

RESEARCH PAPER

Elevated atmospheric CO₂ concentration ameliorates effects of NaCl salinity on photosynthesis and leaf structure of *Aster tripolium* L.

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Abstract

This study investigated the interaction of NaCl-salinity and elevated atmospheric CO₂ concentration on gas exchange, leaf pigment composition, and leaf ultrastructure of the potential cash crop halophyte *Aster tripolium*. The plants were irrigated with five different salinity levels (0, 25, 50, 75, 100% seawater salinity) under ambient and elevated (520 ppm) CO₂. Under saline conditions (ambient CO₂) stomatal and mesophyll resistance increased, leading to a significant decrease in photosynthesis and water use efficiency (WUE) and to an increase in oxidative stress. The latter was indicated by dilations of the thylakoid membranes and an increase in superoxide dismutase (SOD) activity. Oxidative stress could be counteracted by thicker epidermal cell walls of the leaves, a thicker cuticle, a reduced chlorophyll content, an increase in the chlorophyll *a/b* ratio and a transient decline of the photosynthetic efficiency. Elevated CO₂ led to a significant increase in photosynthesis and WUE. The improved water and energy supply was used to increase the investment in mechanisms reducing water loss and oxidative stress (thicker cell walls and cuticles, a higher chlorophyll and carotenoid content, higher SOD activity), resulting in more intact thylakoids. As these mechanisms can improve survival under salinity, *A. tripolium* seems to be a promising cash crop halophyte which can help in desalinizing and reclaiming degraded land.

Key words: *Aster tripolium*, cash crop halophyte, elevated CO₂, gas exchange, oxidative stress, photosynthesis, salt tolerance, ultrastructure, water use efficiency.

Introduction

The CO₂ concentration in the atmosphere has increased due to anthropogenic emissions from 280 ppm to approximately 380 ppm since the beginning of industrialization, and it will continue to rise in future (IPCC, 2007). The well-known consequences are the greenhouse effect and global climate change manifesting itself for example in higher temperatures of the earth's surface and in an increase in extreme weather events such as heat waves or droughts (<http://www.mpimet.mpg.de/fileadmin/grafik/presse/ClimateProjektions2006.pdf>; IPCC, 2007). Due to rising temperatures, higher solar radiation, and decreasing and/or more irregular precipita-

tion patterns, climate change exacerbates soil degradation and desertification, especially in arid and semi-arid areas (Yeo, 1999; van Ittersum *et al.*, 2003; IPCC, 2007). In turn, desertification is often accompanied by soil salinization which today affects 7% of the global total land area (Szabolcs, 1994) and 20–50% of the global irrigated farmland (Tanji, 2002; Hu and Schmidhalter, 2005), leading to growth conditions unacceptable for most conventional crops (Choukr-Allah and Harrouni, 1996).

A promising solution to the problems mentioned above is the desalinization and reclamation of degraded land by

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Abbreviations: ppm, parts per million; sws, seawater salinity; IPCC, Intergovernmental Panel on Climate Change; ROS, reactive oxygen species; SOD, superoxide dismutase; PIPES, piperazine-1,4-bis(2-ethanesulphonic acid); ERL, epoxy resin of low viscosity; LM, light microscopy/microscope; TEM, transmission electron microscopy/microscope; SEM, scanning electron microscopy/microscope; PVP, polyvinylpyrrolidone; EDTA, ethylenediaminetetraacetic acid; A_{\max} , photosynthetic rate at light saturation; E , transpiration; R_s , stomatal resistance; WUE, water use efficiency of photosynthesis; C_i , intercellular CO₂ concentration; I_0 , light compensation point; I_s , saturation irradiance; Φ_c , apparent quantum yield of photosynthetic CO₂ assimilation; SD, standard deviation.

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making sustainable use of naturally salt-tolerant halophytes under seawater irrigation (including drainage mechanisms which avoid salt accumulation in the soil) (Pasternak, 1990; Lieth *et al.*, 1999; Lieth and Mochtchenko, 2002; Koyro, 2003). These plants, also called cash crop halophytes, have a high potential for utilization as they can be used for various economic and ecological purposes (Güth, 2001; Lieth and Mochtchenko, 2002). One of the ecological benefits of sustainable use is the fact that every new large plant population which is created for long-lasting use may sequester CO₂ and thus counteract the greenhouse effect. This is true even for annual or biannual species such as *A. tripolium* if the part of the biomass which is not utilised (in our case the roots) remains permanently in the soil. On the other hand, halophytes are likely to be promising crop plants in a future with rising atmospheric CO₂ concentrations because elevated CO₂ can enhance their salt tolerance and productivity (Ball and Munns, 1992; Urban, 2003). This becomes clear when looking at the effects of NaCl salinity and of elevated CO₂ concentration on plants. The four major constraints of salinity on plant growth are osmotic effects, restriction of CO₂ gas exchange, ion toxicity, and nutrient imbalance (Greenway and Munns, 1980; Koyro, 2003). As a consequence, on the one hand halophytes have to maintain ion homeostasis under saline conditions which can be achieved by selective ion transport and ion compartmentation. On the other hand, osmotic stress due to the low water potential of saline soils forces plants to minimize water loss because growth depends on the ability to find the best trade-off between a low transpiration and a high net photosynthetic rate (Koyro, 2006). However, various halophyte species show a clearly reduced assimilation rate under saline conditions due to stomatal closure (Huchzermeyer and Koyro, 2005). A consequence can be an increase in oxidative burst (Lovelock and Ball, 2002), especially in C₃ plants which are particularly susceptible to photorespiration and thus to oxidative stress. However, the production of reactive oxygen species (ROS) can be regulated by the amount and composition of photosynthetic pigments (Moorthy and Kathiresan, 1999, in Koyro, 2006), and generated ROS can be scavenged by the antioxidative system which includes enzymes such as superoxide dismutase and ascorbate peroxidase (Blokhina *et al.*, 2003).

Compared with salinity, elevated CO₂ concentration has contrary effects on C₃ plants. It often improves photosynthesis while reducing stomatal resistance, thus increasing water use efficiency, but decreasing photorespiration and oxidative stress (Urban, 2003; Kirschbaum, 2004; Rogers *et al.*, 2004). Therefore salt tolerance can be enhanced under elevated CO₂ (Ball and Munns, 1992; Wullschlegel *et al.*, 2002; Urban, 2003). According to literature, this is probably the case for *A. tripolium* as well, a promising potential cash crop halophyte which can be used for food (the leaves have a high nutritional value and can be eaten as salad or vegetable), for fodder, and as an ornamental plant (Güth, 2001; Lieth and Mochtchenko, 2002). It is already being cultivated in pilot schemes in the Netherlands, in Belgium, Portugal, and Pakistan (Güth, 2001; Lieth and Mochtchenko, 2002). It is

known that a high sodium concentration induces stomatal closure in *A. tripolium* due to the inactivation of the K influx channels of the guard cells (Perera *et al.*, 1994, 1995, 1997; Robinson *et al.*, 1997; Véry *et al.*, 1998; Kerstiens *et al.*, 2002), leading to reduced transpiration and lower photosynthesis (Lorenzen *et al.*, 1990; Huiskes, 1996a, b; Ueda *et al.*, 2003). As *Aster* is a C₃ plant, the impaired assimilation rate is likely to cause oxidative stress. There have also been some hints in the literature that *A. tripolium* may benefit from elevated CO₂ (enhanced water relations and/or growth; Lenssen and Rozema, 1990; Rozema *et al.*, 1990; Lenssen *et al.*, 1995), but up to now there has not been any detailed information about the effect of elevated CO₂ on the salinity tolerance of this plant.

