Plant Physiol. Biochem. 1993, **31** (6), 887-892

Accumulation of nitrate in bladder hairs of *Atriplex* species

Helena Freitas* and Siegmar-W. Breckle**

Department of Botany, Faculty of Sciences, University of Coimbra, Coimbra, Portugal, and Department of Ecology**, Faculty of Biology, University of Bielefeld, Bielefeld, Germany*

(Received for publication December 18, 1992; accepted June 4, 1993)

Freitas, H. and Breckle, S.-W. 1993. Accumulation of nitrate in bladder hairs of *Atriplex* species. – Plant Physiol. Biochem. 31: 887-892.

Plants of *Atriplex littoralis* L. and *Atriplex calotheca* (Rafn.). Fries were grown under sodium nitrate and sodium chloride salinity. Concentrations of Na^+, K^+, Cl^-, and NO_3^- , were investigated in leaves and bladder hairs harvested from young leaves. The content of ions in bladder hairs was correlated with concentration in young leaves. Na^+ and Cl^- concentrations were higher in bladders than in leaves. Accumulation of nitrate in tissues and bladders was observed in plants grown under nitrate salinity. This suggests that in the absence of chloride, nitrate may have an osmotic role.

Additional key words – Chenopodiaceae, salt excretion, sodium nitrate.

H. Freitas, Department of Botany, Faculty of Sciences, University of Coimbra, 3000 Coimbra, Portugal; S.-W. Breckle (reprint requests), Department of Ecology, Faculty of Biology, University of Bielefeld, PO Box 100131, D-33501 Bielefeld, Germany.

INTRODUCTION

Chenopodiaceae are known as a plant family with many special ecological adaptations, enabling them to grow on very special stands. Many halophytes, but also ruderals are known from that family. For coping with salinity not only halo-succulence, but also special bladder hairs are important.

Those salt bladders occur in some genera of the Chenopodiaceae, essentially in all *Atriplex* species (Schirmer and Breckle, 1982). These trichomes in *Atriplex* have a large and vacuolate bladder cell attached to a stalk composed of one or more cells which is based on an epidermal cell (Osmond *et al.*, 1969; Mozafar and Goodin, 1970; Thomson, 1975; Schirmer and Breckle, 1982; Reimann and, Breckle, 1988). According to Osmond *et al.*, (1980), in some *Atriplex* species more than 50% of the sodium and chloride transported to the shoot may be excreted through the stalk cell of the epidermal trichomes. The fact that more than half of the total amount of ions of the leaf are present in the bladders, which constitute about 1/3 of the fresh weight of the whole leaf, leads to the conclusion that the bladders play an important role in salt removal (Schirmer and Breckle, 1982; Jeschke and Stelter, 1983), especially in young leaves.

Besides sodium and chloride, other ions may occur in the bladder cell, for instance nitrate (Schirmer and Breckle, 1982). To test this hypothesis for nitrate, the bladder hairs of two *Atriplex* species from European salt marshes were investigated under chloride and nitrate salinity.

MATERIALS AND METHODS

Plant cultivation. Plants of *Atriplex calotheca* (Rafn.) Fries and *Atriplex littoralis* L. were grown from seeds in a growth chamber at 25°C, 55% relative humidity and 400 μmol m^{-2} s^{-1} during 14 h; 15°C, 50% relative humidity and dark during 10 h of the day. Seedlings were planted in pots filled with sand and irrigated every second day with a culture solution containing 2.8 mM K^+, 3.4 mM Ca^{2+}, 0.7 mM Mg^{2+}, 4.6 mM NH_4^+, 10.4 mM NO_3^-, 1.3 mM PO_4^{3-}, 1.4 mM SO_4^{2-}, and FeEDTA and micronutrients (Breckle, 1976). After four weeks of growth different concentrations of NaCl or $NaNO_3$ were added to the culture solution: 10 mM and 50 mM of NaCl or $NaNO_3$ respectively. The plants were treated with such solutions for two weeks.

