

<sup>1</sup>Leibniz-Institute for Agricultural Engineering Potsdam-Bornim, Dept. Horticultural Engineering

<sup>2</sup>Humboldt University Berlin, Section Quality Dynamics / Postharvest Physiology

<sup>3</sup>Institute of Vegetable and Ornamental Crops Großbeeren / Erfurt, Dept. Quality

## Effects of saline irrigation on growth, physiology and quality of *Mesembryanthemum crystallinum* L., a rare vegetable crop.

W.B. Herppich<sup>1</sup>, S. Huyskens-Keil<sup>2</sup>, M. Schreiner<sup>3</sup>

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### Summary

World wide increased desertification due to recent global changes enhances the need of irrigation, which, in turn, provokes the risk of soil salinization. Furthermore, limited fresh water resources may increasingly constrain the use of low quality irrigation water. Hence, intensified use of halotolerant crop plants will be necessary, even in Europe.

Commercial use of halophytes as fresh food is limited. Several facultative halophytic members of Aizoaceae are nowadays used as special crop plants. A rare leafy vegetable species is the common ice plant *Mesembryanthemum crystallinum*, a Crassulacean acid metabolism (CAM) species, which is mostly cultivated in India, California, Australia, and New Zealand. It is also known in Europe as a quickly cooked tender vegetable. With their succulent, mellow, slightly salty tasting leaves and young shoots, *M. crystallinum* is getting interesting as delicious cool flavored salad greens during recent years. However, it is a perishable product and thus, shelf life is short. On the other hand, CAM capacity of *M. crystallinum* can be largely enhanced by saline irrigation. Increased CAM potentially reduces water and carbon losses.

In this project we studied whether moderate salt treatment affects physiology, growth and yield of this rare crop plant. Furthermore, we investigated whether such treatment that enhances the irreversible C<sub>3</sub> to CAM shift in young leaves of this CAM species, potentially prolongs shelf life. Results showed that moderate salt treatment did not negatively influence growth, yield and sensory quality. When in CAM, leaves showed reduced transpiration water losses and CAM also reduced carbon losses during storage.

### Introduction

Recent models of global changes prognosticate a world wide dramatically increase in desertification (SCHNEIDER et al., 2007). This is not only true for the arid and semi arid regions of the world. Desertification may also spread throughout low land regions of Europe that are now still well supplied with precipitation. Even if the velocity of changes might be lower than predicted an enhanced need of irrigation seems to be unavoidable in many cases. This may further limit fresh water resources and increasingly constrains the use of low quality irrigation water (KUNDZEWICZ et al., 2007). Combined with a higher evaporation demand this, in turn, may largely provoke the risk of soil salinization. Hence, intensified use of halotolerant crop plants will be necessary, even in Europe.

However, many important food crop species are more or less sensitive to salinity stress (SHANNON and GRIEVE, 1999). Hence, there will be an increased demand for new crop species or improved varieties. Conventional but also modern molecular based (e.g. marker assisted selection) plant breeding programs are normally slow and it is not clear whether they can reach the goals at all. The same applies to genetic engineering approaches because of the very complex nature of halotolerance (YAMAGUCHI and BLUMWALD, 2005). One relative rapid approach might be the development of naturally halotolerant plants into valuable crop species (GLENN et al., 1999) or the increased

cultivation of halotolerant crop species. In this context, commercial use of halophytes as fresh food is nowadays very limited. Few exceptions are e. g. *Aster tripolium*, *Batis maritima*, *Crambe maritima*, *Portulaca oleracea*, or *Salicornia bigelovii* (NATIONAL RESEARCH COUNCIL, 1990; SHANNON and GRIEVE, 1999; EL-HADDAD and NOAMAN, 2001).

Several facultative halophytic members of Aizoaceae are nowadays used as special crop plants since many years. Since approximately two hundred years *Tetragonia tetragonioides* (Pall.) O. Kunze has been grown as a spinach-like vegetable in New Zealand, Australia, Japan, India, California and Europe (VOGEL, 1996). Young leaves and tender shoots of this species can also be eaten as a fresh and tasty salad. However, in Europe New Zealand Spinach lost most of its economic relevance to spinach during the last decades. As a convenience product deep frozen spinach (*Spinaca oleracea*) is much easier to handle than New Zealand spinach. Although it contains some oxalate (AHMED and JOHNSON, 2000) *Tetragonia* has a low nitrate and a high vitamin C content. In contrast to New Zealand Spinach, *Aptenia cordifolia* (L.f.) N.E.Br., commonly called Baby Sun Rose, is getting increasingly interesting as a delicious salad during recent years, even in Germany (HERPPICH et al., 2004). Leaves of *A. cordifolia* contain small amounts of some soothing and bloodstream stimulating alkaloids such as mesembrin, mesembrinin and tortuosamin (SMITH et al., 1996).

An old, but nowadays rare salad green is the common ice plant *Mesembryanthemum crystallinum* (VOGEL, 1996). This species is an ephemeral or pseudo-biennial prostrate white flowering herbaceous weedy plant with simple, ovate, succulent leaves and stems which are densely covered with bladder cells. These cells give the plant a glistening appearance, and hence the species name „ice-plant“ (ADAMS et al., 1998). It has been shown that plants of this species grow best under moderately saline conditions (WINTER, 1973).

According to VOGEL (1996), *M. crystallinum* is mostly cultivated in India, California, Australia, and New Zealand. With their succulent, mellow, slightly salty tasting leaves and young shoots, this species is a delicious cool flavored salad green. In Europe it is also known as a quickly cooked tender vegetable. Sold in the delicatessen shops *M. crystallinum* can reach reasonable price in Germany (e.g. 25 € per kg in a well-known Berlin department store). However, this product is highly perishable and should be stored in a cool and humid atmosphere or sold packed in plastic bags. Nevertheless, their shelf life is very short and may not extend 2 to 3 days (VOGEL, 1996).