However, to assess its potential as a cash crop in a future with rising atmospheric CO₂ concentrations, detailed scientific information is needed on both its salt tolerance mechanisms and their interaction with elevated CO₂ concentration. In this respect, gas exchange, water relations and connected parameters are of special importance because CO₂ mainly influences salinity tolerance mechanisms connected to the osmotic effects of salinity rather than to ion specific effects. Therefore this study focused particularly on the interactive effects of NaCl salinity and elevated CO₂ on gas exchange and related parameters (such as chlorophyll and carotenoid content) on *A. tripolium*. NaCl salinity and elevated CO₂ concentration also cause anatomical and morphological changes such as dilations of the thylakoid membranes or changes in cell wall composition and/or thickness (Koyro, 2002; Oksanen *et al.*, 2005). In order to find symptoms of disorders and to explain adaptation mechanisms, (ultra)structural changes accompanying physiological alterations in *A. tripolium* were also examined under salinity and elevated CO₂ concentration. Finally, measurements of the superoxide dismutase activity served as an indicator of the plant's response to ROS and therefore of the intensity of oxidative stress.

Materials and methods

Plant material and culture conditions

Stratified seeds of *Aster tripolium* L. (origin: Weser salt marsh near Cuxhaven, northern Germany) were sown on moist seed soil in an environmentally controlled greenhouse (16/8 h light/dark; day temperature 25±2 °C, night temperature 18±2 °C; 65±5% relative humidity). After 2 months, the plants were transferred to two different open-top chambers (Fangmeier *et al.*, 1992) where they were supplied with ambient atmospheric CO₂ concentration (380 ppm) and elevated CO₂ concentration (520 ppm, simulating the future concentration in the atmosphere in 40 to 80 years' time; IPCC, 2007), respectively. In each chamber, the plants were grown in a hydroponics quick check system (modified after Koyro, 2003; see Fig. 1 for more details). They were irrigated with a basic nutrient solution (1 mM KNO₃; 1 mM Ca(NO₃)₂; 1 mM NH₄H₂PO₄; 1 mM (NH₄)₂HPO₄; 1 mM MgSO₄; 0.02 mM Fe-EDTA; 0.025 mM H₃BO₃; 0.05

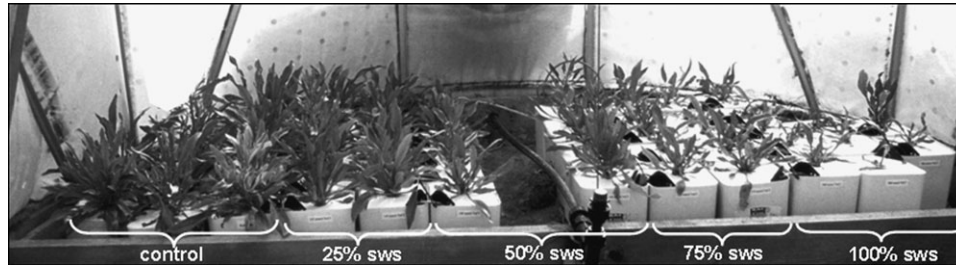


Fig. 1. Quick check system of *Aster tripolium* plants. Each pot contains 6.0 l of nutrient solution. Salinity increases from left to right; sws, seawater salinity.

mM KCl; 0.002 mM MnSO₄; 0.002 mM ZnSO₄; 0.0005 mM CuSO₄; 0.0005 mM MoO₃; modified after Epstein, 1972), and the pots were permanently aerated. After 1 week of acclimation time, the salinity of the nutrient solution was raised stepwise by adding 50 mol m⁻³ NaCl every morning and every evening. There were five salinity levels containing eight to ten plants each (0, 125, 250, 375, and 500 mol m⁻³ NaCl; equivalent to 0, 25, 50, 75, and 100% seawater salinity=sws) which were maintained for 4 weeks. Two independent cultures grown in different years were used for measurements.

As, in contrast to many other halophytes, *A. tripolium* shows maximum growth under non-saline conditions (N Geissler, unpublished results), the 0% sws treatment was considered as a control with which the other salinity levels could be compared.

Gas exchange

Gas exchange was measured porometrically with a Li-Cor 6200 portable photosynthesis system (Li-Cor, Lincoln, NE, USA) on young, but fully emerged leaves which grew completely under the treatment. Two leaves of at least six plants of each treatment and of each culture were used for measurements. Measurements were carried out at natural relative humidity (40–60%) and temperature (26–31 °C) in the open top chambers. A steady-state light response curve was determined at photosynthetic photon flux density levels of 0 and approximately 50, 200, 500, 1000, 1500, and 2000 μmol photon m⁻² s⁻¹ given by a light source (halogen bulb with reflector, 50 W, with dimmer). The net photosynthetic rate, transpiration, stomatal resistance, and intercellular CO₂ concentration were determined under saturating irradiation (1500 μmol photon m⁻² s⁻¹; formulas for calculation in Li-Cor Inc., 1990). The light compensation point (*I*_c), saturation irradiance (*I*_s), and apparent quantum yield of photosynthetic CO₂ assimilation (Φ_c) were calculated with the help of the exponential function explained by Schulte *et al.* (2003).

Chlorophyll and carotenoid content

Fresh material of adult leaves was extracted in 80% ethanol, and chlorophyll *a*, chlorophyll *b*, and carotenoids were determined spectrophotometrically after Lichtenthaler and Wellburn (1983) and Lichtenthaler (1987).

Light microscopy (LM) and transmission electron microscopy (TEM)

Leaf samples for LM and TEM were harvested in the early morning, and sample preparation was modified after Ruzin (1999). Leaf pieces of the controls were fixed for 4 h with 2.5% glutardialdehyde in 0.05 M PIPES buffer (pH 6.8) at room temperature after a vacuum infiltration (1 min at 0.05 MPa and 0.025 MPa respectively, then 5 min at 0.05 MPa). The salt-treated samples were washed for 30 s with aqua bidest before being fixed with 2% glutardialdehyde+1.5% formaldehyde in 0.05 M PIPES buffer. After that, the fixing medium was step by step substituted by 0.05 M PIPES buffer: One-third of the medium was exchanged three times, and after that almost the whole medium was exchanged twice. After a post-fixation with 1% OsO₄ in 0.05 M PIPES buffer at room temperature, the samples were carefully rinsed with water, continuously dehydrated with acetone (Sitte, 1962), infiltrated in ERL-4206 resin (Spurr, 1969) overnight and polymerized at 70 °C for 12 h.

The embedded samples were sectioned with glass knives on an ultramicrotome (Ultracut E, Reichert-Jung). Semi-thin sections for LM were stained with 0.5% toluidine blue+0.5% borax, ultrathin sections for TEM were contrasted with 2% uranylacetate and 5% lead citrate (Reynolds, 1963).

Scanning electron microscopy (SEM)

Leaf pieces were fixed for 10 d with FAA (45% ethanol+5% acetic acid+1.85% formaldehyde) at room temperature and were then dehydrated in ethanol (15 min in 50%, 70%, 80%, 90%, 96%, 100%, and 100% over a molecular sieve each) (modified after Ruzin, 1999). The samples were dried in a Balzers Union Critical Point Dryer, and a thin gold layer was sputtered onto the surface in a Leitz sputtering chamber (2 min, 0.6 mA).