Plant harvest. Four plants per pot were harvested as one sample in order to obtain enough bladder material. For both species four pots per treatment were available except for *A. calotheca* in nitrate treatments. Bladder hairs were removed by brushing the leaf lamina with a hair paint brush in 20 ml of distilled water (Breckle *et al.*, 1990). Brushing was repeated in another 20 ml of distilled water, then the leaves freed from bladders were collected in a moist chamber. The volume of the bladder sample was adjusted to 50 ml and then oven dried at 60°C. Finally, the bladder samples were diluted into 10 ml distilled water and filtered. Two groups of leaves were distinguished: young leaves - with intact bladders - and old leaves - without intact bladders. Bladder hairs were taken only from young leaves; old leaves were harvested intact. Plant height and leaf fresh weight were determined. The dry weight of the leaves was measured after 3 days of oven drying at 60°C. Then 20 mg of crushed material was extracted with 10 ml distilled water at 60°C overnight.

Analyses. In the filtered extracts, sodium and potassium were determined by atomic absorption, chloride amperometrically by a Marius-Micro-Chlorocounter, and nitrate photometrically with dimethylphenol (Steubing, 1965).

RESULTS AND DISCUSSION

Plant growth

In terms of height and weight only small differences of growth were observed between *A. calotheca* and *A. littoralis*, when grown in NaCl or $NaNO_3^-$ solutions. Still, plants of *A. littoralis* grew slightly better in nitrate solutions whereas plants of *A. calotheca* grew better in sodium chloride solutions. The percentage of water content in leaves of both species increased when grown in sodium chloride solutions indicating increasing succulence, whereas under sodium nitrate salinity an increment of this percentage was verified for *A. calotheca* but not for *A. littoralis* (tab. 1). Regulation of salt concentration in leaves can be partially achieved by increased succulence. According to Jennings (1968) Na^+ is the most important ion responsible for succulence. In this experiment Na^+ induction of succulence seems to be enhanced by chloride on plants grown under sodium chloride salinity. Osmond *et al.* (1980) suggested that succulence might also be a response to Cl^- in *Atriplex* species.

Ion concentrations in leaves

The content of ions both of bladder hairs and mesophyll sap are dependent on the culture

Table 1. *Percentage of water content (FW-DW FW^{-1}) and dry weight (g) in young and old leaves of* A. littoralis *and* A. calotheca. Mean (±)SE when $n=4$ and mean when $n=2$.

		A. littoralis		*A. calotheca*	
		(DW)	*(% water)*	*(DW)*	*(% water)*
Young leaves	Control	0.13±0.06	87.2±2.7	0.26	81.7
	10 mM NaCl	0.16±0.05	89.3±1.6	0.26±0.05	88.1±1.3
	50 mM NaCl	0.14±0.03	90.0±1.4	0.20±0.10	89.2±3.0
	10 mM $NaNO_3$	0.21±0.08	87.8±3.4	0.18	89.7
	50 mM $NaNO_3$	0.31±0.08	85.6±2.4	0.30	86.0
Old leaves	Control	0.28±0.06	87.1±2.0	1.00	67.6
	10 mM NaCl	0.61±0.24	90.0±1.4	1.34±0.2	87.2±1.3
	50 mM NaCl	0.80±0.13	89.5±2.8	1.40±0.7	87.6±0.6
	10 mM $NaNO_3$	0.90±0.30	88.0±3.4	1.70	84.8
	50 mM $NaNO_3$	1.31±0.20	85.6±2.0	2.00	84.7

Table 2. *Concentration of Na^+, K^+, Cl^- and NO_3^- (µmol ml^{-1} water content) in young and old leaves of* A. littoralis *under 10 mM and 50 mM of nitrate and chloride salinity.* Mean (±) SE when $n=4$ and mean when $n=2$

		Na^+	K^+	Cl^-	NO_3^-
Young leaves	Control	7.0±2.0	20.0±9.0	4.0±1.0	2.0±1.0
	10 mM NaCl	30.0±10.0	9.0±4.0	10.0±5.0	
	50 mM NaCl	42.0±20.0	8.0±3.0	30.0±5.0	
	10 mM $NaNO_3$	23.0±4.0	9.0±4.0		1.0±0.5
	50 mM $NaNO_3$	30.0±6.0	6.0±2.0		2.0±0.4
Old leaves	Control	4.0±0.6	20.0±8.0	2.0±0.3	1.0±0.4
	10 mM NaCl	10.0±6.0	3.0±3.0	4.0±0.9	
	50 mM NaCl	10.0±3.0	1.0±0.3	7.0±1.0	
	10 mM $NaNO_3$	8.0±4.0	3.0±2.0		0.5±0.2
	50 mM $NaNO_3$	9.0±2.0	1.0±0.4		0.7±0.4

solution. Sodium and chloride concentrations in leaves of both species increased with an increment in salt external concentration (*tab.* 2 and 3). Nitrate increased only in *A. calotheca*. Potassium does not decrease in proportion to the increase in Na^+ content. *Atriplex* species are able to maintain sufficient K^+ uptake and transport to the shoot in the presence of higher Na^+ concentrations (Jeschke and Stelter, 1983).

