ACTA BIOLOGICA CRACOVIENSIA Series Botanica 47/1: 199–204, 2005

SUPEROXIDE DISMUTASE ACTIVITY IN ORGANS OF *MESEMBRYANTHEMUM CRYSTALLINUM* L. AT DIFFERENT STAGES OF CAM DEVELOPMENT

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Received January 7, 2005; revision accepted March 3, 2005

Fluctuations of acidity were found in leaves, shoots and roots of two groups of *Mesembryanthemum crystallinum* plants: watered and salt-treated. This suggests that the functioning of CAM (related to malate accumulation) in the tested organs can differ in "early CAM" in watered plants versus "full CAM" in NaCl-irrigated plants. Among the different organs analyzed, shoots exhibited the highest acidity level at the beginning of the day in both groups of *M. crystallinum* plants. Superoxide dismutase forms (MnSOD, CuZnSOD and FeSOD) differed in activity depending not only on their localization in the plant tissue but also on the stage of CAM development. Plants exhibiting "full CAM" and showing clear diurnal Δ-acidity had much higher total SOD activity during evening hours than in plants following "early CAM" photosynthesis. Shoots showed the highest activity of SOD.

Key words: *Mesembryanthemum crystallinum*, ice plant, oxidative stress, salinity, SOD.

INTRODUCTION

Plants exhibiting CAM usually fix CO_2 during the night into malic or citric acid; after releasing CO_2 during the following day, they refix it to carbohydrates. This strategy allows CO_2 uptake from the atmosphere to occur when evaporation rates are low, and permits daytime photosynthetic carbon fixation to occur behind closed stomata. It results in minimal water loss and reduced photorespiration when a high amount of CO_2 is available (Lüttge, 1993; Cushman and Bohnert, 1997; Miszalski et al., 1998). CAM is very often an ecophysiological adaptation of plants to unfavorable environmental conditions such as drought stress (Lüttge, 1993; Adams et al., 1998). *Mesembryanthemum crystallinum* L. (common ice plant) is a well known facultative halophyte. In its native habitat, the Namibian Desert of Southern Africa, this plant germinates in the short rainy season and changes its mode of photosynthesis from C_3 to CAM in the dry season. Further development of *M. crystallinum* is strictly influenced by progressive drought stress coupled with increasing salinity, and it can complete its life cycle at 500 mM NaCl (Winter, 1973; Winter et al., 1978; Lüttge, 2002).

In recent years, *M. crystallinum* has been used as a model for investigation of the C_3/CAM transition in plants exposed to different factors including salinity (Cushman et al., 1990, Lüttge, 1993; Adams et al., 1998), abscisic acid (Chu et al., 1990), excess light (Broetto et al., 2002) and hydrogen peroxide (Ślesak et al., 2003). Under laboratory conditions the most common factor accelerating the C_3/CAM shift is salinity

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Abbreviations: ABA – abscisic acid; AOS – active oxygen species; BSA – bovine serum albumin; CAM – Crassulacean acid metabolism; DDT – dithiothreitol; EDTA – ethylenediaminetetraacetic acid; EGTA – ethylene glycol-bis(α-aminoethyl ether)-N,N,N',N'-tetraacetic acid tetrasodium salt; NBT – nitro blue tetrazolium; PAGE – polyacrylamide gel electrophoresis; PAR – photosynthetically active radiation; PEPC – phopsphoenolpyruvate carboxylase; RH – relative humidity; SDS – sodium dodecyl sulphate; SOD – superoxide dismutase (EC 1.15.1.1); TEMED – N,N,N',N'-tetramethylethylenediamine; Tricine – N-Tris[hydroxymethyl]methylglycine; Tris – Tris(hydroxymethyl)aminomethane.

PL ISSN 0001-5296

stress achieved after irrigation of plants with a solution of 100–400 mM NaCl (Cheng and Edwards, 1991). One of the major effects of salinity is generation of active oxygen species (AOS) such as superoxide radicals ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) (Corpas et al., 1993; Dat et al., 2000). These molecules can be very toxic due to their high reactivity, and can cause oxidative stress at the cellular level (Hernández et al., 1995). However, they can also act as secondary messengers controlling many processes in plant cells (Dat et al., 2000).

