Attività di Ricerca svolta dal Dr. Maciej Kocurek durante il suo soggiorno presso l'IBAF

Isoprenoids emission rates from plants in III phase of CAM metabolism.

INTRODUCTION

Crassulacean acid metabolism (CAM) is one of the most intriguing plant adaptations to environmental stress, is expressed by an estimated 6% of vascular plant species (Smith and Winter, 1996; Crayn et al., 2004; Silvera et al., 2005). CAM is appeared to varying degrees in species from over 300 genera from 34 families that are mostly found in tropical and subtropical habitats subject to periodic water limitation (Holtum et al. 2007; Griffiths et al. 2008). The simplest defnition of CAM, first described for species of the family *Crassulaceae*, is that there is nocturnal uptake of CO_2 via open stomata, fixation byphosphoenolpyruvate carboxylase (PEPC) and vacuolarstorage of CO_2 in the form of organic acids (malate and citrate), and (I) daytime remobilization of vacuolar organic acids, decarboxylation and refixation plus assimilation of CO_2 behind closedstomata in the Calvin-cycle (phase III). Between these twophases there are transitions when stomata remain open forCO_2 uptake for a short time during the very early lightperiod (phase II) and reopen again during the late lightperiod for CO_2 uptake with direct assimilation to carbohydratewhen vacuolar organic acid is exhausted (phase IV)[Osmond 1978, Lütge 2002].This model may change related with the intensity of the stress factor which is hydration.Strong water stress may cause permanent closing of the stomata, and a high intensity of re-fixation CO_2 derived from the Krebs cycle may occurred. Inversely, in well watered CAM plants is possible that stomata remain closed during the dark period but somenocturnal synthesis of organic acid fed by respiratory CO_2occurs.Then stomata are open during the light period with uptake of atmospheric CO_2 and direct Calvin-cycleCO_2 reduction (C_3-photosynthesis) in addition to assimilation of CO_2 remobilized from nocturnally stored organic acid (Griffiths 1988, Lütge 2004). Expression of the CAM cycle may be also modulated by developmental and other environmental factors: abiotic (Lütge 1993, Broetto et al. 2002, Dodd et al. 2003) and biotic (Kuźniak et al. 2010, Libik-Konieczny et al. 2011).

The common ice plant (*Mesembryanthemumcrystallinum*), originating from the Namib Desert, is widely distributed in seasonally arid habitats throughout the world. This facultative halophyte is characterized by a developmentally programmed switch from C_3 photosynthesis to CAM. The induction of CAM in *M. crystallinum*, its enzymatic set, is considerably accelerated by salinity and drought (Adams et al., 1998). In contrast to many facultative CAM plants in which induction is reversible (Griffiths 1988, Borland and Griffiths1996), the induction of CAM in adult leaves of *M. crystallinum* is constitutive (Vernon et al. 1988).

In recent years, *M. crystallinum* has been used as a model for studying many physiological changes in both modes of photosynthetic carbon assimilation pathway as well as for investigation of the C_3/CAM transition in plants exposed to different factors including salinity (Cushman et al. 1990, Lüttge 1993), abscisic acid (Chu et al. 1990), excess light (Broetto et al. 2002) and hydrogen peroxide (Ślesak et al. 2003).

However, until now there has been no study about the volatile organic compounds (VOCs) that are emitted by *M. crystallinum* in its various life cycles, C_3, C_3/CAM transition and CAM. Many plants emit a part of the photosynthetically assimilated carbon into the atmosphere in the form of VOCs. VOCs constitute an important factor of atmospheric chemistry because they are involved in ozone and aerosols formation (Andreae and Crutzen 1997). Under normal conditions, isoprenoids (isoprene and monoterpenes) are the most abundant VOCs though methanol, acetaldehyde and C-6 compounds (hexanal, hexenal, hexanol and hexenol) are also emitted in great quantities (Fall 2003). Under stress conditions the emission of these compounds generally increases. The study of how emissions change depending of stress conditions has become a useful "in vivo" indicator of plant vitality and of the plant response to abiotic stresses. Therefore we haveanalysed the VOCs emitted from C_3 or CAM-induced M. *crystallinum* in order to evaluatethe possible role that VOCs may have role of adaptation of this plant to salinity.