The content of Na^+ increased similarly in all plants with increased external concentrations of sodium nitrate and sodium chloride in the culture solution(*tab.* 2 and 3). So, as ion uptake has to maintain the charge balance in the high concentration range (Glass, 1989), the uptake of nitrate and chloride must be similar. However, nitrate is metabolized and thus its concentration in leaves is lower than chloride's. According to Osmond *et al.* (1980), the metabolic incorporation of nitrate in leaves of *Atriplex* sp. is associated with substantial carboxylate synthesis-oxalate is the principal acid involved. As the reduction of NO_3^- is correlated with the synthesis of organic acids the uptake of Na^+ maintains the osmotic balance (Raven and Smith, 1976; Smirnoff and Stewart, 1985). Despite substantial nitrate reduction, considerable amounts of this ion are accumulated in leaves of *A. calotheca* where it may have an osmotic role (*tab.* 3). In young leaves of *A. calotheca* the ratio Na^+/NO_3^- is 3.5 in the control, 3.3 in 10 mM $NaNO_3$ and 6.0 in 50 mM $NaNO_3$. So, the accumulation of sodium in leaves is nearly balanced by nitrate. In young leaves of *A. littoralis* the same ratio Na^+/NO_3^- is 3.5 in the control, 23.0 in 10 mM $NaNO_3^-$ and 15.0 in 50 mM $NaNO_3^-$, thus sodium accumulation must be balanced by organic anions. Nitrate accumulation is common in Chenopodiaceae (Smirnoff and Stewart, 1985). According to these authors nitrate is an important osmoticum in some halophytes, particularly Chenopodiaceae, grown in the absence of sodium chloride. Under such circumstances nitrate is accumulated instead of chloride. The energy expense of nitrate transport and accumulation in the plant is much lower than its reduction (Salsac *et al.*, 1987). Furthermore, the accumulation of nitrate may constitute a reserve of nitrate when the uptake rate exceeds its reduction and transport. This stock can be very important especially in unstable habitats such as the salt marsh, where *Atriplex* species are very common and fluctuations of nutrients in the soil solution are frequent (Jefferies and Perkins, 1977; Stewart *et al.*, 1979). Compared with nitrate, chloride concentrations in leaves are much higher (*tab.* 2 and 3). The accumulation of chloride and sodium in leaves of both species was very similar, with the ratio Na^+/Cl^- varying among 1.0 in the control and 2.0 in sodium chloride salinity. Chloride accumulation on *Atriplex* plants is well known (Osmond *et al.*, 1980; Albert, 1982).

Ion concentrations in bladders

Bladder hairs in all cases exhibit higher ion concentrations than the leaf mesophyll cells. This is principally apparent for Na^+ and Cl^- (*fig.* 1 and 2).

The percentages of chloride inside bladder hairs of both species and nitrate in bladders of *A. littoralis* (*fig.* 3), which represents the contribution of bladders to total ion content of

Table 3. *Concentration of Na^+, K^+, Cl^- and NO_3^- (μmol ml^{-1} water content) in young and old leaves of* A. calotheca *under 10 mM and 50 mM of nitrate and chloride salinity.* Mean ± SE when $n=4$ and mean when $n=2$.

		Na^+	K^+	Cl^-	NO_3^-
Young leaves	Control	7.0	10.0	4.0	2.0
	10 mM NaCl	25.0±10.0	7.0±3.0	20.0±10.0	
	50 mM NaCl	33.0±10.0	8.0±4.0	22.0±3.0	
	10 mM $NaNO_3$	20.0	6.0		6.0
	50 mM $NaNO_3$	30.0	6.0		5.0
Old leaves	Control	2.0	4.0	2.0	0.5
	10 mM NaCl	6.0±4.0	0.9±0.2	4.0±1.0	
	50 mM NaCl	12.0±1.0	2.0±2.0	6.0±2.0	
	10 mM $NaNO_3$	5.0	0.9		0.4
	50 mM $NaNO_3$	6.0	0.7		0.8

