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Molybdenum as an essential element for improving total yield in seawater-grown *Salicornia europaea* L.

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ABSTRACT

The growth of crop plants on full-strength seawater constitutes a major challenge because of the high salt content and the limited availability of essential microelements such as molybdenum. For cultivation of the halophyte *Salicornia* as seawater-grown crop, the effect of molybdate application on total yield production and the activities of the two molybdenum containing enzymes, nitrate reductase (NR) and xanthine dehydrogenase (XDH) was investigated. Increasing molybdate levels in the growth medium supplemented with nitrate or ammonium enhanced yield during multiple shoot removal. Similarly, NR and XDH activities were enhanced with increasing molybdate, indicating that the activity of both enzymes may play an important role in facilitating yield accumulation. Notably, XDH activity in the roots was high and the levels of ureides were low, whereas in the shoot tips ureides were higher and XDH activity was lower. Considering that XDH is a key enzyme in the biosynthesis of the low C/N ratio ureides, these suggest a source–sink relationship between the roots and shoot tips for efficient transport of root-generated ureides to the young growing shoot tips. Our results imply that the supply of molybdenum to *Salicornia* grown in seawater enhances plant biomass accumulation by increasing the activities of NR and XDH, thereby stimulating a more efficient remobilization of ureides to the newly grown shoot tips after periodic shoot removal.

1. Introduction

Species of the halophyte genus *Salicornia* (Chenopodiaceae) that thrive naturally along the coastal salt marshes from the Arctic to the Mediterranean are recognized as being among the most salt tolerant of the terrestrial plants (Davy et al., 2001). During the past few decades, a number of studies have evaluated the potential of *Salicornia* species as oil seed crops irrigated by using seawater, especially in warm coastal desert climates (Glenn et al., 1998; Eganathan et al., 2006). Up to date, *Salicornia bigelovii* has been cultivated successfully near the desert coast lines of Mexico and Eritrea (Lu et al., 2001). More recently, *S. bigelovii* was introduced as a specialty crop for the fresh vegetable market in Europe and the US and a breeding program was initiated for productivity improvement (Zerai et al., 2010). The marketable value of the leafless shoots coupled with the possibility using seawater to irrigate this species suggests that with suitable agromanagement this plant could be developed into a lucrative commercial crop (Glenn et al., 1999).

The two inorganic N-forms readily available for uptake by plants, NO_3^- and NH_4^+, switch on N-assimilation specific pathways to synthesize fundamental cell components such as amino acids, nucleic acids, proteins and photosynthetic pigments (Pilbeam and Kirkby, 1992). The key and rate-limiting enzyme for NO_3^- assimilation is nitrate reductase (NR, EC 1.6.6.1). This enzyme catalyzes the first step in the NO_3^- reduction pathway to yield NO_2^-, which is then further reduced to NH_4^+. The latter ion is incorporated into organic N-compounds by the activity of the enzymes glutamine synthetase and glutamate synthase (the GS/GOGAT pathway). The resulting amino acids, glutamine and glutamate then serve as substrates to produce additional amino acids (Tischner, 2000). While NO_3^- transport and assimilation occur practically throughout the entire plant, NH_4^+ is taken up directly into the roots and the resulting assimilation products are transported to the growing plant parts (Lips et al., 1990; Tischner, 2000). Generally, plants may transport N in the form of amides (glutamine and asparagine) and/or ureides (allantoin and allantoate). Ureides are more rich in N (4N:4C) in comparison to amides and amino acids and, therefore, their involvement in the transport of assimilated ammonia from the root to the shoot minimizes loss of carbon originating from photosynthesis (Zrenner et al., 2006). Particularly in high saline environments, where photosynthates biosynthesis is reduced, the type of transportable N-compounds may be critical to guarantee

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the nitrogen and carbon economy of the plants (Sagi et al., 1998; Brychkova et al., 2008a).

Xanthine dehydrogenase (XDH, EC 1.2.1.37) is an important enzyme in purine catabolism where it catalyzes the oxidative hydroxylation of hypoxanthine to xanthine and xanthine to uric acid that ultimately yields the purine degradation products, ureides (allantoin and allantoate) (Brychkova et al., 2008a,b). Recently, *AtXDH1* transcript was shown to be induced by drought, salinity and abscisic acid treatments (Hesberg et al., 2004; Yesbergenova et al., 2005), indicating a role for XDH in purine catabolism in plants subjected to environmental stresses.

Extensive studies with nodulated legumes describe the ureides allantoin and allantoic acid as important N-storage and transport compounds (Zrenner et al., 2006; Smith and Atkins, 2002), while the metabolic role of ureides for N-assimilation in non-legumes is less well-known (Mazzafera et al., 2008). Nevertheless, it was recently demonstrated that ureides accumulate in non-legumes plants exposed to stress conditions such as salinity, high NH_4^+ concentrations, dark-induced senescence and normal senescence (Sagi et al., 1998; Brychkova et al., 2008a).