Plants are equipped with an effective system for scavenging AOS, including antioxidative enzymes (e.g., superoxide dismutases, catalase, peroxidases) and different low molecular substances (e.g., glutathione, ascorbate, carotenoids). The functioning of these scavengers can maintain the proper level of active oxygen species.

Superoxide dismutases (SODs) constitute the first line of defense against AOS, since they catalyze the dismutation of superoxide radical to molecular oxygen and hydrogen peroxide (McCord and Fridovich, 1969; Alsher et al., 2002). Several SOD forms (MnSOD, FeSOD, Cu/ZnSOD) are known to occur in different plant cell compartments. MnSOD is present mainly in mitochondria and peroxisomes (Salin and Bridges, 1981; Streller et al., 1994, del Río et al., 1983), FeSOD appears to be located exclusively in chloroplasts (Salin and Bridges, 1980, Niewiadomska et al., 1997), while Cu/ZnSOD occurs within chloroplasts, cytosol and mitochondria (Foster and Edwards, 1980; Sakamoto et al., 1995)

In *M. crystallinum*, all three SOD forms have been identified (Mn-, Fe- and Cu/ZnSOD (Miszalski et al., 1998). An additional form named MnSOD-like form (MnSODII) has been detected in roots and callus tissue of the ice plant (Ślesak and Miszalski, 2003; Libik et al., 2005). It has been reported that in leaves of *M. crystallinum* during the C_3-CAM shift, accelerated by NaCl stress, SOD activity increased (Miszalski et al., 1998). Different organs of *M. crystallinum* exhibit differing levels of CAM, and shoots are predominant in CAM induction (Libik et al., 2004). The present work examines whether there are correlations between the levels of SOD activity in different organs of this plant at different stages of the CAM mode of photosynthesis.

MATERIALS AND METHODS

PLANT MATERIAL AND CULTURE CONDITION

Mesembryanthemum crystallinum plants were grown from seeds in phytotron growth chambers in soil culture under a 12 h photoperiod at 25/17˚C (day/night), 60/80% RH, and irradiance of 200–250 µmol quanta $m^{-2}s^{-1}$ (PAR; λ = 400–700 nm). Two types of plants were used for the experiments: 5-week-old plants irrigated with water (early CAM) and 5-week-old plants irrigated for 1 week with 400 mM NaCl (full CAM). Plant organs were separated into mature leaves (first and second leaf pairs) (Ml), young leaves (third and fourth leaf pairs) (Yl), shoots (Sh) and roots (Rt) (Fig. 1). The organs were weighed, frozen in liquid nitrogen and stored at -80˚C for further analyses.

TITRATABLE ACIDITY

Samples of the different organs were preheated in 80% methanol (0.3 g tissue in 5 ml methanol) and then allowed to cool to room temperature. The supernatant (1 ml) was extracted for titration with 1 M NaOH to an end point of pH 7.0 using bromo-thymol blue as an indicator. Titratable acidity was expressed as µmol H^+ g^{-1} (fresh weight). $\Delta[H^+]$ equivalents are the difference between the level of acidity at dawn (6.00 a.m.) and at dusk (6.00 p.m.).

PROTEIN EXTRACTION

To isolate fractions of soluble proteins, plant material (1 g fresh weight) was homogenized at 4˚C using a mortar and pestle in 2.5 ml extraction buffer (100 mM Tricine, adjusted with Tris to pH 8.0, containing 3 mM $MgSO_4$, 1 mM DDT, 3 mM EGTA). Insoluble material was removed by centrifugation for 3 min at 3000 × g.

The protein concentration was determined according to Bradford (1976), using the BioRad protein assay, with BSA as a standard. Soluble protein fractions were stored at -80˚C until further use.