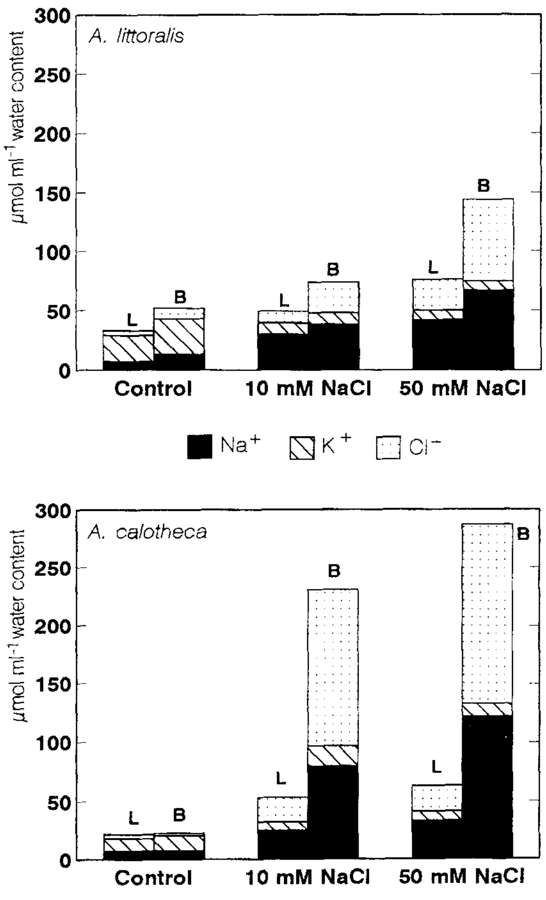

Figure 1. *Na^+, K^+ and Cl^- concentrations (mean of 4 samples) in young leaves* (**L**) *and bladder hairs* (**B**) *of* A. calotheca *and* A. littoralis *under 10 mM and 50 mM of sodium chloride salinity.*

leaves, suggest a transport of these ions from leaves to the bladders. For chloride treatments similar results were presented by Osmond *et al.* (1969), Schirmer and Breckle (1982), Jeschke and Stelter (1983) and Reimann and Breckle (1988). Nitrate concentrations in bladders have previously been studied in *Halimione portulacoides* by Baumeister and Kloos (1974), who found insignificant excretion of NO_3^-. In our experiment, plants of *A. littoralis* grown under nitrate salinity exhibited substantial excretion of nitrate into bladders (*fig.* 2). Plants of *A. calotheca* submitted to the same salinity showed insignificant excretion of nitrate. The percentage of nitrate in bladders of *A. littoralis* is about 70% in the control and higher than 90% in both nitrate treatments (*fig.* 3). This high excretion of nitrate may be important tc prevent toxic levels in the leaves. In bladders of *A. calotheca* the percentage of nitrate is about 55% in the control, 60% in 10 mM $NaNO_3$ and 40% in 50 mM $NaNO_3$. Nitrate is accumulated in leaves of *A. calotheca*; it has an osmotic role and it may be used as a nutrient resource.

In the sodium chloride salinity the ratio $Na^+ + K^+/Cl^- + NO_3^-$ inside leaves and bladder hairs was around 1. Excess uptake of cations over anions was observed in plants grown under nitrate salinity. This caution excess is usually balanced by oxalates (Osmond, 1967; Mozafar and Goodin, 1970). The cation-anion ratio indicates that in *A. calotheca* the accumulation of anions inside bladders is more effective for chloride, whereas in *A. littoralis* there is also a significant excretion of nitrate into bladder hairs. This different response could be due to a different production of organic anions by the two