*M. crystallinum* is a CAM plant (WINTER and VON WILLERT, 1972), i.e. it possesses the ability to fix internal or external CO<sub>2</sub> at night into organic acids, which are stored in the vacuoles. During the daytime organic acids are set free into the cytoplasm and decarboxylated to give free CO<sub>2</sub> which is then finally fixed in the normal C<sub>3</sub> photosynthesis. This resulted in the well known diurnal pattern of CO<sub>2</sub> uptake which is mostly governed by the related changes in internal CO<sub>2</sub> concentration (C<sub>i</sub>). C<sub>i</sub> also controls stomatal opening. As a consequence, stomata are at least partially closed during daytime, largely reducing transpiration and, hence, water losses in CAM plants. It has been shown that even mild salt treatment largely enhances the CAM capacity of the ice plant (WINTER, 1973; HERPPICH

et al., 1992; 1995; 1996; 1997).

From the above it may be concluded that irrigation of *M. crystallinum* plants with saline water may stimulate growth and enhance CAM capacity, and may, thus, increase the keeping quality of the harvested product. Hence, in the presented project we studied whether moderate salt treatment affects physiology, growth and yield. Furthermore, we investigated whether such treatment that enhances the irreversible C<sub>3</sub> to CAM shift in young leaves of this CAM species (CUSHMAN et al., 1990; HERPPICH et al., 1992), potentially prolongs shelf life.

## Materials and methods

### Plant material

Plants of *Mesembryanthemum crystallinum* were grown from seeds on soil (Fruhstorfer Erde Typ-P, Industrie-Erdenwerk-Archut GmbH Co. KG, Lauterbach, Germany) in a glasshouse under semi-controlled conditions ( $T_{D/N} = 18/14^{\circ}\text{C}$ ;  $rF_{D/N} = 40/55\%$ ) and natural light (mean photosynthetic photon fluence rate (PFR) approximately  $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; max PFR  $< 1300 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Nine days after germination seedlings were pricked out into plastic pots ( $\varnothing 9 \text{ cm}$ ). At day 24 after germination plants were finally transplanted in large plastic pots („BC 29“;  $\varnothing 28 \text{ cm}$ ,  $V = 10 \text{ l}$ ). Plants were watered daily with tap water and regularly fertilized by top dressing ( $0.88 \text{ g Ca(NO}_3)_2$  and  $0.25 \text{ g MgSO}_4$  per plant). Ninety days after germination each plant was approximately  $69 \text{ cm}$  wide and  $16 \text{ cm}$  high. It developed 12 shoots with approximately 50 shoot tips.

### Experimental design and methods

On days 64 and 85 after germination 12 plants each were randomly selected for the presented experiments. Six plants (+ NaCl) were watered with NaCl-solution ( $150 \text{ mmol l}^{-1}$ ) every 2<sup>nd</sup>. The remaining six plants (- NaCl) were further irrigated with tap water. These 12 pots were placed in 4 rows, alternating salt-treated and untreated plant. On day 69 after germination the measurements were performed with the first set of plants. On days 90, 104 and 133 after germination plants of the second batch were used for the investigations. This means that in this second set leaves and shoot tips were repeatedly harvested as recommended for the practical use (VOGEL, 1996).

### Free acidity

For the analysis of the diurnal changes in the free acid content the largest fully developed leaves of each shoot of each plant (3 plants per treatment) were harvested early in the morning (approx. 7 am) and in the afternoon (approx. 3 pm). The leaves of each harvesting time were combined to give two mixed-samples per plant (approximately  $15 \text{ g}$ ), weighed (fresh mass, FM), roughly chopped, mixed with  $50 \text{ ml}$  distilled water and finally homogenised for 2 min ( $20000 \text{ rpm}$ , Ultra-Turrax, IKA® Werke GmbH & Co. KG, Staufen, Germany). In a graduated flask the volume of the homogenate was replenished to  $250 \text{ ml}$  with distilled water, the mixture was shaken and filtered. Finally,  $50 \text{ ml}$  of the filtrate was titrated with NaOH solution ( $1 \text{ mol l}^{-1}$ ) to a pH of 8.1. The free acid concentration ( $\text{mmol l}^{-1}$ ) of the samples ( $n = 6$  per treatment) were calculated from the added volume of the NaOH solution.

### Leaf sodium content

Approximately  $6 \text{ g}$  of leaf material per plant was harvested in the morning and in the afternoon, weighed, and dried at  $60^{\circ}\text{C}$  in the oven for 48 h and re-weighed (dry mass, DM). Afterwards the dry material was ground, dried at  $85^{\circ}$  for additional 4 h and then ashed

at  $500^{\circ}\text{C}$  in a muffle kiln for 4 h. Afterwards, the ash was dissolved in  $5 \text{ ml}$  water, mixed with  $5 \text{ ml}$   $6 \text{ mol l}^{-1}$  HCl-solution and stirred for 30 min. The solution was brought to  $100 \text{ ml}$  with distilled water and filtered. The sodium concentration of the solution was determined with an atomic absorption spectrometer (Vario 6, Analytik Jena, Jena, Germany).

### Transpiration measurements

Approximately  $100 \text{ g}$  leaves and shoot tips per plant and treatment were harvested in the morning (7 am), weighed, and placed loosely into pre-weighed open containers. These containers were stored at  $8^{\circ}\text{C}$  and 70% relative air humidity (corresponding to a water vapour pressure deficit,  $\Delta w$ , of  $0.32 \text{ kPa MPa}^{-1}$ ) in a climate chamber (Type VB1014, Vötsch Industrietechnik GmbH, Balingen-Frommern, Germany) for 3 days.

Every day, the containers with leaves were weighed at 08:45. From the mass losses over time and the respective initial fresh mass mean transpiration rates ( $J_{H_2O}$ ) were calculated as  $\text{mmol h}^{-1} \text{ g}_{\text{FM}}^{-1}$ . Average leaf conductance ( $g_{H_2O}$ ;  $\text{mmol h}^{-1} \text{ g}_{\text{FM}}^{-1}$ ) for water vapour was calculated as the ratio of  $J_{H_2O}$  and  $\Delta w$  (VON WILLERT et al., 1995).

### CO<sub>2</sub> and O<sub>2</sub> gas exchange

For the determination of leaf gas exchange approx.  $40 \text{ g}$  of freshly harvested pre-weighed plant material per plant and treatment was sealed in an air-tight plastic container ( $18.5 \text{ cm} \times 15.5 \text{ cm} \times 9 \text{ cm}$ ). These containers were stored at  $8^{\circ}\text{C}$  in a climate chamber (Type VB1014, Vötsch Industrietechnik GmbH, Balingen-Frommern, Germany) for 3 days. Each container was fitted with a standard septum through which gas samples ( $5 \text{ ml}$ ) were taken with a syringe every day at the same time (8 am).

