

## Adaptive responses to high salinity of two subspecies of *Aster tripolium* on different nitrogen sources

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**ABSTRACT** The effects of NaCl salinity, different N-sources (nitrate or ammonium), as well as pH on the major enzymes of N-metabolism and total antioxidant capacity were investigated in two subspecies of *Aster tripolium* L., *A. tripolium* ssp. *tripolium*, a maritime halophyte, and *A. tripolium* ssp. *pannonicus*, endemic on the continental alkaline salty meadows. Differences in their biochemical and physiological responses to the experimental conditions are in agreement with their evolutionary adaptation either to the fluctuating coastal circumstances or to the more constant salinity level on the alkaline salty meadows. Accordingly, *A. tripolium* ssp. *tripolium* reacted more sensitively to salinity while *A. tripolium* ssp. *pannonicus* showed less physiological flexibility and more stable performance.

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### KEY WORDS

*A. tripolium* ssp. *tripolium*  
*A. tripolium* ssp. *pannonicus*  
glutamine synthetase  
nitrate reductase  
N-sources (nitrate, ammonium)  
total antioxidant capacity

Soil salinity is an important agricultural problem. One possible way to use affected fields is planting salt tolerant crops such as *Aster tripolium* L..

*Aster tripolium* (Asteraceae/Compositae) is a typical halophyte species with two horizontally isolated subspecies (Borhidi 1995). The two subspecies are very different concerning their habitats, morphology and physiology. The ssp. *tripolium* is a maritime halophyte, while ssp. *pannonicus* is common on the continental alkaline salty meadows. Morphological differences are obvious in leaf size and shape, leaf colour, leaf number and growth habitus. Both ssp. accumulate inorganic ions even at low external concentrations. Physiologically sea aster was more intensively studied (Shennan et al. 1987ab) because of its recently increasing commercial importance as halophyte crop. Its value is the mild salty taste and high protein content of the succulent leaves. The ssp. *pannonicus* inhabits salty meadows rich in NaHCO<sub>3</sub> thus having high pH.

### Materials and Methods

Two subspecies of *Aster tripolium* were examined in our experiments: ssp. *tripolium* and ssp. *pannonicus*. Plants were grown hydroponically in complete modified Hoagland nutrient solution of different pH values (from 4 to 10). In one container, 7 plants of each of the subspecies were placed and were grown for 6 weeks under controlled conditions in greenhouse at an additional light intensity of 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for 12 hours. Day/night temperature was 24/18°C. The pH values were controlled and adjusted daily by addition of HCl or NaOH. On the basis of protein content data (Bradford 1976), pH 5 and 8 were selected for further experiments when plants were grown at 0, 50, 100, 200 and 300 mM NaCl concentrations added to the complete nutrient solution. The nutrient solutions also

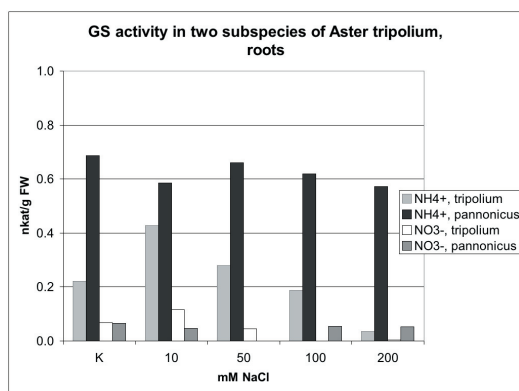
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altered in their nitrogen source which was nitrate or ammonium, respectively. Chlorophyll (Lichtenthaler 1987), protein content, cation concentrations, nitrate reductase (Mauriño et al. 1986), glutamine synthetase (Rhodes et al. 1975) activity and total antioxidant capacity with FRAP method (Benzie and Strain 1996) were measured in both leaves and roots. Cation concentrations were determined by atomic absorption spectrophotometry (Hitachi, Type Z-8200).

### Results and Discussion

*Aster tripolium* ssp. *tripolium* obviously suffered under low salt conditions combined with high pH values as shown by the low pigment concentrations. In both subspecies, qualitative and quantitative alterations were observed in protein concentrations with increasing salinity and pH values. At low pH and medium salt concentrations, ssp. *tripolium* had higher protein levels than ssp. *pannonicus*, while at high pH values ssp. *pannonicus* had higher performance. Very high Na<sup>+</sup> concentrations were accumulated in the leaves in both pH regions. Calcium is known to play a special role in tolerance under salinity. Surprisingly, in ssp. *pannonicus* Ca<sup>2+</sup> accumulation increased under the highest NaCl concentrations (300 mM) while in contrast, ssp. *tripolium* showed a decreasing tendency in calcium accumulation under increasing external salinity.