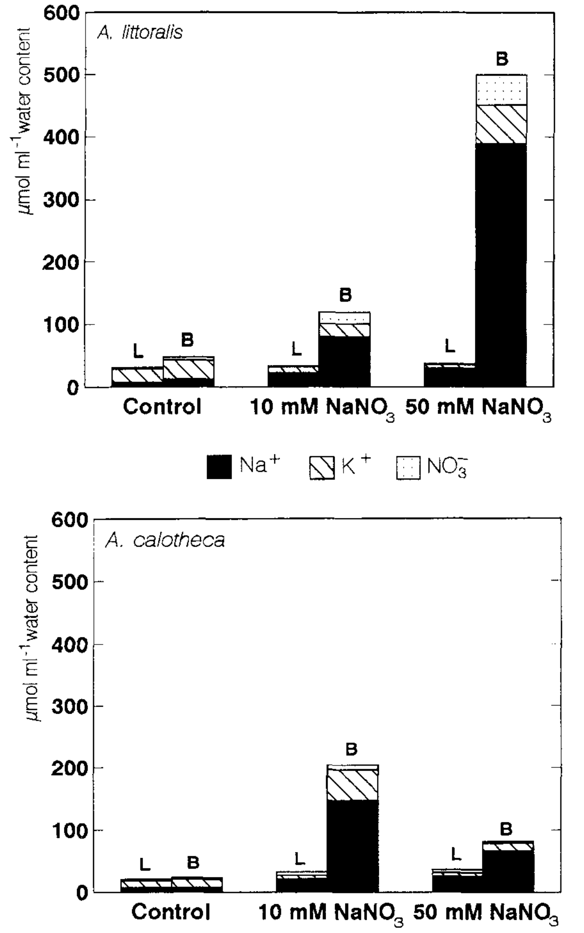

Figure 2. *Na^+, K^+, and NO_3^- (mean of 4 samples) in young leaves (***L***) and bladder hairs (***B***) of* A. calotheca and *A. littoralis* under 10 and 50 mM of sodium nitrate salinity.

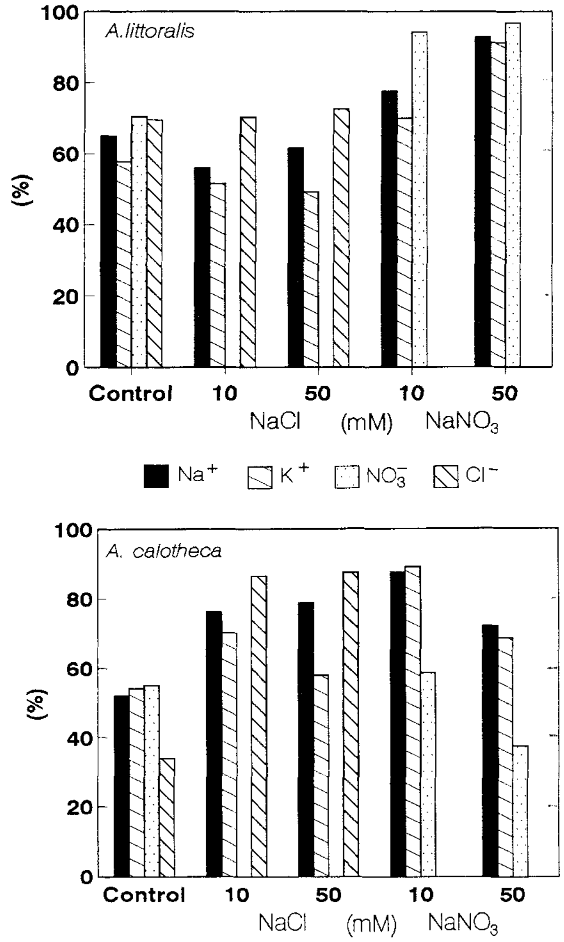

Figure 3. *Contribution of bladders to the total ion content (Na^+, K^+, Cl^- and NO_3^-) of leaves of* A. calotheca *and* A. littoralis.

species. In leaves of *A. littoralis* it is probable a higher production of organic anions because of nitrate reduction. This synthesis is followed by an increment of sodium uptake. The excretion of nitrate into bladders is possibly associated with the excretion of sodium, thus contributing to the osmotic balance of *A. littoralis* bladder hairs.

The ability of *Atriplex* species to regulate the transport and tolerance of high quantities of ions is a whole-plant phenomenon and bladder hairs are an important component of it.

CONCLUSION

Bladder hairs are very important in the ionic regulation of *Atriplex* leaves (Schirmer and Breckle, 1982; Jeschke and Stelter, 1983; Freitas and Breckle, 1992). The results presented here show sodium and chloride excretion into bladders of *A. calotheca* and *A. littoralis* grown under sodium chloride salinity. In plants of *A. littoralis* grown under nitrate salinity a high uptake of sodium was balanced by sodium and nitrate excretion into bladders. This excretion of nitrate contributes to the osmotic balance of *A. littoralis* bladders. Moreover, it may prevent toxic levels of nitrate in leaves.

Nitrate accumulation in leaves of *A. calotheca* grown under nitrate salinity was important to balance sodium uptake. Furthermore, this accumulation of nitrate may constitute a nutrient stock in leaves of *A. calotheca*. The accumulation of sodium in leaves of *A. littoralis* grown under nitrate salinity is balanced by organic anions.