MATERIAL AND METHODS

Plants of *Mesembryanthemum crystallinum* L. were grown from seeds (collection of the Botanical Garden, Darmstadt, Germany) in soil culture under irrigation with tap water in and under a natural photoperiod in a green house. Irradiance was 250–500 μmol quanta m^{-2} s^{-1}. Relative air humidity ranged between 20 and 40%. After the appearance of the third leaf pair, 3 weeks after sowing, one set of plants was treated with 0.4 kmol m^{-3}NaCl (salt-treated), while another set of plants was irrigated further with tap water (controls). Twelve-day treatment of *M. crystallinum* with saline solution induced CAM.The difference between malate concentration at the beginning and at the end of the day (Δ malate) is routinely assumed a hallmark of CAM. Malate concentration in the leaf cell sap was determined using a reflectometer (RQflex 10, Merck) according to the manufacturer's instruction manual.

After 14 d of water- (control) and salt-treatment (CAM) the plants were used in the experiments. A portable infrared gas analyzer (LI-6400; Li-Cor, Lincoln, NE, USA) was used to determine CO_2 and H_20 exchange: photosynthesis (A), stomatal conductance (g_s), transpiration and intercellular CO_2 concentration all along the day in *M. crystallinum* plants in C_3 and CAM states. Measurements were carried under natural light conditions. The leaf temperature during measurements was 25°C -45 and the relative humidity was between 15 and 30%.To collect VOCs, the outlet of the leaf cuvette was connected to a tube filled with 200 mg Tenax. A pump was used to draw

through the tube 5 L of the air flowing over the leaf inside the cuvette, at a rate of 200 ml min^{-1}. Then, trapped compounds were thermally desorbed at 275°C for 10 min and cryofocused in a cold trap for 3 min. at -10°C. The cold trap was then flash heated at 300°C and the compounds were injected with a flow of helium into a GC-MS (Agilent 5975C). Isoprenoid emission was measured by comparing sample peak with peaks from a gaseous standard (100 ppb). Collection of VOCs from C_3 and CAM *M. crystallinum* plants was performed from 8 a.m. to 6 p.m. covering the II and III fazes of CAM in salt treated plants and this same time in the case of C_3 ones.

RESULTS

Malate concentration

Night/day fluctuations of malate concentration in the cell sap of C_3 and CAM *M. crystallinum* plants are shown in Figure 1. In C_3 plants malate concentration was homogeneous along the day. In the case of plants subjected to stress by salnity treatement, typical CAM rhythm of diurnal malate fluctuation was detected. Daily changes covering high concentrations of malate at dawn and decreasing during the day were undoubtedly the result of induction of CAM metabolism. Similar fluctuations were observed in the following days during the collection of VOCs.

Gas exchange

The diurnal variation of A and g_s for *M. crystallinum* performing C_3 and CAM modes of photosynthesis are shown in Figure 2. Daily course A and g_s for *M. crystalinum* working as a C_3 plant showed the typical course for this phenomenon: closed stomata at night, their gradual opening during the day for drive photosynthesis. Then, there was a midday depression and the maximum opening of stomata in the afternoon which allowed achieve an A value of almost 5 μmol m^2 s^{-1}.

In the case of plants treated with salt, the daily fluctuations strongly correspond to the CAM cycle. Clearly visible were all four phases of CAM. The first phase lasted from 8 p.m. to 8 pm. The next phase (II) ended at noon. Phase III passed in IV at about 5.30 pm. The determination of the occurrence of a particular stage of Cam enabled the collection of VOCs divided into phase II and III.

VOCs analysis

The collection of VOCs emitted by *M.crystallinum* was performed during the early morning (8 – 12 am), corresponding with the second phase and after midday during third phase of CAM metabolism in CAM plants, when stomata were cloused and CO_2 fixation took place through Rubisco. The list of the volatiles emitted by *M. crystallinum* is listed in Table 1. The compounds emitted by plants in III fase of CAM and in related time by C_3 was very similar. Emissions of VOCs was generally higher in plants representing C_3 but in the case of butanone, methoxypropylacetate, octanal, ethylhexanol, tetradecane, geranylacetone and benzylalcohol revealed a higher eflux of these components in III phase of CAM. However, in the case of C_3 plants from 12 am to 4.30 pm. stomatal conductance were about 10 times higher compared to the CAM. This

means that the possibility of VOCs emission was significantly lower in the case of CAM plants. It seems that at least some VOCs are involved in the adaptation of *M. crystallinum* to salinity but their emission are limited by stomata closure. Further analysis of the chromatograms is necessary in order to have a complete vision of the volatiles emitted by *M. crystallinum* in both metabolisms II fase of CAM and related C_3 period.