The CO<sub>2</sub> and O<sub>2</sub> concentration of these samples were measured with a Checkmate 900 head space gas analyser (PBI Dansensor, Ringsted, Denmark). From the variation in the CO<sub>2</sub> and O<sub>2</sub> concentration of the samples and the respective fresh mass the actual respiration rates  $J_{\text{CO}_2}$  and  $J_{\text{O}_2}$  were calculated ( $\text{mmol h}^{-1} \text{ kg}^{-1}$ ). Furthermore, the respiratory quotient (RQ) was determined as the ratio of  $J_{\text{CO}_2}$  and  $J_{\text{O}_2}$ .

### Chlorophyll fluorescence analysis

On days 88 to 94 d and 104 to 118 after germination photosynthetic activity of salt-treated and untreated plants was investigated by means of chlorophyll fluorescence analysis. Using a MiniPAM Fluorometer (Heinz Walz GmbH, Effeltrich; Germany) the steady state fluorescence signals  $F_1$  and  $F_0$ , respectively, and the maximum fluorescence signals  $F_m'$  and  $F_m$  were automatically (rec-modus) recorded under natural light conditions, i.e. without additional actinic irradiation, in 30-min intervals over several 24 h-periods. The 2030-B leaf-clip holder kept attached to the respective leaf for the whole measurement time without any visible damage to the leaf tissue.

From the recorded fluorescence signal measured on illuminated leaves the actual photochemical efficiency of photosynthesis ( $\Phi_{\text{PSII}}$ ) was calculated as  $\Phi_{\text{PSII}} = \Delta F/F_m' = (F_m' - F_1)/F_m'$ . The photosynthetic electron transport rate through photosystem II (ETR) was determined as  $\text{ETR} = \Phi_{\text{PSII}} * \text{PPFD} * a * f$  (e.g. HERPPICH et al., 1998). The absorptivity  $a$  of the succulent leaves in the visible range was taken as 0.68 (ELLER et al., 1983) and the light distribution factor between photosystem I and II,  $f$ , was set to 0.5 (KRALL and EDWARDS, 1992). In addition, non-photochemical quenching (NPQ), an indicator of total non-radiative dissipation of absorbed light energy, was determined as  $\text{NPQ} = (F_m - F_m')/F_m'$  (BILGER and BJÖRKMAN, 1990). The fluorescence signals recorded on dark adapted leaves ( $F_m$  and  $F_0$ )

during the night were used to calculate the maximum photochemical efficiency of photosystem II,  $F_v/F_m (= (F_m - F_0)/F_m)$ , which is an indicator of the potential maximum performance of photosynthesis. This parameter is also a valuable measure of the intactness of the photosynthetic apparatus (VON WILLERT et al., 1995).

### Statistical analysis

All data were statistically analysed (ANOVA) with WinSTAT (R. Fitch Software, Staufeu, Germany). Significant differences were determined by the Duncan's multiple range test ( $P < 0.05$ ). The mean variability was indicated by the standard deviation.

## Results

### Biomass, NaCl content and CAM capacity

Saline irrigation of *M. crystallinum* affected growth only in plants older than 105 days (Fig. 1A). Up to this date salt-treated and well-irrigated plants showed exact the same increase in fresh mass. At the last harvest date however, fresh mass of salt-treated plants was significantly higher than that of well-watered. As expected, repeated moderate salt treatment resulted in significantly enhanced leaf  $\text{Na}^+$  content (Fig. 1B). The initial mean  $\text{Na}^+$  content of leaves of well-watered plants ( $0.44 \pm 0.03 \text{ mmol g}_{\text{DM}}^{-1}$ ) increased by only about 50% at the end of the experiment. Intermediate watering with  $150 \text{ mmol l}^{-1}$  NaCl-solution led to approximately 60% higher leaf  $\text{Na}^+$  contents. In contrast, salt treatment did not significantly affect mean leaf water content (Fig. 1C) or leaf dry matter content (Fig. 1D). Nevertheless, salinity-treated plants tended to have slightly higher water content at a lower dry mass content at the end of the experiment. Irrespective of the treatment all plants showed a clear nocturnal accumulation of free acidity ( $\Delta\text{H}^+$ ) indicating that mature plants utilize CAM under all conditions (Fig. 2). Intermediate saline irrigation only very slightly affected the capacity of the plants to perform CAM. However, development of plants led to a significant increase in the maximum morning content of free acidity (Fig. 2B).

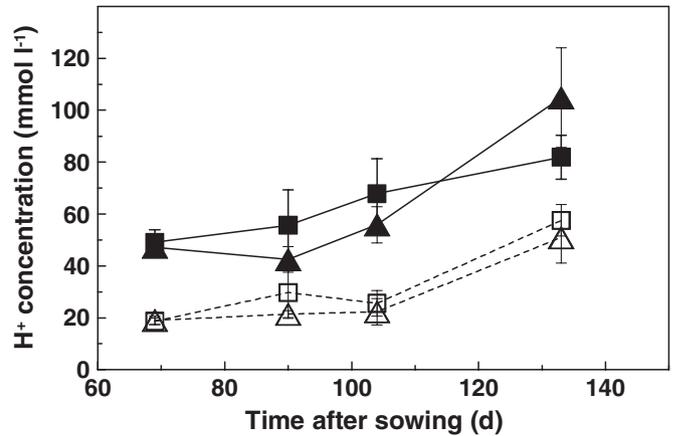


Fig. 2: Free acid concentration of salt-treated (squares) and well-irrigated (triangles) plants of *M. crystallinum* (mean  $\pm$  SD;  $n = 6$ ) harvested in the morning (closed symbols) and in the afternoon (open symbols) during the entire growth period.

Nevertheless, mean diurnal acid difference, i.e. CAM capacity, as well as mean maximum morning content declined again with further aging in salt-treated plants of *M. crystallinum*.