Acknowledgements. This work has been supported by a travel grant from the Deutscher Akademischer Austauschdienst to H. Freitas. We acknowledge the comments made by Dr Jürgen Hagemeyer and Christine Reimann.

REFERENCES

Albert R., 1982. – Halophyten. In *Pflanzenökologie und Mineralstoffewechsel*, Kinzel H., ed., Verlag Eugen Ulmer, Stuttgart, 33-204.

Baumeister V. W. and Kloos G., 1974. – Über die Salzsekretion bei *Halimione portulacoides* (L.) Aellen. *Flora*, **163**, 310-326.

Breckle S.-W., 1976. – Zur Ökologie und zu den Mineralstoffverhältnissen absalzender und nichtabsalzender Xerohalophyten. *Diss. Bot.* **35**. Vaduz.

Breckle S.-W., Freitas H. and Reimann C., 1990 – Sampling *Atriplex* bladders : a comparison of methods. *Plant Cell Env.* **13**, 871-873.

Freitas H. and Breckle S.-W., 1992 – Importance of bladder hairs for salt tolerance of field-grown *Atriplex* species from a Portuguese salt marsh. *Flora*, **187**, 283-297.

Glass A. D. M., 1989. – *Plant nutrition*. Jones and Bartlett, Boston.

Jefferies R. L. and Perkins N., 1977. – The effects on the vegetation of the additions of inorganic nutrients to salt marsh soils at Stiffkey, Norfolk. *J. Ecol.*, **65**, 867-882.

Jennings D. H., 1968. – Halophytes, succulence and sodium in plants – a unified theory. *New Phytol.*, **67**, 899-911.

Jeschke W. D. and Stelter W., 1983. – Ionic relations of garden orache, *Atriplex hortensis* L.: growth and ion distribution at moderate salinity and the function of bladder hairs. *J. Exp. Bot.*, **34**, 795-810.

Mozafar A. and Goodin J. R., 1970. – Vesiculated Hairs: A mechanism for salt tolerance in *Atriplex halimus* L. *Plant Physiol.*, **45**, 62-65.

Osmond C. B., 1967. – Acid metabolism in *Atriplex*. *Aust. J. Biol. Sci.*, **20**, 575-587.

Osmond C. B., Lüttge U., West K. R., Pallaghy C. K. and Shacher-Hill B., 1969 – Ion absorption in *Atriplex* leaf tissue II: secretion of ions to epidermal bladders. *Aust. J. Biol. Sci.*, **22**, 797-814.

Osmond C. B., Björkman D. and Anderson D. J., 1980. – *Physiological processes in plant ecology. Towards a synthesis with Atriplex*. Ecol. Studies. **36**, Springer-Verlag, Berlin.

Raven J. A. and Smith F. A., 1976. – Nitrogen assimilation and transport in vascular land plants in relation to intracellular pH regulation. *New Phytol.*, **76**, 415-431.

Reimann C. and Breckle S.-W., 1988. – Salt secretion in some *Chenopodium* species. *Flora*, **180**, 289-296.

Salsac L., Chaillou S., Morot-Gaudry J. F., Lesaint C. and Jolivet E., 1987. – Nitrate and ammonium nutrition in plants. *Plant Physiol. Biochem.*, **25**, 805-812.

Schirmer U. and Breckle S.-W., 1982. – The role of bladders for salt removal in some *Chenopodiaceae* (mainly *Atriplex* species). In *Contributions to the ecology of halophytes*, Sen D. N. and Rajpurohit K. S., ed., DR Junk Publishers, The Hague, 215-231.

Smirnoff N. and Stewart G. R., 1985. – Nitrate assimilation and translocation by higher plants: Comparative physiology and ecological consequences. *Physiol. Plant.*, **64**, 133-140.

Steubing, 1965. – *Pflanzenökologisches Praktikum. Methoden und Geräte zum Bestimmung wichtiger Standortsfaktoren*, Verlag Paul Parey, Berlin.

Stewart G. R., Larher F., Ahmad I. and Lee J. A., 1979. – Nitrogen metabolism and salt tolerance in higher plant halophytes. In *Ecological processes in coastal environments*, Jefferies R. L. and Davy A. J., ed., Blackwell Scientific Publications, Oxford.

Thomson W. W., 1975. – The structure and function of salt glands. In *Plants in saline environments*, Poljakoff-Mayber A. and Gale I., ed., Berlin, 118-146.