Determination of superoxide dismutase (SOD) activity

For the determination of SOD activity, 0.5 g leaf material (taken from the second youngest fully emerged leaf) were ground in liquid nitrogen in a mortar and homogenized with 2 ml 50 mM potassium phosphate buffer (pH 7.4) containing 1 mM Na₂-EDTA*2H₂O, 1% (w/v) PVP, and 10 mM ascorbic acid. After centrifugation, the supernatant was dialysed overnight against 700 ml extraction buffer at 4 °C

and then used as the enzyme source. SOD activity was measured according to Rios-Gonzalez *et al.* (2002). 1 ml 50 mM potassium phosphate buffer (pH 7.8) which contained 0.1 mM $\text{Na}_2\text{-EDTA}\cdot 2\text{H}_2\text{O}$, 13 mM L-methionine, 0.17 mM nitro blue tetrazolium chloride and 7 μM riboflavin was mixed with 10 μl enzyme extract in small glass bowls. The samples were shaken for 25 min under a UV lamp (350 nm), and the extinction was measured at 560 nm.

Statistics

Statistical analysis was carried out by one-way or multi-way (in case several variables directly influenced one another) analysis of variance using SPSS software. Differences between means ($P \leq 0.05$) were assessed by Tukey's *post hoc* test (differences between more than two salinity levels) and by Student's *t* test (differences between two salinity levels and between the CO_2 treatments).

Results

Gas exchange

Under ambient atmospheric CO_2 concentrations, seawater salinity significantly reduced the net assimilation rate at light saturation (A_{max}) by two-thirds (Fig. 2A; Table 1A), and photosynthesis was saturated at lower light intensities. The intercellular CO_2 concentration (C_i) decreased in this C_3 plant to the conspicuously low value of less than 150 ppm at sea water salinity (Table 1A). Stomatal resistance (R_s) increased significantly so that transpiration (E) was reduced, but this effect was not strong enough to prevent a significant decrease in water use efficiency (WUE) by 30%. The results of the stomatal resistance are in accordance with the structural investigations which showed that the stomata were more closed in the salt treatments (Fig. 2B). Salinity also led to a higher light compensation point (I_c) and to a transient decrease (decrease at medium salinity,

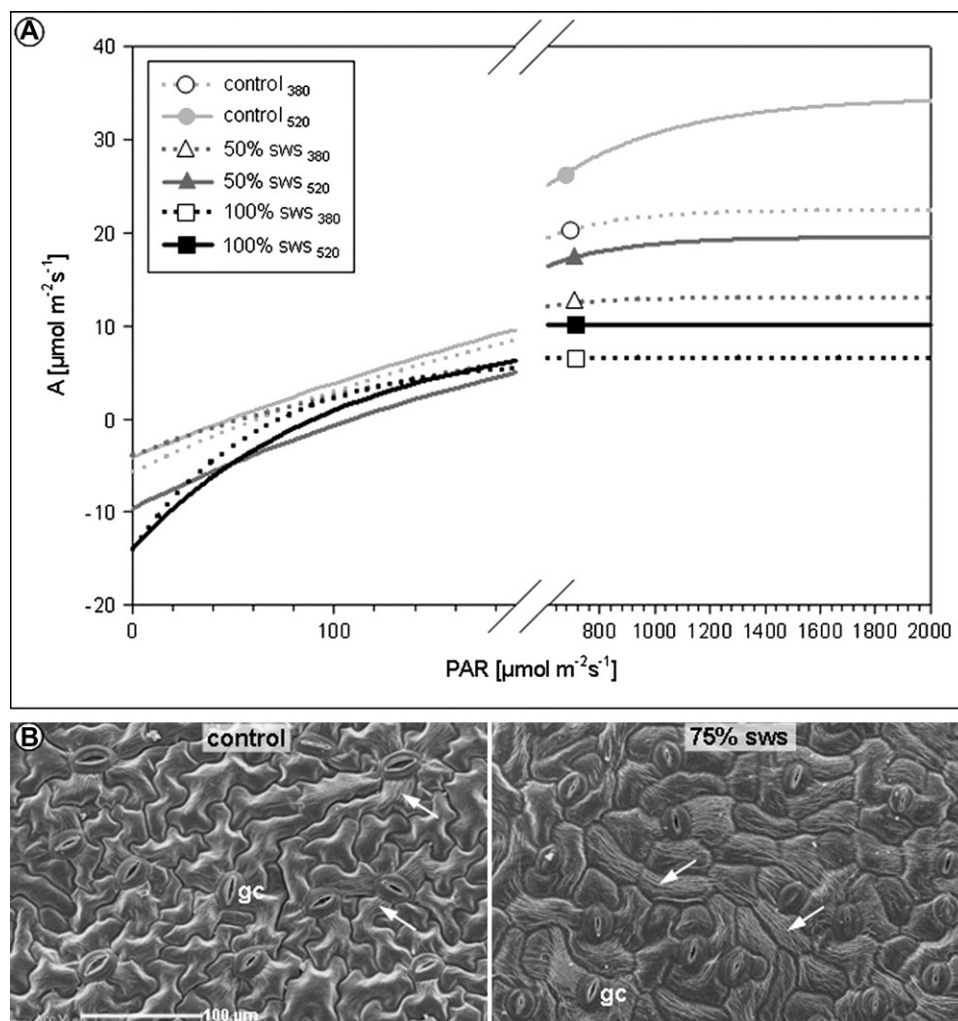


Fig. 2. Effect of NaCl salinity on photosynthesis. (A) Light saturation curves at different salinities under ambient CO_2 ($_{380}$) and elevated CO_2 ($_{520}$). Curves were drawn with the help of SigmaPlot Software, based on all 12 measurements at 0, 50, 200, 500, 1000, 1500, and 2000 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$, respectively. (B) Stomata in the adaxial epidermis of controls and of plants grown at 75% sws (ambient CO_2 concentration; SEM photographs). sws, seawater salinity; A , net photosynthetic rate; PAR , photosynthetic active radiation; gc , guard cells of stomata; arrows indicate cuticular ridges.

Table 1. (a) Gas exchange parameters at different salinities under ambient and elevated CO₂

All parameters were measured at light saturation. *A*, net assimilation rate; *R_s*, stomatal resistance; *E*, transpiration; *WUE*, water use efficiency of photosynthesis; *C_i*, internal CO₂ concentration; sws, seawater salinity. Values represent mean ±SD values of 12 measurements per treatment. Different letters indicate significant differences ($P \leq 0.05$) between salinity levels (within one CO₂ treatment), an asterisk indicates significant difference between CO₂ treatments (within one salinity level).

Salinity treatment	CO ₂ treatment	<i>A</i> (μmol m ⁻² s ⁻¹)	<i>R_s</i> (s cm ⁻¹)	<i>E</i> (mol m ⁻² s ⁻¹)	<i>WUE</i> (μmol mmol ⁻¹)	<i>C_i</i> (ppm)
Control	ambient	21.6±6.1 a	1.7±0.8 a	4.3±0.9 a	5.1±1.6 a	172.9±31.1 a
50% sws	ambient	11.4±6.0 b	4.5±2.2 b	2.8±1.4 b	4.3±0.9 b	144.3±48.5 b
100% sws	ambient	7.6±4.6 c	9.7±5.4 c	2.3±1.6 b	3.5±0.4 c	132.0±30.2 b
Control	elevated	33.6±6.3 a*	1.2±0.7 a	6.1±1.5 a*	5.8±1.5 a	262.8±33.2 a*
50% sws	elevated	20.8±7.9 b*	3.1±2.1 b	4.0±1.9 b	5.4±1.1 a*	231.9±49.6 b*
100% sws	elevated	13.0±4.8 c*	6.9±4.9 c*	2.5±1.3 c	5.6±0.3 a*	184.0±52.6 c*

(b) Gas exchange parameters of the light saturation curves

I_c, light compensation point; *I_s*, saturation irradiance; Φ_c , apparent quantum yield of photosynthetic CO₂ assimilation; sws, seawater salinity. SD values and significances were not determined because *I_c*, *I_s*, and Φ_c were calculated once for each treatment with the help of an exponential function (Schulte *et al.*, 2003), based on all 12 measurements at 0, 50, 200, 500, 1000, 1500, and 2000 μmol photon m⁻² s⁻¹, respectively.