SOD ACTIVITY ANALYSIS

To determine SOD activity, native PAGE on 12% gel at 4˚C and 180 V was performed with Laemmli's (1970) buffer system without SDS. Fractions of soluble proteins were obtained as described above. SOD bands were visualized using the activity staining procedure described by Beauchamp and Fridovich (1971). The gels were incubated in staining buffer (potassium phosphate buffer, pH 7.8, containing 500 mM KH_2PO_4, 500 mM Na_2HPO_4, 10 mM EDTA, 28 mM TEMED, 22 mM riboflavin and 0.25 mM NBT) for 30 min in the dark at room temperature. After incubation the gels were exposed to white light until SOD activity bands became visible.

For densitometric determination of SOD activity, gels were scanned in a fluorescent chamber and the gel images were analyzed using BioPrint ver. 99 (Vilber-Lourmat, France). The activity of all isoforms was expressed in arbitrary units corresponding to the area under the densitometric curve.

STATISTICAL ANALYSIS

Two-way ANOVA was used to test the significance of any effects on SOD activity. Two-way ANOVA followed by Duncan's multiple range test was used to determine individual treatment effects at $P \leq 0.05$.

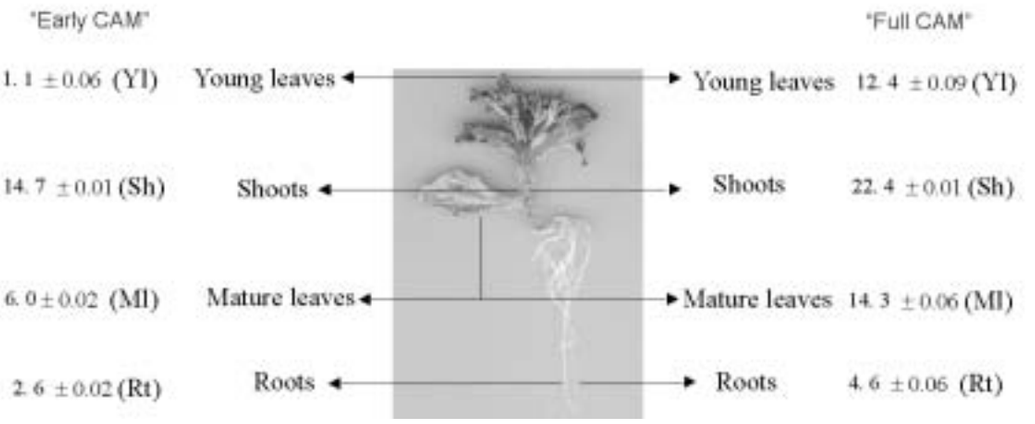

Fig. 1. $\Delta[H^+]$ equivalents (differences between acidity levels at 6.00 a.m. and at 6.00 p.m.) in organs of *M. crystallinum* plants at different stages of CAM development. Numbers are in units of Δ µmol $[H^+]$ g^{-1} $_{\text{fresh weight}}$ (n = 3).

RESULTS AND DISCUSSION

The transition of the metabolic pathway from C_3 to CAM in the facultative CAM plant *M. crystallinum* is associated with an increase in the activity of key CAM-related enzymes such as PEPC, and nocturnal opening of stomata with concomitant CO_2 uptake leading to diurnal fluctuations of tissue acidity and malate content (Lüttge, 2004). In our experiment, induction of CAM was measured as changes in acidity at dawn (6.00 a.m.) and dusk (6.00 p.m.) in different organs of the ice plant.

All analyzed tissues of *M. crystallinum* were found to be able to accumulate organic acids regardless of the metabolic stage. However, $\Delta[H^+]$ was much higher in plants exposed to salinity ("full CAM") than in plants irrigated with water ("early CAM") (Fig. 1). This result agrees with previous findings that malate can be accumulated in different organs of both watered and NaCl-treated plants (Libik et al., 2004).