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Table 1: Volatile organic compounds (VOCs) emission rates from *M. crystallinum* plants performing C_3 and CAM metabolism. Means ± SE are presented (n=5).

	M. crystallinum			
Compound [nmol m^{-2} s^{-1}]	C_3		III fase CAM	
	mean	SE	mean	SE
methyl-2-buten-1-ol	0.0162	±0.0030	0.0094	±0.0019
Dimethylsilanediol	0.0124	±0.0025	0.0101	±0.0036
2-butenal-3-methyl	0.0386	±0.0062	0.0000	±0.0000
hexanal	0.0270	±0.0023	0.0094	±0.0032
Butanone	0.1288	±0.0144	0.1663	±0.0461
Methoxypropylacetate	0.0104	±0.0017	0.0263	±0.0051
Acetylcyclohexene	0.0292	±0.0036	0.0123	±0.0047
2-heptanone	0.0648	±0.0117	0.0544	±0.0107
2-1hydroxy-1-methylethylcyclopropylethanone	0.0802	±0.0089	0.0276	±0.0054
Benzaldehyde	0.0106	±0.0011	0.0039	±0.0004
trans-2-methyl-4-hexen-3-ol	0.0212	±0.0026	0.0093	±0.0014
phenol	0.0602	±0.0109	0.0230	±0.0034
5-hepten-2-one, 6-methyl	0.0711	±0.0088	0.0544	±0.0096
propanoic acid-3-ethoxy-ethylester	0.0485	±0.0065	0.0397	±0.0064
octanal	0.0327	±0.0035	0.0361	±0.0057
ethylhexanol	0.0114	±0.0022	0.0143	±0.0037
benzylalcohol	0.0286	±0.0034	0.0619	±0.0096
citronellol	0.0079	±0.0010	0.0056	±0.0014
nonanal	0.1831	±0.0270	0.1316	±0.0356
3 dodecane	0.0083	±0.0010	0.0027	±0.0007
tetradecane	0.0180	±0.0033	0.0194	±0.0039
geranylacetone	0.0846	±0.0171	0.0926	±0.0199
nonadecanal	0.0140	±0.0014	0.0041	±0.0014
octylether	0.0123	±0.0016	0.0072	±0.0015

Figure 1: Night/day fluctuations of malate concentration in the cell sap of C_3 and CAM *M. crystallinum* plants during light period.Means ± SD are presented (n=3).

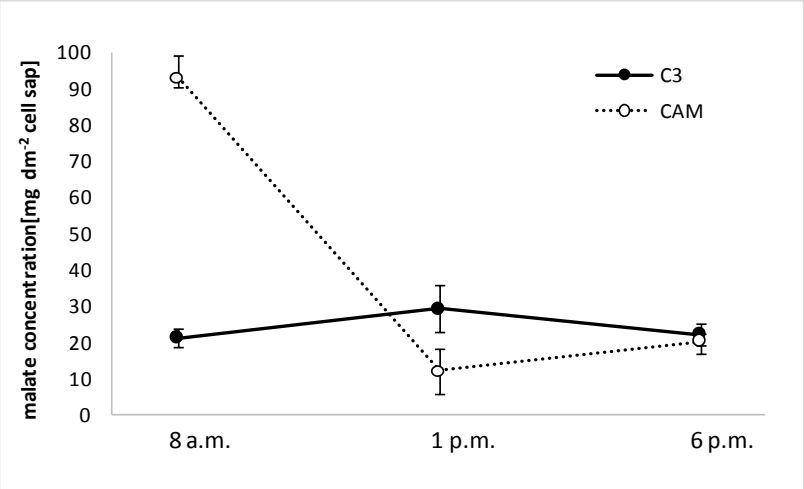

Figure 2: Typical daily variation of photosynthesis and stomatal conductance of *M. crystallinum* plants in C_3 (dotted line) and CAM (solid line) metabolism.