### Leaf conductance and respiration

Salt treatment significantly reduced the average leaf conductance for water vapour (Fig. 3A) compared to well-irrigated plants. Leaf conductance generally declined with plant development. However, this effect was more pronounced in well-watered plants finally leading to lower leaf conductance in these plants at the end of the harvest season. After 3 days of storage leaf conductance was low in all plants irrespective of the treatment. In this case the effect of plant development was no longer significant.

The results of mean leaf conductance clearly reflected the fact that

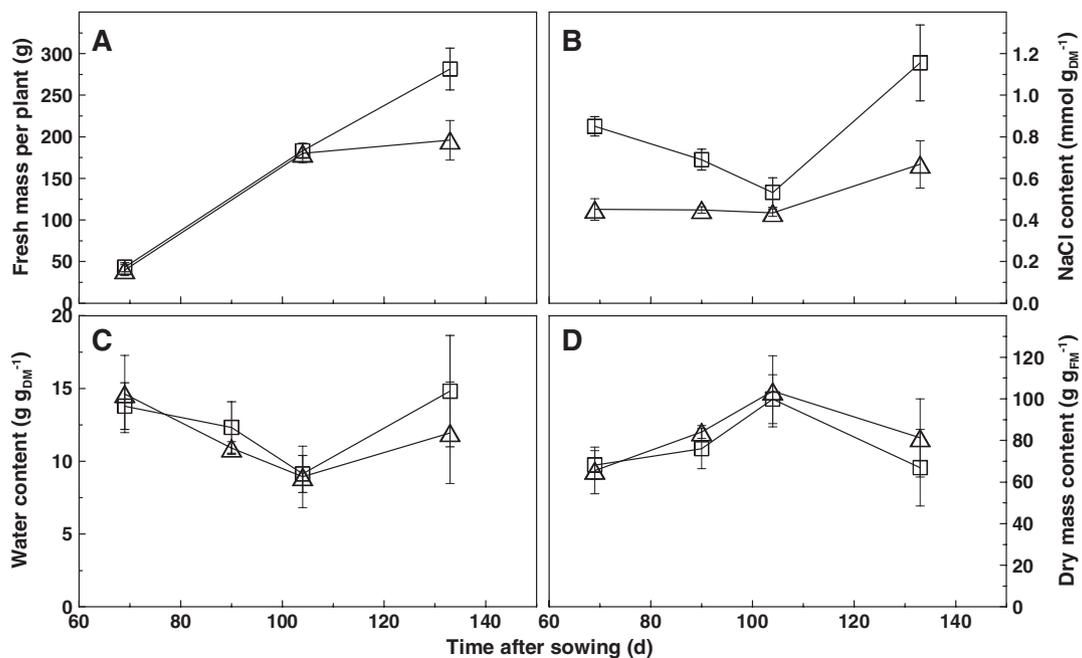
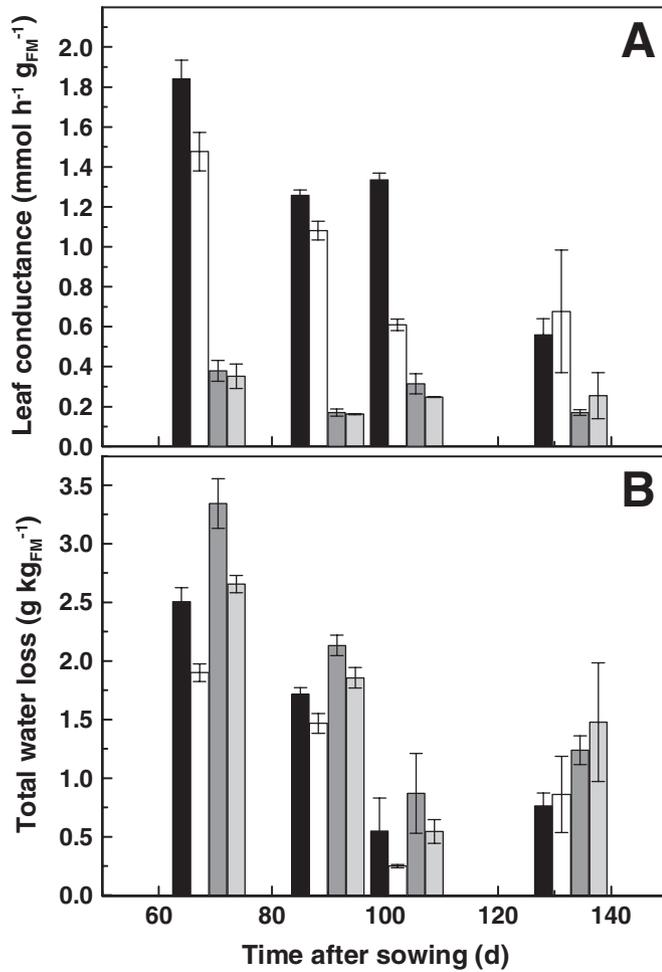