Salinity treatment	CO ₂ treatment	<i>I_c</i> (μmol m ⁻² s ⁻¹)	<i>I_s</i> (μmol m ⁻² s ⁻¹)	Φ_c (mol CO ₂ mol ⁻¹ photons)
Control	ambient	61.1	683.4	0.08
50% sws	ambient	55.0	544.9	0.06
100% sws	ambient	72.8	219.4	0.10
Control	elevated	48.8	1049.9	0.08
50% sws	elevated	108.0	730.4	0.07
100% sws	elevated	90.4	332.8	0.10

increase at high salinity) in photosynthetic efficiency (Φ_c) (Table 1B).

Under elevated atmospheric CO₂ concentrations, net photosynthesis was significantly ameliorated and was saturated at higher light intensities, and the plants showed a significantly higher intercellular CO₂ concentration (*C_i*; Fig. 2A; Table 1A). *R_s* was lower and *E* was higher in the salt-treated plants than under ambient CO₂. *WUE* could be held constant at all salinity levels due to the increase in photosynthesis. Elevated CO₂ concentration also led to a higher *I_c* of the salt treatments (Table 1B). No distinct differences in Φ_c were observed between the CO₂ treatments.

Chlorophyll and carotenoid content

Under ambient atmospheric CO₂ concentrations, rising NaCl salinity caused a continual decrease in chlorophyll *a*, chlorophyll *b*, and carotenoid content which was significant for chlorophyll *b* (Fig. 3A). The chlorophyll *alb* ratio showed a significant increase under saline conditions (Fig. 3C). Elevated CO₂ concentration led to a significantly higher content of all three pigments in the high salt treatments (Fig. 3B). The chlorophyll *alb* ratio was not influenced by elevated CO₂ (Fig. 3D).

Leaf anatomy

The leaves of the control plants were bifacial flattened leaves with an adaxial palisade parenchyma (one or two cell layers) and an abaxial spongy parenchyma (Fig. 4A). A few

leaves exhibited an inflated, thick spongy parenchyma, the adaxial part of which formed a dense tissue with only few intercellular spaces. Under saline conditions, the vertical extension of the cross-sectioned leaves increased up to two times due to changes of the mesophyll (Fig. 4B; Table 2). The palisade parenchyma of many leaves developed an additional cell layer, and an inflated spongy parenchyma of stronger development was present in the majority of the leaves (Fig. 4C). Furthermore, the mesophyll cells were larger under saline conditions; in particular, the vertical length of the palisade parenchyma cells increased (Table 2). The rise in leaf thickness was accompanied by a decline in intercellular spaces in all mesophyll tissues (Fig. 4B, C; Table 2).

Elevated CO₂ concentration had only minor effects on leaf anatomy which are therefore not discussed in this paper.

Ultrastructure of the outer epidermal cell walls of the leaves, palisade parenchyma cells, and vascular bundles

Both saline conditions and elevated atmospheric CO₂ concentration led to an increase in the thickness of the outer epidermal cell walls of the leaves; under elevated CO₂ the walls of the salt-treated plants were twice as thick as the ones of the controls grown at 380 ppm CO₂ (Fig. 5). Furthermore, cuticle thickness increased under salinity plus elevated atmospheric CO₂ concentration. It more than doubled in the salt treatments under elevated CO₂ concentration compared with the controls under ambient CO₂. Salinity also altered the surface structure of the epidermis

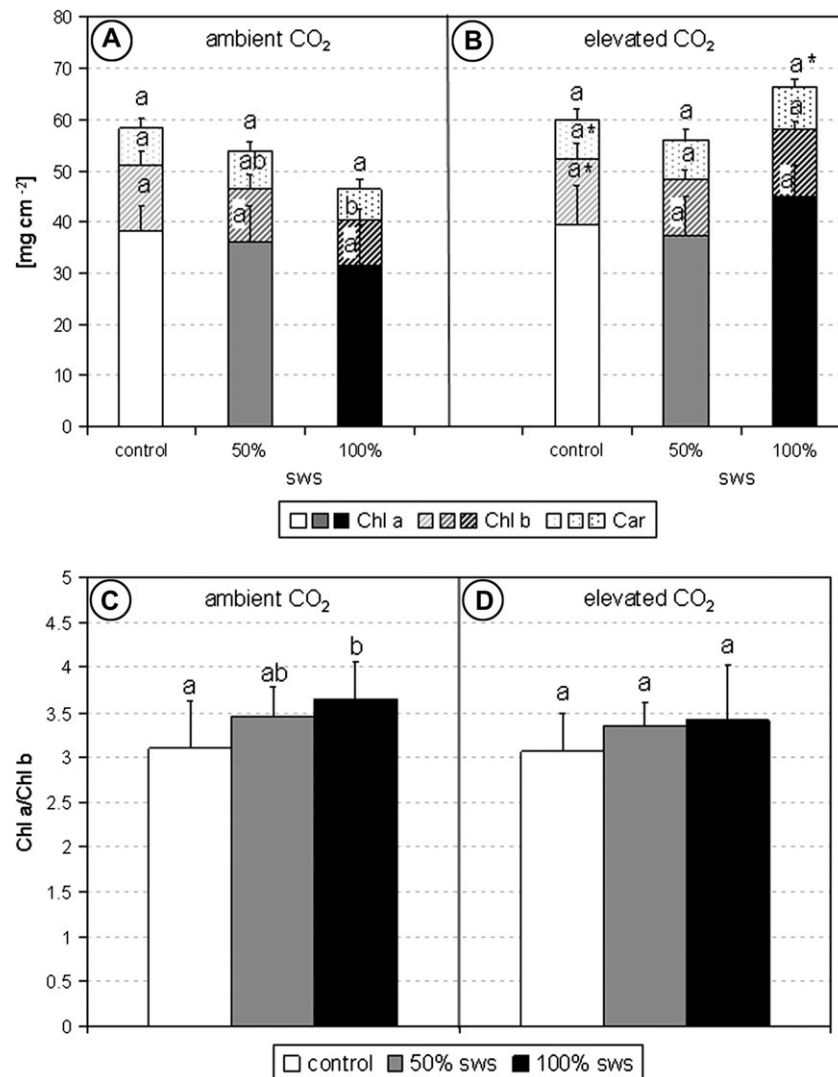


Fig. 3. Pigment composition of adult leaves at different salinities. (A) Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoid (Car) content under ambient CO₂; (B) chlorophyll *a*, chlorophyll *b* and carotenoid content under elevated CO₂; (C) chlorophyll *a/b* ratio under ambient CO₂; (D) chlorophyll *a/b* ratio under elevated CO₂. Values represent mean \pm SD values of nine measurements per treatment. Different letters indicate significant differences ($P \leq 0.05$) between salinity levels (within one CO₂ treatment), an asterisk indicates a significant difference between CO₂ treatments (within one salinity level). sws, seawater salinity.