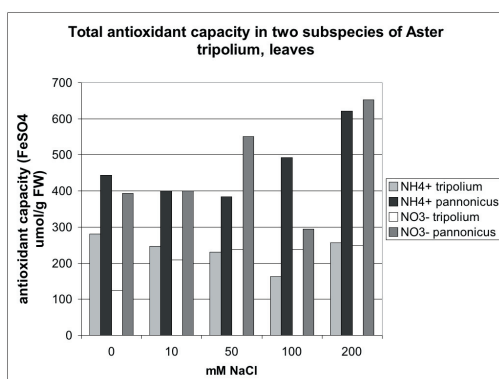
In the following pH 5 was selected and the nitrogen source was nitrate or ammonium, respectively. Our intention was to investigate enzymes involved in nitrogen metabolism as a function of sodium concentration. Nitrate reductase (NR), glutamine synthetase (GS) activity and total antioxidant capacity were measured in both leaves and roots. Control (0), 10, 50, 100 and 200 mM NaCl was added to the nutrient solutions with both nitrogen sources. One of our interesting results was that young leaves of *Aster tripolium* ssp. *tripolium*



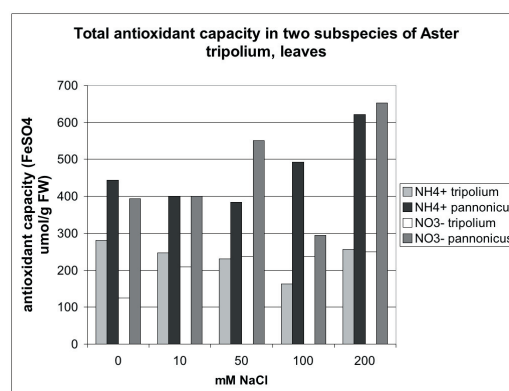
**Figure 1.** Effects of salinity and N-source in the nutrient solution on glutamine synthetase activity in roots of *Aster tripolium* ssp. tripolium and *A. t.* ssp. pannonicus.

treated with ammonium and relatively low NaCl concentrations became chlorotic, while on high salt concentrations showed the signs of ammonium toxicity. Toxicity symptoms: roots turn brown and appear unhealthy, with necrotic root tips; plant growth is decreased; necrotic lesions occur on stems and leaves. Ammonium toxicity is common in soilless, highly acidic media.

In plants, grown on solution containing ammonium, in the root of both subspecies, GS activity was higher in control plants (especially in ssp. pannonicus) than in plants grown on nitrate. In the case of ssp. tripolium GS activity had an optimum as a function of Na<sup>+</sup> concentration while in ssp. pannonicus the activity remained steadily high (Fig. 1). In the leaf, increasing GS activity was observed. When the nitrogen source is nitrate we can say that the scene of nitrate processing is not in the root: GS activity is about threefold higher in leaves than in roots, and much lower than in the roots of plants grown on ammonium. It seems that ammonium uptake is enhanced by NaCl. NR activity in the root decreased with



**Figure 3.** Effects of salinity and N-source in the nutrient solution on the total antioxidant capacity in the leaves of *Aster tripolium* ssp. tripolium and *A. t.* ssp. pannonicus.



**Figure 2.** Effects of salinity and N-source in the nutrient solution on nitrate reductase activity in the leaves of *Aster tripolium* ssp. tripolium and *A. t.* ssp. pannonicus.

increasing Na<sup>+</sup> concentrations when getting ammonium and did not change characteristically on nitrate. In leaves of plants grown on NH<sub>4</sub><sup>+</sup> the activity remained low in both subspecies but it was two-threefold higher on NO<sub>3</sub><sup>-</sup> nutrition and the activity increased with increasing sodium concentrations (Fig. 2). The total antioxidant capacity was more a question of subspecies than nitrogen source: in ssp. pannonicus it was 150-200% higher than in ssp. tripolium, and it was increasing at high exogenous sodium concentrations (Fig. 3). In the case of ssp. tripolium there were not so characteristic changes.

We conclude that *Aster tripolium* ssp. tripolium reacts more sensitively to the different conditions while ssp. pannonicus have steadier performance, which can be regarded as adaptive responses to their environmental conditions.

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