The ability to accumulate organic acids was highest in shoots, and it depended not only on the type of metabolism. Even in plants not exposed to salinity, the highest $\Delta[H^+]$ equivalent were found in shoots, indicating that CAM developed first in these organs (Fig. 1). The much higher $\Delta[H^+]$ in shoots, and the ability of roots to accumulate such a high amount of organic acids, would mean that CO_2 can also be transported to photosynthetic tissues from the soil via the vascular system, and not from the stomata as was described for tobacco (Hibberd and Quick, 2002).

Little is known about the signal transduction pathway which must serve to link stress recognition to gene expression in the leaf. However, it is evident that the ice plant must be capable of monitoring the soil water status either directly or indirectly, since it is able to respond to water stress via induction of the CAM pathway. Thus, our findings confirm previously provided evidence (Eastmond and Ross, 1997) that the roots of *M. crystallinum* are capable of perceiving signals of low soil water availability and transport this information to the leaves, triggering the physiological expression of the CAM pathway.

In plants the role of SODs during environmental adversity has received much attention, since active oxygen species are produced during many stress conditions (Bowler et al., 1992; Scandalios, 1993). Changes in SOD activity in response to salinity in different plant material such as leaves, roots, shoots or callus have been reported previously (Hernández et al., 1999; Fadzilla et al., 1997; Olmos et al., 1994). In *M. crystallinum* leaf tissue, the presence of three main SOD forms – MnSOD, FeSOD and CuZnSOD – localized in mitochondria, chloroplasts and cytosol, respectively, has been shown (Miszalski et al., 1998). In this experiment, different organs of the ice plant also exhibited the activity of three SOD forms. Organs of "early CAM" plants showed similar patterns of SOD activity at dawn and dusk (Fig. 2a), but total SOD activity was highest during evening hours (Tab. 1). The highest diurnal fluctuations in activity in all analyzed organs were exhibited by MnSODI and also FeSOD, but the latter was much more active only in leaves. Roots and shoots exhibited the highest absolute activity of SOD, with CuZnSOD being the predominant form. Leaves of "early CAM" plants, both mature and young, showed lower activity of total SOD as a consequence of much lower CuZnSOD activity than in the other organs (Tab. 1).

Organs of "full-CAM" plants exhibited differences in the pattern of SOD activity at dawn and dusk (Fig. 2b). During the morning hours (6.00 a.m.) the pattern of SOD activity in different organs of salt-treated plants resembled that already described for "early CAM" plants, where the decrease in CuZnSOD activity

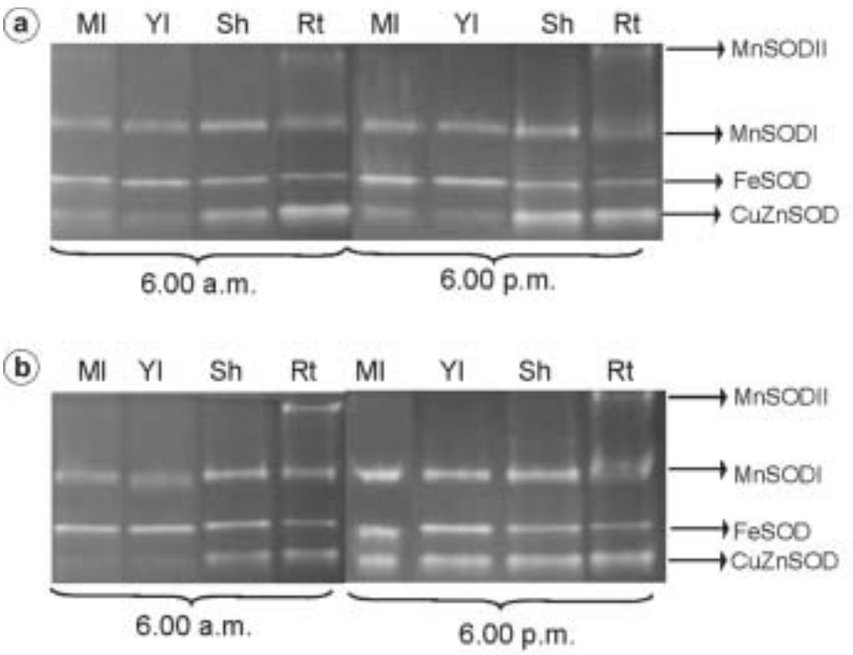

Fig. 2. SOD activity in extracts from organs of *M. crystallinum* plants at different stages of CAM development. (**a**) "Early CAM", (**b**) "Full CAM". Representative gels are shown.