(Fig. 2B). While the cells of the controls were irregularly shaped and had narrow cuticular ridges, the salt-treated cells showed fewer bulges and had a larger amount of cuticular ridges which were wider and flatter. The surface structure of the epidermis was not altered by elevated CO₂ concentration.

Regarding the palisade parenchyma, NaCl salinity affected chloroplast ultrastructure. The chloroplasts of the control plants (at 380 ppm CO₂) were more or less oval-shaped and contained only a few small starch grains (Fig. 6A). Their thylakoid membranes were clearly differentiated into stacks of grana and stroma thylakoids (Fig. 7A, B). Under saline conditions, the chloroplasts were partially horseshoe-shaped, and they contained a larger number of starch grains (Fig. 6B). Their thylakoid membranes showed dilations, the spaces between the membranes looked swollen, and undulated thylakoid areas developed (Fig. 7C, D).

In particular, the number of grana stacks was considerably reduced. Under elevated atmospheric CO₂ concentrations, the control chloroplasts contained numerous large starch grains (Fig. 6C). In the salt treatments, less starch was observed than in the controls (Fig. 6D). Due to excessive starch accumulation, some of the chloroplasts developed a round shape or lateral protuberances. General chloroplast ultrastructure was not affected by elevated CO₂, but a larger number of nearly intact chloroplasts with only minor dilations of the thylakoids were found under saline conditions (Fig. 7E).

NaCl salinity also affected the number of vesicles in the mesophyll cells. The salt treatments exhibited a larger number of vesicles than the controls (Table 2). In some areas of their cytoplasm, vesicles were observed, some of which were fused with the plasma membrane (Fig. 6E) instead of chloroplasts.

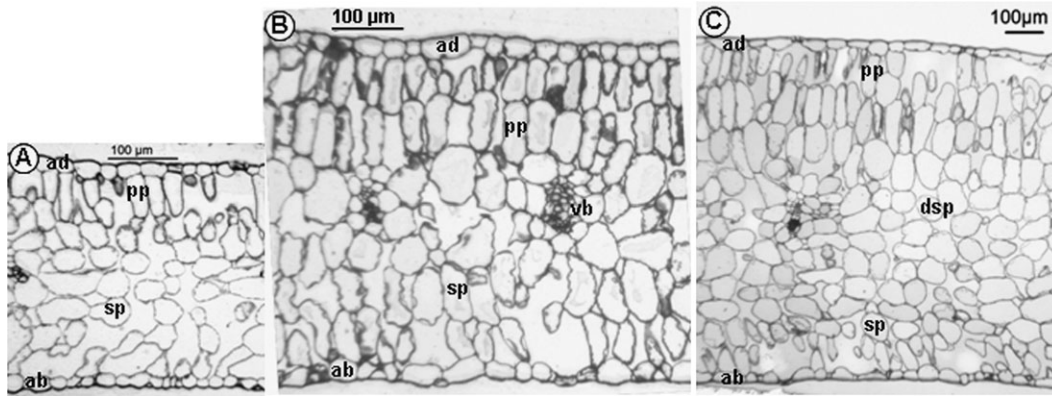


Fig. 4. Cross-sections of leaves (LM photographs). (A) Control; (B) 75% sws (seawater salinity); (C) 75% sws with strongly developed, inflated spongy parenchyma. ad, adaxial epidermis; pp, palisade parenchyma; sp, spongy parenchyma; dsp, dense adaxial part of spongy parenchyma; ab, abaxial epidermis; vb, vascular bundle.

Table 2. Anatomical and ultrastructural characteristics of control plants and salt treatments under ambient CO₂ concentration sws, seawater salinity. Values represent mean \pm SD values of three leaves per treatment (in case of single cells of 10 cells each). Different letters indicate significant differences ($P \leq 0.05$) between salinity levels.

	Control	75% sws
Vertical extension of palisade parenchyma (μm)	117.0 \pm 12.2 a	329.0 \pm 64.4 b
Vertical extension of spongy parenchyma (μm)	247.7 \pm 35.1 a	450.1 \pm 78.4 b
Vertical extension of whole leaf (μm)	410.1 \pm 49.7 a	837.6 \pm 66.9 b
Vertical extension of single palisade parenchyma cells (μm)	59.4 \pm 13.7 a	112.0 \pm 26.2 b
Vertical extension of single spongy parenchyma cells, in dense adaxial part (μm)	45.2 \pm 11.7 a	75.2 \pm 15.7 b
Vertical extension of single spongy parenchyma cells, in abaxial part (μm)	47.9 \pm 11.4 a	80.0 \pm 24.9 b
Inter cellular spaces in palisade parenchyma (% per area)	41.1 \pm 4.9 a	16.6 \pm 5.5 b
Inter cellular spaces in spongy parenchyma, in dense adaxial part (% per area)	19.5 \pm 6.3 a	8.6 \pm 4.2 a
Inter cellular spaces in spongy parenchyma, in abaxial part (% per area)	47.0 \pm 10.1 a	31.3 \pm 10.3 a
Number of vesicles (1000 μm^{-2})	3.0 \pm 1.4 a	7.7 \pm 1.0 b

The ultrastructure of the vascular bundles was not appreciably affected by salinity and elevated CO₂. For the sake of completeness it should be mentioned that in all treatments numerous transfer cells with protuberances of the cell walls, plastids and a large number of mitochondria were present in the phloem (Fig. 7F).

Superoxide dismutase (SOD) activity

Salinity led to a tendential increase in relative SOD activity (Table 3). Under elevated CO₂ concentrations, the activity of the salt treatments was significantly higher (factor 1.6) compared to ambient CO₂, and it was twice as high (a significant effect) as the one of the controls at elevated CO₂.

Discussion

Regarding the four major constraints of NaCl salinity on plant growth (see Introduction), the osmotic effect is the primary one affecting the plant (Munns, 2002). As expected (see above) *A. tripolium* reacts to this constraint by closing its stomata in order to reduce transpiration and thus dehydration. Several xeromorphic changes of the

leaves which were also observed in other plant species (Hajibagheri *et al.*, 1983, in Koyro, 2002; Koyro, 2000a, b, 2006; Daoud *et al.*, 2003; Debez *et al.*, 2003) perform the same function, namely (i) the thickened outer epidermal cell walls, the thickened cuticle, and the wider and flatter cuticular ridges on a larger number of cells; (ii) the thicker leaves due to an increase in vertical extension of the mesophyll cells and a larger amount of mesophyll cell layers; and (iii) the decrease in intercellular spaces.