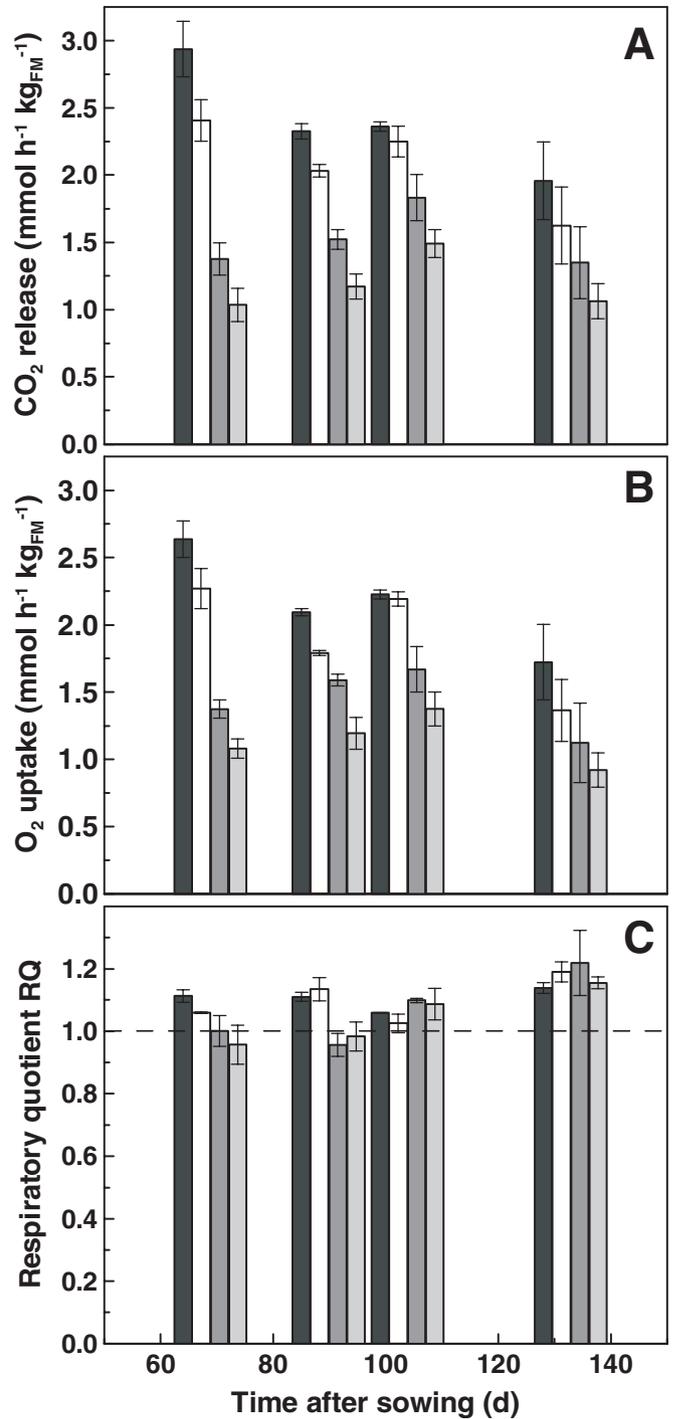
Fig. 1: Average (mean  $\pm$  SD;  $n = 6$ ) fresh mass (A), NaCl content (B), water and dry mass content (C) of salt-treated (squares) and well-irrigated (triangles) plants of *M. crystallinum* during the entire growth period.



**Fig. 3:** (A) Average (mean  $\pm$  SD;  $n = 3$ ) leaf conductance for water vapour transfer and (B) total water loss of freshly harvested (open and closed bars) and 3 d-stored (dark grey and light grey bars) well-irrigated (closed and dark grey bars) and salt-treated (open and light grey bars) plants of *M. crystallinum* (i.e. closed bar = freshly harvested + well-irrigated; open bar = freshly harvested + salt-treated; light grey bar = 3 d-stored + salt-treated; dark grey bar = 3 d-stored + well-irrigated).

moderately salt treated plants lost significantly less water (approximately 20%) than well-watered plants, both during the initial and the late storage, except again at the last harvest date (Fig. 3B). Nevertheless, water losses generally declined by approximately 70% with plant development.

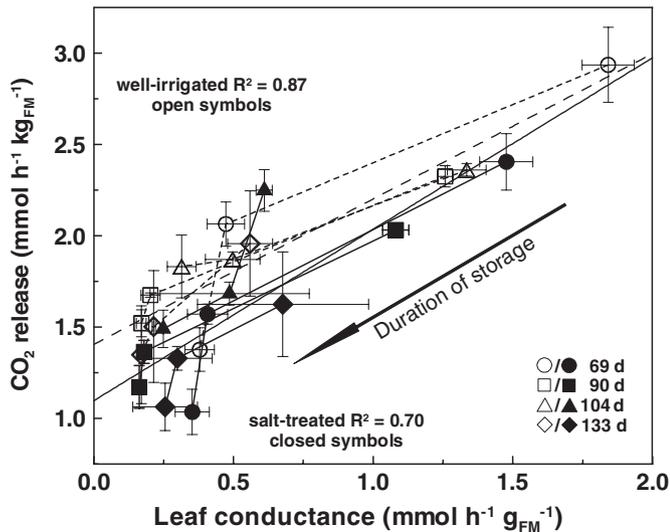
Both CO<sub>2</sub> release and O<sub>2</sub> uptake of harvested leaves of *M. crystallinum* were always approximately 10 to 15% higher in well-watered than in salt-treated plants (Fig. 4 A, B). Gas exchange in general significantly declined during the 3 days of storage. Nevertheless, this decline did not affect the effect of the treatment. The respiratory quotient (RQ), a helpful indicator of the average respiratory source, of freshly harvested leaves was always significantly higher than 1 ( $1.10 \pm 0.05$ ; Fig. 4C). During storage the RQ declined significantly at the early harvest or in younger plants, respectively, when the quotient was slightly lower than 1 ( $0.97 \pm 0.02$ ). This effect was largely reduced and the RQ of stored leaves increased during further plant development. No clear effect of the treatment could be detected. A comparison of CO<sub>2</sub> release and leaf conductance to water vapour revealed that the observed seemingly decline in leaf respiration during storage was nearly exclusively due to a reduced leaf conductance for gas transfer. Both CO<sub>2</sub> release and leaf conductance were highly



**Fig. 4:** Average rates (mean  $\pm$  SD;  $n = 3$ ) of CO<sub>2</sub> release (A) and O<sub>2</sub> uptake of freshly harvested (open and closed bars) and 3 d-stored (dark grey and light grey bars) well-irrigated (closed and dark grey bars) and salt-treated (open and light grey bars) plants of *M. crystallinum* (i.e. closed bar = freshly harvested + well-irrigated; open bar = freshly harvested + salt-treated; light grey bar = 3 d-stored + salt-treated; dark grey bar = 3 d-stored + well-irrigated). (C) Means of the respiratory quotient as calculated from the rates of CO<sub>2</sub> release and O<sub>2</sub> uptake.

linearly related (Fig. 5) in well-watered ( $R^2 = 0.87$ ) and salt-treated ( $R^2 = 0.70$ ) plants. This evaluation further illustrates that the reduction of CO<sub>2</sub> release with plant development also mainly resulted from a decline in leaf conductance with plant maturation. Again, the initial

CO<sub>2</sub> release and leaf conductance for water vapour of samples harvested at different times after sowing were significantly linearly related ( $R^2 = 0.87$ ). Nevertheless, at a given leaf conductance well-irrigated plants always exhibit a significantly higher CO<sub>2</sub> release and hence a higher respiration activity. Furthermore, after 3 days of storage rates of CO<sub>2</sub> release and O<sub>2</sub> uptake (data not shown) tended to decline without concomitant corresponding reduction in leaf conductance indicating a direct effect of storage on respiration.