was evident in mature as well as young leaves (Tab. 1). During evening hours (6.00 p.m.) the activity of all identifiable forms was high, especially in shoots. The strong increase in activity of SOD forms during evening hours in "full-CAM" plants, especially of MnSODI and CuZnSOD, suggests that salinity could induce oxidative stress in mitochondria and in the cytosol.

Total SOD activity was higher during the evening (6.00 p.m., dusk) hours, except in roots, than in the morning hours (6.00 a .m., dawn) in both "early CAM" and "full-CAM" plants (Tab. 1), suggesting that there is strong diurnal fluctuation of SOD activity independent of the influence of salt.

TABLE 1. Densitometric analysis of SOD activity-stained polyacryalamide gels after electrophoretic separation of extracts from organs of *M. crystallinum*. Statistical analysis is based on three replicates. Values with the same letter do not differ statistically at $p \leq 0.05$

	"Early CAM"								"Full CAM"							
	6.00 a.m.				6.00 p.m.				6.00 a.m.				6.00 p.m.			
	Ml	Yl	Sh	Rt	Ml	Yl	Sh	Rt	Ml	Yl	Sh	Rt	Ml	Yl	Sh	Rt
MnSODII	0	0	0	590	0	0	0	545	0	0	0	1070	0	0	0	810
Δ activity	0 (Ml)		0 (Yl)		0 (Sh)		-45 (Rt)		0 (Ml)		0 (Yl)		0 (Sh)		-260 (Rt)	
MnSODI	885c	879c	990b	761c	1136b	1235b	1770a	885c	861c	835c	1241b	1114b	1228b	1409a	1798a	899c
Δ activity	251 (Ml)		356 (Yl)		780 (Sh)		124 (Rt)		376 (Ml)		574 (Yl)		557 (Sh)		-215 (Rt)	
FeSOD	898c	945b	812c	802c	1321a	1024b	875c	815c	941b	950b	949b	796c	1563a	1453a	1241b	1070b
Δ activity	423 (Ml)		79 (Yl)		63 (Sh)		13 (Rt)		622 (Ml)		503 (Yl)		292 (Sh)		274 (Rt)	
CuZnSOD	794c	738c	1241b	1702a	911b	812c	1775a	1292b	543c	538c	816b	836b	1785a	1794a	1721a	1563a
Δ activity	117 (Ml)		74 (Yl)		543 (Sh)		-410 (Rt)		1242 (Ml)		1256 (Yl)		905 (Sh)		727 (Rt)	
Total SOD	2577c	2562c	3043b	3855b	3368b	3101b	4420a	3537b	2345c	2323c	3006b	3816b	4576a	4656a	4760a	4342a

In line with findings by Ślesak and Miszalski (2003), roots of *M. crystallinum* growing in vivo exhibited the activity of an additional form of SOD called MnSODII (Fig. 2). This form has been detected in both "early CAM" and "full-CAM" plants. However, its activity as well as that of other SOD forms was much higher in plants treated with salt. This result demonstrates that treatment with salt increases the level of total SOD activity, suggesting that NaCl causes oxidative stress not only in leaves, as previously described (Miszalski et al., 1998), but also in other plant organs.

ACKNOWLEDGMENTS

This work was partially supported by the Polish State Committee for Scientific Research, grant no. 3P04C 064 23 and EUCropStress project no. QLAM-2001-00424. We thank Dr. Ireneusz Ślesak (Cracow, Poland) for critically reading the manuscript.

REFERENCES

ALSCHER RG, ERTUK N, and HEALTH LS. 2002. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *Journal of Experimental Botany* 53: 1331–1341.