The last two factors led to a higher mesophyll resistance for CO₂ which, in turn, together with the rising stomatal resistance, caused a reduction of the intercellular CO₂ concentration. This reinforces, especially in C₃ plants like *Aster*, the oxygenase reaction of Rubisco, thus leading to higher photorespiration at the expense of photosynthesis (Sobrado, 2005; Debez *et al.*, 2006). The impaired assimilation rate also correlates with the ultrastructural changes of the chloroplasts (see below). As there was a higher reduction in net photosynthesis than in transpiration, water use efficiency of photosynthesis (*WUE*) declined significantly. Obviously, the reduction in transpiration is not sufficient to maintain a positive water balance at higher salinities. These results contrast with the studies of Lorenzen *et al.* (1990) and Huiskes (1996b) who report a rising *WUE* in salt-treated

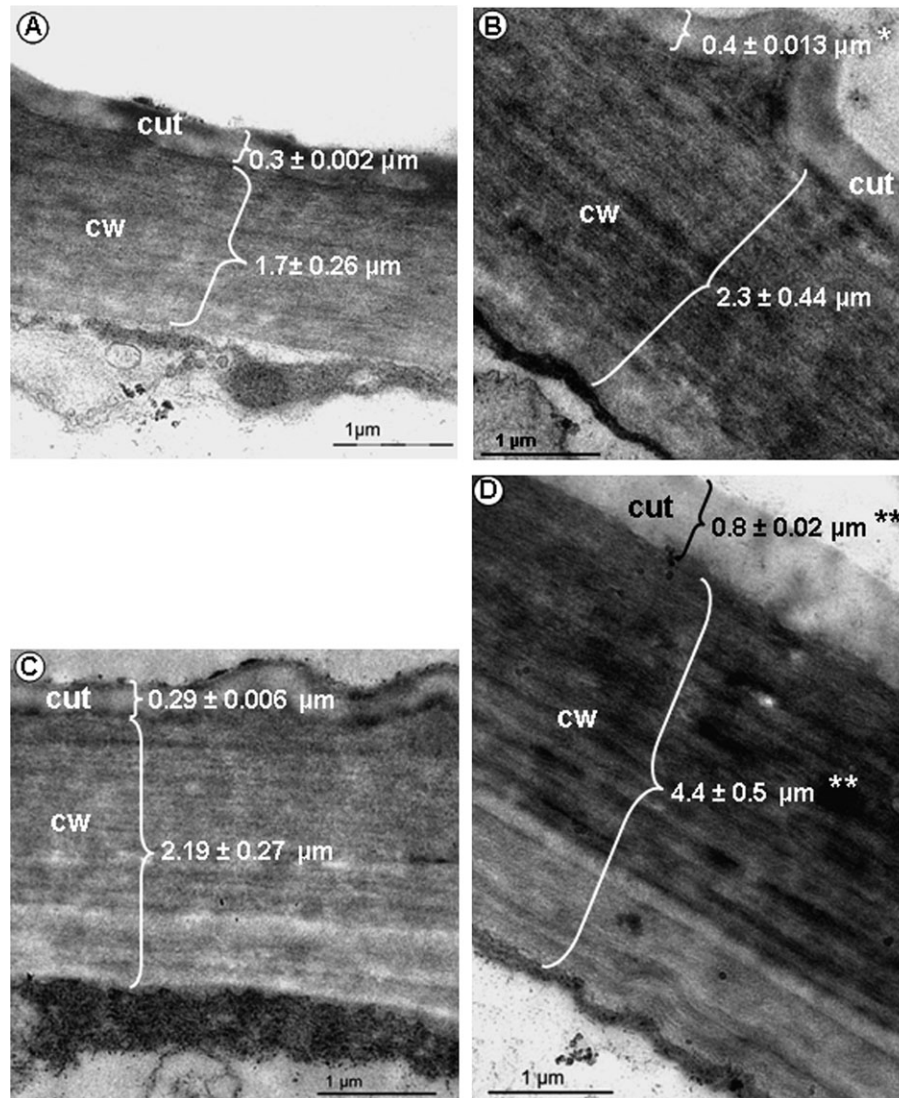


Fig. 5. Outer cell wall of the adaxial epidermis with cuticle (TEM photographs). (A) Control, ambient CO₂; (B) 75% sws (seawater salinity), ambient CO₂; (C) control, elevated CO₂; (D) 75% sws, elevated CO₂. cw, cell wall; cut, cuticle. Values represent mean \pm SD values of three leaves per treatment. An asterisk indicates a significant difference ($P \leq 0.05$) between salinity treatments under ambient CO₂, two asterisks indicate a significant difference both between salinity treatments under elevated CO₂ and between the CO₂ treatments.

Aster plants. However, those results were obtained under very low light intensities ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$) so that they are comparable with our study only to a limited extent. Our own measurements of greenhouse cultures under low light intensities (data not shown) showed similarities with the study of Huiskes (1996b): they showed a more prominent decrease in transpiration, in relation to the decrease in photosynthesis, and thus a transient increase in *WUE* with rising salinity. This consideration indicates that high light intensities have a negative impact on the gas exchange of *A. tripolium* and it simultaneously confirms the thesis that salinity enhances photorespiration and thus oxidative stress.

Another important point is that a reduced assimilation rate often leads to oxidative stress due to an energy surplus of the photosystems. (Schulze *et al.*, 2002; Ben Amor *et al.*, 2005). Two concrete indications of a higher exposure of *A. tripolium* cells to ROS under saline conditions were observed.

Firstly, the superoxide dismutase showed a higher activity in the salt treatments, similar to several other studies on plant responses to various abiotic stresses (Mittova *et al.*, 2002; Ben Amor *et al.*, 2005; Kukreja *et al.*, 2005; Stepien and Klobus, 2005). This crucial antioxidant enzyme (Halliwell, 1987; Asada, 1992; Ben Amor *et al.*, 2005) is present not only in the cytosol, but also in the stroma of the chloroplasts (<http://www.plantstress.com/Articles/Oxidative%Stress.htm>), i.e. at the site of photosynthesis and ROS formation. Therefore, a higher enzyme activity is a good indicator of an increase in oxidative stress.

Secondly, dilations of the thylakoid membranes were observed. This kind of damage has often been observed in salt-stressed plants (Kurkova *et al.*, 2002; Rahman *et al.*, 2002; Mitsuya *et al.*, 2003a; Fidalgo *et al.*, 2004; Paramanova *et al.*, 2004), has been discussed by many authors as a consequence of oxidative stress (Mitsuya *et al.*, 2003a, b;