**Fig. 5:** Respiratory CO<sub>2</sub> release rates as a function of the respective leaf conductance for water vapour transfer of salt-treated (closed symbols) and well-irrigated (open symbols) plants of *M. crystallinum* as measured 69 (circles), 90 (squares), 104 (triangles) and 133 days after sowing.

### Photosynthesis

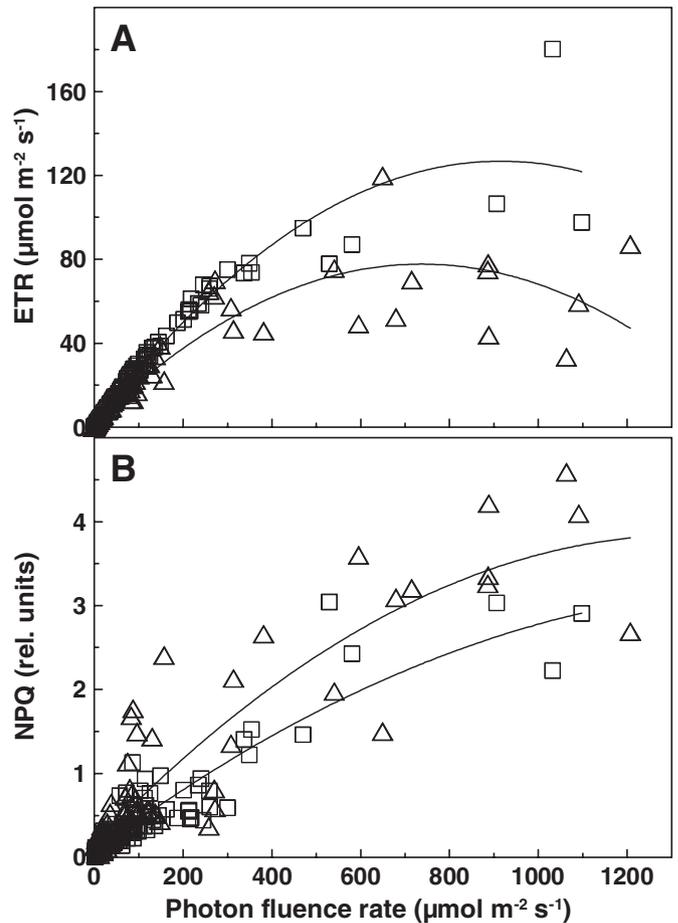
Determined 88 to 94 d after germination, salt-treated plants of *M. crystallinum* had significantly higher maximal mean  $F_v/F_m$  ( $0.809 \pm 0.011$  versus  $0.786 \pm 0.002$ ) (data not shown). Also, saturated photosynthetic electron transport rates (ETR, measured at  $T = 26 \pm 8$  °C) of salt-treated plants were higher ( $107 \pm 27 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) than in well-watered ( $68 \pm 24 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). This was valid irrespective of the photosynthetic fluence rates during measurement (Fig. 6A). The lower gross photosynthesis in the well-watered plants mainly resulted from a higher activity of the non-photochemical fluorescence quenching mechanisms (Fig. 6B). These results fit very well with the higher CAM capacity of salt-treated plants at this age.

In contrast, investigated 104 to 118 d after germination, photosynthetic electron transport at a constant, and, according to the results given in Fig. 6A, nearly saturating PFR of  $368 \pm 57 \mu\text{mol m}^{-2} \text{s}^{-1}$  was increased in well-watered plants (Fig. 7) if compared with salt-treated plants at the same temperature range ( $115 \pm 29 \mu\text{mol m}^{-2} \text{s}^{-1}$  versus  $84 \pm 14 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). In the latter, ETR started to decline above a threshold of 25°C while in well-watered plants gross photosynthesis nearly linearly increased with temperature. Again these results reflect the analysis of CAM capacity. During the later harvest period the salt-treated plants showed a lower nocturnal acid accumulation than well-watered plants and hence a smaller internal photosynthetic CO<sub>2</sub> source.

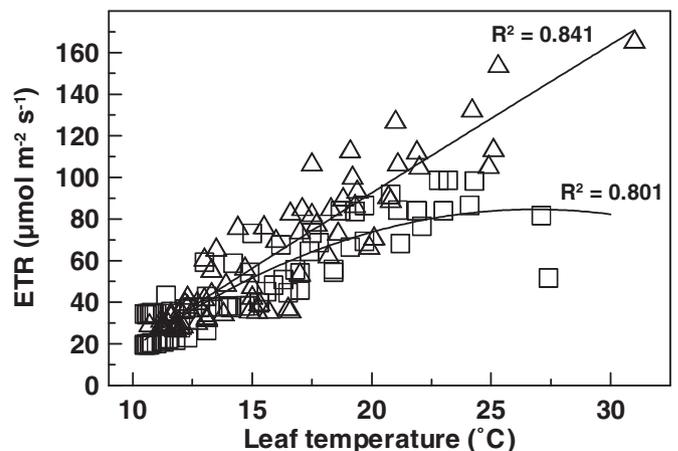
### Discussion

#### Effects of intermittent low-salt treatment on CAM capacity

One major goal of this study was to show that even a practically meaningful intermittent low-salt treatment could enhance the CAM



**Fig. 6:** Dependence of (A) photosynthetic electron transport rates, ETR, and (B) non-photochemical quenching, NPQ, on incident photosynthetic active photon fluence rates of approximately 90 days old salt-treated (squares) and well-irrigated (triangles) plants of *M. crystallinum*.



**Fig. 7:** Photosynthetic electron transport rates, ETR, as a function of leaf temperature of approximately 116 days salt-treated (squares) and well-irrigated (triangles) plants of *M. crystallinum*.

capacity in leaves of *M. crystallinum* without negatively affecting productivity. The presented results clearly indicated that repetitive irrigation with saline water did not negatively affect plant growth in *M. crystallinum*. In fact, in this facultative halophytic species the salt treatment applied even acts as a NaCl-nutrition. Our results are

in accordance with those published by WINTER (1973). This author found that only NaCl-concentrations above 300 mmol l<sup>-1</sup> lead to a reduction of growth in hydroponically cultured plants of *M. crystallinum*, while maximum growth was obtained with a 100 mmol l<sup>-1</sup> NaCl-solution. Furthermore, neither water content nor dry matter content seemed to be affected by the irrigation regime used in our experiments. Hence, moderately saline or low quality irrigation water certainly does not exert any stress on but even increase the physiological activity and yield of this rare crop.

