency than salt treatment (Tables 3 and 5) where it may be replacing the osmotic and cation charge-balancing roles of nitrate. Deficiencies of anions like phosphate and nitrate could stimulate malate synthesis to replace these roles. This could explain the higher background levels of malate in response to phosphate deficiency before and after the application of NaCl (Fig. 1a), but cannot account for the diel fluctuations in acid contents in response to phosphate and nitrate deficiencies.

If accumulation of phosphate is caused by nitrate deficiency, is it possible that the accumulation of phosphate in response to salt treatment is also due to a nitrate deficiency? If this is so, phosphate and nitrate accumulation must respond differently to low water potential. Evidence does suggest that phosphate and nitrate uptake are subject to very different metabolic controls (Pan, 1987) which could allow phosphate uptake to continue in conditions of drought which would prevent nitrate accumulation. Salinity, (Aslam, Haffaker & Rains, 1984) and drought (Shaner & Boyer, 1976; Hanson & Hitz, 1982; Kirby & Armstrong, 1980) inhibit nitrate uptake. Nitrate deficiency is also known to result from a phosphate deficiency (Schjorring, 1986). Hence nitrate may be deficient not only when it is withdrawn from the growing medium, but also in conditions of low water potential and phosphate deficiency.

Could the reduction in nitrate accumulation which may occur with these treatments and the induction of CAM be related? The increase in CAM activity with leaf age (von Willert *et al.*, 1976a, b) correlates with a reduction in leaf nitrogen content during leaf development (Thomas & Stoddart, 1980) and a reduction in nitrate reductase activity (Chang, Vines & Black, 1981). It is possible that the stimulation of CAM with age is enhanced by salinity (von Willert & Kramer, 1972) and nitrogen deficiency which both promote ageing and senescence, and that CAM is mediated via changes in nitrogen status within the leaf. The continuation of CAM activity however, requires a greater amount of nitrate than that available after a period of prolonged nitrate deficiency (Table 4).

The influence of ion compartmentation on CAM induction has been considered by von Willert *et al.* (1977). Interactions between ions are complex and incompletely understood, but nitrate could influence this process. Low nitrate concentrations are known to stimulate the vacuolar uptake of P_i from the

cytosol of barley root cells (Lamaze, Sentenac & Grignon, 1987) leading to lower cytosolic P_i levels. Such an interaction could influence carbohydrate partitioning and CAM activity. Nitrate could also exert an effect on the tonoplast ATPase which is known to be inhibited by nitrate (Smith *et al.*, 1984). Nitrate deficiency could relieve this inhibition allowing the movement of malate and H^+ ions into the vacuole which could precipitate CAM activity. This is highly speculative and depends on whether changes in cellular nitrate levels, presumably at the expense of the vacuole, would influence the tonoplast ATPase which is located on the cytosolic side of the membrane (Rea & Sanders, 1987). The greater stimulation of CAM in the presence of nitrate compared with ammonium as the sole nitrogen source in *Kalanchoe blossfeldiana* (Ota, 1988a) leads to doubt that stimulation of CAM results from a specific absence of nitrate, though it does suggest some specific effect of nitrate and ammonium ions rather than an influence of nitrogen status alone.

A further possibility is that a retardation of growth is responsible for the higher levels of carbohydrate in response to phosphate and later on, to nitrate deficiencies (Tables 2, 5), and for the higher levels of P_i in response to nitrate deficiency (Tables 3, 5). This could give rise to low solute and water potentials normally implicated in the induction process. Pinitol, present in response to nitrate and phosphate deficiencies in the absence of salt treatment (Fig. 2, Table 3), and implicated as a compatible solute (Paul & Cockburn, 1989), could participate in osmotic balancing in response to the accumulation of solutes. This latter explanation may be less contentious than CAM induction as a specific response to nitrate or nitrogen status. The induction of CAM as a result of decreasing water potential could still arise as a consequence of ions accumulating at specific locations and specific effects of phosphate and nitrate on this process cannot be ruled out. The confirmation of possible roles for specific ions in CAM induction will require detailed information of ion location and effects of ion location on metabolism.

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