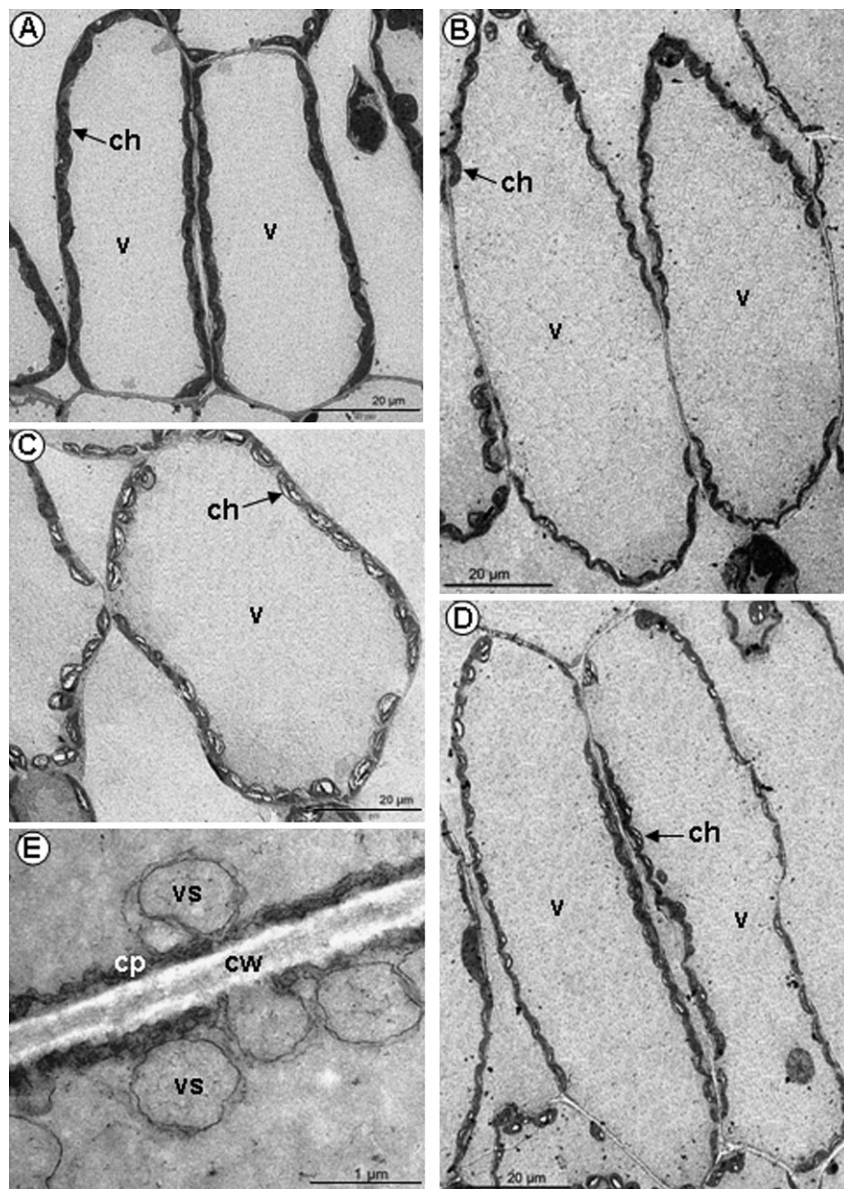


Fig. 6. Palisade parenchyma cells (TEM photographs). (A) Control, ambient CO₂; (B) 75% sws (seawater salinity), ambient CO₂; (C) control, elevated CO₂; (D) 75% sws, elevated CO₂; (E) vesicles in a cell grown at 75% sws under ambient CO₂. ch, chloroplast; v, vacuole; cw, cell wall; cp, cytoplasm; vs, vesicle.

Fidalgo *et al.*, 2004; Yamane *et al.*, 2004; Oksanen *et al.*, 2005) and indicates that the increase in SOD activity is not high enough to eliminate ROS sufficiently in *A. tripolium*. Oxidative stress can also be reduced by mechanisms which counteract the energy surplus of the photosystems. One of these mechanisms is the higher photorespiration under saline conditions which can diminish the Mehler reaction. (Wingler *et al.*, 2000). The reduction of oxidative stress seems to be crucial for the survival of *A. tripolium* under saline conditions. This is indicated by several reactions of *Aster* to salinity which ameliorate oxidative stress while counteracting the energy surplus of the photosystems. The thicker outer epidermal cell walls, the thicker cuticle, and the wider cuticular ridges can avoid an overreduction of the photosynthetic electron transport chain because they reflect a higher

proportion of the incoming radiation (Thomas, 2005). The reduced chlorophyll content of the leaves can fulfil a similar function (less energy conduction by the light-harvesting complex, see below). On the one hand, it reduces the assimilation rate of *A. tripolium* (Lorenzen *et al.*, 1990), but on the other hand (and in case of *A. tripolium* more important), it decreases the light absorption of the leaves (Wang *et al.*, 2003; Christian, 2005). This thesis is supported by the fact that, at medium salinity, the reduced photosynthetic efficiency (Φ) was linked to a lower light compensation point. At high salinity, Φ rose again, probably due to an increasing dark respiration (data not shown). Photosynthetic efficiency could also be reduced by the higher chlorophyll *a/b* ratio in the leaves (Moorthy and Kathiresan, 1999; in Koyro, 2006) because chlorophyll *b* is mainly located in the

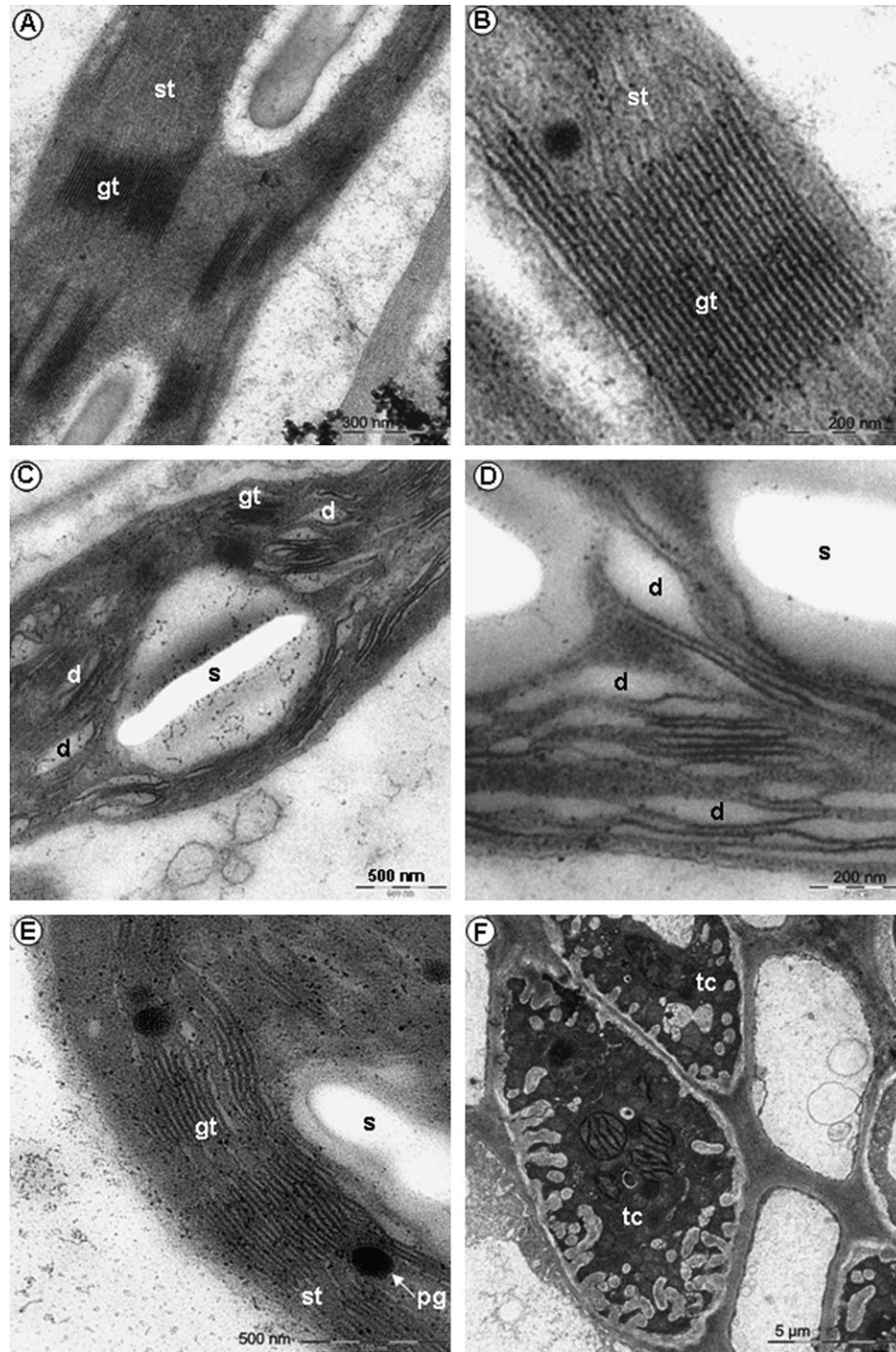


Fig. 7. Chloroplasts and vascular bundle (TEM photographs). (A–D) Chloroplasts. (A, B) Control, ambient CO₂; (C, D) 75% sws (seawater salinity), ambient CO₂; (E) 75% sws, elevated CO₂; (F) transfer cells in phloem parenchyma. st, stroma thylakoids; gt, grana thylakoids; s, starch grain; d, dilations; pg, plastoglobulus; tc, transfer cell.

light harvesting complex (LHC). As a direct consequence, the disproportionate reduction of chlorophyll *b* compared to chlorophyll *a* seems to be accompanied by a reduction of grana stacks, the location of the LHC, in the salt-treated plants.