This conclusion is further substantiated by the study of the photosynthesis using chlorophyll fluorescence analysis. Photosynthetic performance, a sensitive indicator of general metabolic activity, was significantly higher in salt-treated plants when investigated about 90 d after sowing. This applied both for the maximum efficiency of photosystem II, i.e. the potential capacity under optimal conditions (VON WILLERT et al., 1995; MAXWELL and JOHNSON, 2000), and for the actual photosynthetic electron transport rates, i.e. that part of the maximum efficiency actually used by plants (HERPPICH, 2001). This higher photosynthetic activity of salt-treated plants might be explained by a general physiological stimulation in response to salinity. It may also reflect increased chloroplastic carbon dioxide availability during times of higher irradiance due to the higher CAM capacity and thus increased nocturnal acid accumulation of those plants. The presented results seemingly contrast those reported earlier (e.g. KEILLER et al., 1994; BAKER et al., 2004). Those authors found a clear reduction of actual photosynthetic activity if plants of *M. crystallinum* were salt-treated. However, in these investigations NaCl concentrations of 400 mmol l<sup>-1</sup> were used which represent true stress conditions even for a halophytic plant (WINTER, 1973). On the other hand, maximum photochemical efficiency was not affected by the salt treatment, as reported by BAKER et al. (2004). Nevertheless, BAKER et al. (2004) also reported that older leaves were more affected by the salt treatment than younger and denoted it to a probable acceleration of senescence. This interpretation would also nicely explain the finding in the presented study that in older plants salt-treated leaves showed a lower photosynthetic activity than un-treated.

The applied intermittent salt treatment resulted in the expected increased nocturnal accumulation of free acid ( $\Delta\text{-H}^+$ ) and, hence, enhanced CAM capacity. However, the obtained differences between the  $\Delta\text{-H}^+$  of well-watered and salt-treated plants were not very pronounced. Instead, well-watered plants also showed clear and distinct night time acid accumulation. As all these plants were always abundantly watered to yield high productivity this result unequivocally indicates that no environmental stress is necessary to induce the C<sub>3</sub> to CAM shift in fully developed plants of *M. crystallinum*. Hence, our study confirmed earlier investigations (VON WILLERT et al., 1977; CUSHMAN et al., 1990; CHU et al., 1991; HERPPICH et al., 1992) postulating plant development being the crucial parameter controlling the ability of plants to perform CAM. In contrast, WINTER and HOLTUM (2007) recently concluded from „continuously monitoring net CO<sub>2</sub> exchange of whole shoots from the seedling stage until seed set“ that „the induction of the CAM pathway for carbon acquisition in *M. crystallinum* is under environmental control“. This conclusion was based on whole-plant gas exchange measurements only. However, it is well established that the CAM capacity in plants of *M. crystallinum* strictly depends on leaf age. Hence, the large young still developing non-CAM as well as senescent, out-of-CAM leaves might have „diluted“ the weak nocturnal CO<sub>2</sub> uptake of mature leaves. Furthermore, nocturnal accumulation of organic acid is the decisive feature making up CAM at all while night-time CO<sub>2</sub> uptake is an important but secondary pattern. It is well known that CAM gas exchange can be easily shifted to what is called a typical C<sub>3</sub> pattern without clearly affecting night time acid accumulation in flexible CAM plants (HERPPICH, 1997; DE MATTOS and LÜTTGE, 2001).

The relatively small partial increase in CAM capacity due to the salt treatment may also contrast the popular hypothesis that it is the indirect drought stress resulting from a NaCl mediated lower water availability which intensifies CAM in *M. crystallinum* (PIEPENBROCK and SCHMITT, 1991; WINTER and GADEMANN, 1991). The mean water content of salt-treated plants tended to be higher than in well-irrigated plants in nearly all cases. Furthermore, early morning leaf water content was slightly higher than that of the afternoon samples in most plants irrespective of the treatment (data not shown). This may reflect some transpiration water loss or the enhance ability to take up water as a result of the CAM induced increase in osmotic content (HERPPICH, 2004). Hence, the presented results support the thesis that CAM induction in well-irrigated and CAM enhancement in salt-treated plants was not due to any physiological drought stress (HERPPICH and HERPPICH, 1997b).

Interestingly, salt treatment enhances CAM only in plants younger than 133 d. In fact, nocturnal acid accumulation, and, hence, CAM capacity was significantly reduced in salt-treated *M. crystallinum* plants at the end of the harvest season. An acceleration of plant development by the salt-treatment untimely leading to senescence would be a plausible explanation. Nevertheless, HERPPICH et al. (1992) found that in young, still developing plants salt stress (300 mmol<sub>NaCl</sub> l<sup>-1</sup>) reversibly retarded development in this species. This was evident when salt stress was relieved from those plants at different stages of life cycle. Hence, low-salt treatment of mature plants as used in our study might show different effects than in young still developing plants.

#### Effects of intermittent low-salt treatment on keeping quality

The second aim of the presented investigation was to proof that a higher CAM capacity can improve the keeping quality of harvested leaves of the salad green *M. crystallinum* under practical low temperature (8°C) conditions thereby simulating sale conditions in cooled, mist-equipped display cases or short-term storage in cold rooms. The results of our postharvest gas exchange measurements conclusively show that this was indeed the fact. Plants with a higher  $\Delta\text{-H}^+$  had always lower values of leaf conductance. Hence, these plants suffered lower water losses, both at the beginning and at the end of a three-day storage period. As this was also valid when well-irrigated plants showed the higher CAM capacity at the last harvest date, the reduced stomatal conductance was not an effect of salt treatment as might be concluded from earlier studies (WINTER and GADEMANN, 1991; DAI et al., 1994). In contrast, it was directly linked to CAM capacity. However, as was the magnitude of  $\Delta\text{-H}^+$ , leaf conductance and water losses were strongly dependent on plant age largely declining with progressing development.