ADAMS P, NELSON DE, YAMADA S, CHMARA W, JENSEN RG, BOHNERT HJ, and GRIFFITHS H. 1998. Growth and development of *Mesembryanthemum crystallinum* (Aizoaceae). *New Phytologist* 138: 171–190.

BEAUCHAMP C, and FRIDOVICH I. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry* 44: 276–287.

BOWLER C, MONTAGU M, and INZÉ D. 1992. Superoxide dismutase and stress tolerance. *Annual Review of Plant Physiology and Plant Molecular Biology* 43: 83–116.

BRADFORD MM. 1976. A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248–254.

BROETTO F, LÜTTGE U, and RATAJCZAK R. 2002. Influence of light intensity and salt-treatment on the mode of photosynthesis and enzymes of the antioxidant response system of *Mesembryanthemum crystallinum*. *Functional Plant Biology* 29: 13–23.

CORPAS FJ, GOMEZ M, HERNÁNDEZ JA, and DEL RÍO LA. 1993. Metabolism of activated oxygen in peroxisomes from two *Pisum sativum* L. cultivars with different sensitivity to sodium chloride. *Journal of Plant Physiology* 141: 160–165.

CHENG S-H, and EDWARDS GE. 1991. Induction of Crassulacean acid metabolism in the facultative halophyte *Mesembryanthemum crystallinum*. *Plant Cell and Environment*: 14: 271–278.

CHU C, DAI Z, KU MSB, and EDWARDS G. 1990. Induction of Crassulacean acid metabolism in the facultative halophyte *Mesembryanthemum crystallinum* by abscisic acid. *Plant Physiology* 93: 1253–1260.

CUSHMAN JC, and BOHNERT HJ. 1997. Molecular genetics of Crassulacean acid metabolism. *Plant Physiology* 113: 667–676.

CUSHMAN JC, MICHALOWSKI CHB, and BOHNERT HJ. 1990. Developmental control of Crassulacean acid metabolism inducibility by salt stress in common ice plant. *Plant Physiology* 94: 1137–1142.

DAT J, VANDENABEELE S, VRANOVÁ E, VAN MONTAGNU M, INZÉ D, and BREUSEGEM F. 2000. Dual action of the active oxygen species during plant cell responses. *Cell Molecular Life Science* 57: 779–795.

DEL RÍO LA, LYON DS, OLAH I, GLICK, and SALIN ML. 1983. Immunocytochemical evidence for a peroxisomal localization of manganese superoxide dismutase in leaf protoplasts from higher plant. *Planta* 158: 216–224.

EASTMOND PJ, and ROSS JD. 1997. Evidence that the induction of crassulacean acid metabolism by water stress in *Mesembryanthemum crystallinum* (L.) involves root signaling. *Plant Cell and Environment* 20: 1559–1565.

FADZILLA NM, FINCH RP, and BURDON RH. 1997. Salinity, oxidative stress and antioxidant responses in shoot cultures of rice. *Journal of Experimental Botany* 48: 325–331.

FOSTER JG, and EDWARDS GE. 1980. Localization of superoxide dismutase in leaves of C_3 and C_4 plants. *Plant Physiology* 107: 757–750.

HERNÁNDEZ JA, OLMOS E, CORPAS FJ, SEVILLA F, and DEL RÍO LA. 1995. Salt-induced oxidative stress in chloroplasts of pea plants. *Plant Science* 105: 151–167.

HERNÁNDEZ JA, CAMPILLO A, JIMENEZ A, ALARCON JJ, and SEVILLA F. 1999. Response of antioxidant systems and leaf water relations to NaCl stress in pea plants. *New Phytologist* 141: 241–251.

HIBBERD J, and QUICK WP. 2002. Characteristics of C_4 photosynthesis in stem and petioles of C_3 flowering plants. *Nature* 415: 451–454.

LAEMMLI UK. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* 227: 680–685 .

LIBIK M, PATER B, ELLIOT S, ŚLESAK I, and MISZALSKI Z. 2004. Malate accumulation in different organs of *Mesembryanthemum crystallinum* L. following age-dependent or salinity triggered CAM metabolism. *Zeitschrift für Naturforschung* 59c: 223–228.