Apart from structural alterations related to photosynthesis and gas exchange (see above), structural characteristics of *A. tripolium* related to other adaptation mechanisms were also found, such as a larger number of starch grains being present in the salt-treated chloroplasts compared to the

controls. As the samples were harvested in the morning, this is probably not related to photosynthetic activity, but to hampered translocation and/or sink activities. According to Keiper *et al.* (1998) salinity can inhibit starch-degrading enzymes or induce the conversion of saccharides into starch (Parida *et al.*, 2004). Possibly, salt-treated *A. tripolium* plants accumulate a starch reservoir which can be released and metabolized under better growth conditions and decreasing stress.

Table 3. Superoxide dismutase activity of control plants and of plants grown at 75% sws (seawater salinity) under ambient and elevated CO₂

Values represent mean \pm SD values of six measurements per treatment. Different letters indicate significant differences ($P \leq 0.05$) between salinity levels (within one CO₂ treatment), an asterisk indicates a significant difference between the CO₂ treatments (within one salinity level).

Salinity treatment	Ambient CO ₂	Elevated CO ₂
Control	9.4 \pm 1.8 a	8.5 \pm 0.6 a
75% sws	11.5 \pm 2.0 a	18.0 \pm 4.6 b*

Indications of adaptation to ion toxicity and nutrient imbalance (enhanced selective ion transport) had already been shown for *A. tripolium* (N Geissler, unpublished results). Mechanisms such as ion compartmentation into the vacuoles prevent an excessive NaCl accumulation in the stroma of the chloroplasts and are therefore of importance to the maintenance of photosynthesis. The larger mesophyll cells of the salt treatments exhibit larger vacuoles and therefore have a higher capacity to sequester NaCl into these compartments (Ayala *et al.*, 1996). Furthermore, as in other plant species, the mesophyll cells and the sieve tubes of the phloem contained a larger number of vesicles which increase the membrane surface area and are often discussed in connection with processes such as transport, storage, and NaCl compartmentation (Koyro, 1997, 2002; Kurkova *et al.*, 2002; Mitsuya *et al.*, 2002). Transfer cells which were found in the phloem parenchyma fulfil a similar function (Koyro, 2002; Boughanmi *et al.*, 2003; Offler *et al.*, 2003).

Elevated atmospheric CO₂ concentration seemed to ameliorate some of the negative effects of NaCl salinity on *A. tripolium*. Elevated CO₂ caused an increase in intercellular CO₂ concentration and a significantly higher net assimilation rate, leading to an improved *WUE* of the salt treatments. From these results, it can be inferred that the plants had a better supply of energy-rich organic substances while their water relations were improved (N Geissler, unpublished results).

Responses of gas exchange and growth to elevated CO₂ concentration are variable; they depend on plant species and abiotic factors such as salinity and humidity (Drake, 1992; Ball and Munns, 1992; Arp *et al.*, 1993). In the case of *A. tripolium*, the observed decrease in stomatal resistance shows that the priority for this plant (at least under our experimental conditions) does not seem to be the reduction of water loss but the maximization of photosynthesis and energy gain (see below). The significantly higher chlorophyll content also contributes to this strategy. These results also show that *Aster*, at least for the duration of this study, does not acclimate to elevated CO₂ like many other plants (Urban, 2003; Long *et al.*, 2004). Acclimation depends on the source-sink ratio within the plant and will often be alleviated if carbon sinks are enlarged under elevated CO₂ concentration (Engloner *et al.*, 2003; Ainsworth and Rogers, 2007); since the salt-treated *Aster* plants invest in energy-dependent

salinity tolerance mechanisms (see below) and thus enlarge their C sinks, it is not surprising that they do not show any acclimation.

Maximizing CO₂ uptake and photosynthesis under elevated CO₂ concentration is a reasonable strategy because the impaired net assimilation rate and the accompanying oxidative stress limit the survival of *A. tripolium* on saline habitats, and the facilitated CO₂ uptake reduces photorespiration and an overreduction of the photosystems, reducing oxidative stress (Baczek-Kwinta and Kościelniak, 2003; Oksanen *et al.*, 2005; Ainsworth and Rogers, 2007). Elevated atmospheric CO₂ concentration therefore ameliorates metabolic processes in *A. tripolium* which are typically associated with C₃ metabolism and are disadvantageous on saline habitats compared to C₄ plants (which show hardly any photorespiration even at ambient CO₂ concentration; Ainsworth and Rogers, 2007). This fact can enhance the survival of *Aster* on saline habitats under elevated CO₂.

The plants seemed to use the additional energy supply under elevated CO₂ concentration for increasing the investment in salinity tolerance mechanisms, for example, for reducing oxidative stress and water loss. The SOD activity of the salt treatments was significantly enhanced under elevated CO₂, so that ROS could be scavenged more effectively. Similar results were obtained for other species by Marabottini *et al.* (2001), Schwanz and Polle (2001), and Rao *et al.* (1995). In accordance with other studies (Tipping and Murray, 1999; Tingey *et al.*, 2003; Oksanen *et al.*, 2005), the outer epidermal cell walls and the cuticle could also be thickened because a larger number of carbon skeletons is available. Due to the thicker cell walls and cuticle, transpiration was decreased. Furthermore, a higher proportion of the incoming radiation could be reflected so that, on the one hand, an increase in internal leaf temperature (due to lower transpiration) could be prevented and, on the other hand, oxidative stress could be diminished because the amount of usable light was reduced. According to Thomas (2005), an increase in leaf reflection is a phenomenon often observed under elevated CO₂ concentration. The amount of usable light could also be reduced by the higher content of carotenoids which contribute to non-photochemical quenching (Lu *et al.*, 2003; Buchannan *et al.*, 2005; Christian, 2005).

A larger number of chloroplasts with only minor dilutions of the thylakoid membranes in the salt treatments are a concrete indication of less oxidative stress under elevated atmospheric CO₂ concentration. Oksanen *et al.* (2001) noticed similar reactions in ozone-stressed *Populus tremuloides* and *Betula papyrifera*. This fact is of major importance because the chloroplasts, as the site of photosynthesis, fulfil a key function in the adaptation to salinity, and chloroplast integrity correlates with the higher assimilation rate of *A. tripolium*. Another difference in the chloroplasts of the plants grown under elevated CO₂ compared with those under ambient CO₂ was that the number of starch grains declined along with rising salinity. This indicates that the salt-treated plants do not accumulate a starch storage under elevated CO₂ (see above), but they

metabolize a larger amount of starch because of better conditions of growth.

In summary, it can be concluded from our results that elevated CO₂ concentration enhances the energy and water supply of *Aster tripolium*, ameliorates oxidative stress, and thus enhances the survival of this plant in saline habitats. Therefore, *Aster* seems to be a promising cash crop halophyte for a future with rising atmospheric CO₂ concentrations. Its sustainable use can help in desalinizing and reclaiming degraded land and sequestering CO₂, thus counteracting the greenhouse effect. However, more research about the reaction of *A. tripolium* to other factors accompanying climate change (such as elevated temperature) and their interaction with salinity tolerance would be desirable.

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