During storage, the differences in leaf conductance between treatments declined. At the third day after harvest stomata seemed to be more or less completely closed. Hence, the remaining mean value of leaf conductance of  $0.22 \pm 0.06$  mmol kg<sup>-1</sup> h<sup>-1</sup> nearly represented the water vapour conductance of the cuticle. Assuming a degree of succulence (VON WILLERT et al., 1995) of 4 g<sub>H<sub>2</sub>O</sub> dm<sup>-2</sup> (HERPPICH et al., 1992) this value equals a leaf conductance of approximately 0.05 mmol m<sup>-2</sup> s<sup>-1</sup>. Although this value is very low (approx. 10 times lower) if it is compared with mesophytic C<sub>3</sub> plants (VON WILLERT et al., 1995), it lies well in the range of cuticular conductance reported for other succulents (HERPPICH, 1997).

The observed reduction of leaf conductance during postharvest storage and during plant development largely affected CO<sub>2</sub> and O<sub>2</sub> gas exchange. In fact, the close linear relationship between these parameters clearly indicated that most of the variation in CO<sub>2</sub> loss and O<sub>2</sub> uptake was not due to a direct reduction of mitochondrial activity by water loss but simply resulted from the increase in diffusion resistance. This effect was obviously independent of the

treatment. Nevertheless, the evaluation of the data indicated that well-watered plants showed higher CO<sub>2</sub> release rates at a given level of leaf conductance. Thus, this may be due to their lower CAM performance resulting in a lower capacity to retain respiratory CO<sub>2</sub> by a re-fixation into malic and/or citric acid (HERPPICH, 2004). Since O<sub>2</sub> uptake is also higher it is more probably that well-watered plants exhibit higher respiratory activity. However, this seems to contrast the finding that the oxidative capacity of isolated mitochondria showed the tendency to increase with the shift from C<sub>3</sub> to CAM in *M. crystallinum* (PECKMANN et al., 2004). On the other hand, well-watered plants had already developed a significant CAM capacity very similar to that of salt-treated plants in our study. Furthermore, although respiration activity of seedlings of this species was reported to increase at higher nutrient solution salinity (VON WILLERT, 1974), low salt concentrations (50 mmol<sub>NaCl</sub> l<sup>-1</sup>) resulted in an intermitted reduction of respiration. Also, KOYRO (2006) found that dark respiration declined with increasing NaCl-salinity in the halophytic cash crop *Plantago coronopus*. Hence, it is not clear whether the reduction of dark respiration with the salt-treatment as observed in our study is due to CAM induction or is a NaCl-effect. Nevertheless, our data show that intermittent salt-treatment can indeed significantly reduce the postharvest carbon loss and thus, increase keeping quality of this highly perishable produce.

Furthermore, we can speculate that a generally increased CAM with its higher ability to refix mitochondria derived CO<sub>2</sub> in the detached harvested leaves could be the reason for the reduced carbon loss at the end of the storage period. The unavoidable water losses after detaching lead to drought stress, which, in turn, has been shown to amplify CAM capacity (PIEPENBROCK and SCHMITT, 1991), sometimes interpreted as drought-induction of CAM (PIEPENBROCK et al., 1994). The given assumption may be further substantiated by the fact that the reduction of carbon loss is not fully reflected by the same variation in O<sub>2</sub> uptake rates leading to a significantly reduced RQ with means less than 1, at least in younger plants. Although we can not exclude that the change in the RQ were due to a reduced availability of organic acids in storage, this hypothesis seems to be not highly probable in *M. crystallinum* (PIEPENBROCK and SCHMITT, 1991; PIEPENBROCK et al., 1994; ADAMS et al., 1998). Nevertheless, a RQ above 1 observed under most conditions may indicate that malic and citric acid are preferred substrates of dark respiration.

An important quality aspect that might be influenced by the cultivation method is taste, especially affected by the salt content. As a facultative halophytic species plants of *M. crystallinum* might accumulate large amounts of NaCl in their leaves and shoot if they are salt-treated (HERPPICH et al., 1992; ADAMS et al., 1998). High salt content is highly undesired and might render the plants useless for human consumption. However, a mean Na<sup>+</sup>-content of 0.81 mmol g<sub>DM</sub><sup>-1</sup> in salt-treated and 0.50 mmol g<sub>DM</sub><sup>-1</sup> in well-watered plants was very low and a daily uptake up to 150 mmol NaCl is quite safe within the human diet (ELMADFA and LEITZMANN, 1998). These values lie in the range reported for soil grown plants of *M. crystallinum* watered with tap water (HERPPICH et al., 1992). The sodium content found in leaves of salt-treated plants in this investigation is, thus, approximately 10 times less than found earlier if plants were continuously irrigated with a 300 mmol l<sup>-1</sup> NaCl-solution (HERPPICH et al., 1995). Furthermore, the effect of salt-treatment on sensorial quality of *M. crystallinum* plants used in these experiments was also evaluated by a small, untrained consumer panel. The panelists indicated no negative impact such as an unacceptable salty or stringent taste (data not shown).

## Conclusion

In general, the knowledge of preharvest treatments affecting post-harvest physiology and quality dynamics is an essential tool for a

comprehensive product physiological oriented quality management. The results of this study provide clear evidence that intermittent low-salt treatment was effective to prevent quality loss and to optimize quality assurance of *M. crystallinum* leaves due to the enhancement of CAM capacity.

Furthermore, the presented results clearly show that fully-developed well-watered, soil-grown plants of *M. crystallinum* do not need any stress to induce CAM.

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Address of the authors:

W.B. Herppich (corresponding author), Leibniz-Institute for Agricultural Engineering Potsdam-Bornim, Dept. Horticultural Engineering, Max-Eyth-Allee 100, D-14469 Potsdam, Germany, wherppich@atb-potsdam.de  
 S. Huyskens-Keil, Humboldt-Universität zu Berlin, Section Quality Dynamics/Postharvest Physiology, Lentzeallee 75, D-14195 Berlin, Germany  
 M. Schreiner, Institute of Vegetable and Ornamental Crops Großbeeren/Erfurt, Dept. Quality, Theodor-Echtermeyer-Weg 1, D-14979 Großbeeren, Germany