LIBIK M, KONIECZNY R, PATER B, ŚLESAK I, and MISZALSKI Z. 2005. Differences in the activities of some antioxidant enzymes and in H_2O_2 content during rhizogenesis and somatic embryogenesis in callus cultures of the ice plant. *Plant Cell Reports* 23: 834–841.

LÜTTGE U. 1993. The role of crassulacean acid metabolism (CAM) in adaptation of plants to salinity. *New Phytologist* 125: 59–71.

LÜTTGE U. 2002. Performance of plants with C_4-carboxylation modes of photosynthesis under salinity. In: Läuchli A, Lüttge U [eds.]. *Salinity: environment-plants-molecules*, 113–135. Kluwer, Dordrecht.

LÜTTGE U. 2004. Ecophysiology of Crassulacean acid metabolism (CAM). *Annals of Botany* 93: 629–652.

MCCORD JM, and FRIDOVICH I. 1969. Superoxide dismutase: an enzymatic function for erythrocuprein (hemocuprein). *Journal of Biological Chemistry* 244: 6049–6055.

MISZALSKI Z, ŚLESAK I, NIEWIADOMSKA E, BACZEK-KWINTA R, LÜTTGE U, and RATAJCZAK R. 1998. Subcellular localization and stress responses of superoxide dismutase isoforms from leaves in the C_3-CAM intermediate halophyte *Mesembryanthemum crystallinum* L. *Plant, Cell and Environment* 21: 169–179.

NIEWIADOMSKA E, MISZALSKI Z, and PILIPOWICZ M. 1997. The presence of FeSOD in the *Aceraceae* family. *Phyton* 37: 215–218.

OLMOS E, HERNÁNDEZ JA, SEVILLA F, and HELLIN E. 1994. Induction of several antioxidant enzymes in the selection of a salt-tolerant cell line of *Pisum sativum*. *Journal of Plant Physiology* 144: 594–598.

SAKAMOTO A, OKUMURA T, MANINAK H, and TANAKA K. 1995. Molecular cloning of the gene (SodCc1) that encodes a cytosolic copper zinc-superoxide dismutase from rice (*Oryza sativa* L.). *Plant Physiology* 107: 651–652.

SALIN ML, and BRIDGES SM. 1980. Isolation and characterization of an iron-containing superoxide dismutase from an Eucaryote *Brassica campestris*. *Archives of Biochemistry and Biophysics* 201: 369–374.

SALIN ML, and BRIDGES SM. 1981. Localization of superoxide dismutase in chloroplasts from *Brassica campestris*. *Zeitschrift für Pflanzenphysiologie* 99: 37–45.

SCANDALIOS JG. 1993. Oxygen stress and superoxide dismutases. *Plant Physiology* 101: 7–12.

STRELLER S, KRÖMER S, and WINGSLE G. 1994. Isolation and purification of mitochondrial Mn-superoxide dismutase from Gymnosperm *Pinus sylvestris* L. *Plant Cell Physiology* 35: 859–867.

ŚLESAK I, KARPIŃSKA B, SURÓWKA E, MISZALSKI Z, and KARPIŃSKI S. 2003. Redox changes in the chloroplasts and hydrogen peroxide are essential for regulation of C_3-CAM transition and photooxidative stress responses in the facultative CAM plant *Mesembryanthemum crystallinum*. *Plant Cell Physiology* 44: 573–581.

ŚLESAK I, and MISZALSKI Z. 2003. Superoxide dismutase-like protein from roots of the intermediate C_3-CAM plant *Mesembryanthemum crystallinum* L. in *in vitro* culture. *Plant Science* 164: 497–505.

WINTER K. 1973. Zum Problem der Ausbildung des Carssulaceaen – Säurestoffwechsels bei *Mesembryanthemum crystallinum* unter NaCl-Einfluß. *Planta* 109: 135–145.

WINTER K, LÜTTGE U, WINTER E, and 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.