Halophyte species require considerable amounts of electrolytes, typically Na^+ and Cl^-, for optimal growth and development. However, seawater irrigation induces severe salt stress in numerous halophyte species (Glenn et al., 1999). In response to this stress, changes occur at physiological, biochemical and molecular levels, and their effects are expressed at both the cellular and whole plant levels. Thus, salt tolerance will be achieved by a comprehensive mechanism of functional adaptation that results in enhanced plant growth and development (Hasegawa et al., 2000).

Several research studies have highlighted the roles of both enzymes, NR and XDH in the adaptation of various plant species to abiotic stresses, such as those caused by salinity, NH_4^+ toxicity, drought, dark senescence and oxidative stress (Omarov et al., 1998; Sagi et al., 1998; Yesbergenova et al., 2005; Brychkova et al., 2008a). However, only limited information describes the role of NR and XDH in halophyte plants, especially when irrigated with seawater. Both, NR and XDH belong to a small group of four molybdo-enzymes identified in plants. All of these enzymes are homodimeric proteins that require the insertion of a molybdenum cofactor (MoCo) into the apoprotein for proper functioning. The MoCo itself consists of molybdenum (Mo) covalently bound via two dithiolate sulfurs to a molybdopterin unit. NR needs only a di-oxo cofactor, while XDH, which belongs to the mono-oxohydrolases, also requires a third sulfur ligand (Kaiser et al., 2005; Schwarz and Mendel, 2006).

The concentrations of the trace element Mo in seawater is generally below 10 $\mu g\,L^{-1}$ (Kulathilake and Chatt, 1980). Mo availability to the plant is not simply a function of the elements concentration, but may be strongly impaired by the concurrent appearance of high sulfate concentrations (Albasel and Pratt, 1989; Howarth and Cole, 1985). Thus, the high content of sulfate in seawater may result in reduced Mo availability to the plant and limited MoCo biosynthesis available for NR and XDH activities (Howarth et al., 1988; Sagi et al., 1997, 1998) when plants are grown on seawater. Supplementing Mo to seawater-grown salt-loving plants such as the halophyte *Salicornia* may therefore be a key to enhance their N-metabolism and subsequently increase plant growth and biomass productivity. This study investigated the effect of Mo enrichment (as molybdate) on the yield production of *Salicornia* cultivated with seawater.

2. Materials and methods

2.1. Plant material and experimental set-up

Seeds of a *Salicornia europeae* L. ecotype collected in the Dead Sea area of Israel were used in these experiments, which were carried out in a temperature-controlled greenhouse; summer temperatures were kept below 30 °C, while winter temperatures were over 20 °C. The photoperiod was fixed to 15 h day-length by using 100 W standard light bulbs to prevent flowering under short-day light conditions. Plants were grown in 20-L baskets filled with perlite (Agrekal Habonim Industries Ltd., Moshav Habonim, Israel; www.agrekal.co.il) placed in aerated hydroponic units filled with equal amounts of seawater-nutrient solution.

Table 1
Composition of the nutrient solutions with either NH_4^+ or NO_3^- as the sole N-source.

Chemical formula	NH_4^+-nutrient solution (mM in the final solution)	NO_3^--nutrient solution (mM in the final solution)
$CaCl_2$	1.25	1.25
KNO_3	–	5.0
K_2SO_4	2.5	–
$(NH_4)_2SO_4$	5.0	–
$MgSO_4$	1.0	1.0
KH_2PO_4	0.5	0.5
H_3BO_3	23.5	23.5
$MnCl_2$	4.55	4.55
$ZnCl_2$	0.44	0.44
$CuCl_2$	0.18	0.18
Fe-sequestren	25 $mg\,L^{-1}$	25 $mg\,L^{-1}$

The seawater-nutrient solution was prepared by dissolving 33 $g\,L^{-1}$ Red Sea Salt® (Red Sea Fish Pharm Ltd., Eilat, Israel; www.redseafish.com) according to the manufacturer's instructions, supplemented with a Hoagland nutrient solution modified to contain either 5 mM nitrate or 5 mM ammonium as the sole N-source, without the addition of molybdenum (Hoagland and Arnon, 1938, Table 1). The concentration of Mo in the Red Seawater was 9.2 μg/L as detected by ICP-MS model Elan DRC-e production Sciex (Perkin-Elmer) against SRM ocean water NASS-5 from NRC Ottawa (Certificate Mo value 9.6 μg/L).

To prevent nitrification of NH_4^+, 7.5 ppm dicyandiamide (Sigma Chemicals (D-8275), St. Louis, MO, USA) was added to the nutrient solution, thus resulting in NO_3^- levels below the detectable level when determined according to Cataldo et al. (1975). Molybdenum treatments were given as sodium molybdate ($Na_2MoO_4{\cdot}2H_2O$) dissolved in the seawater-nutrient solution at concentrations of 0, 3 or 6 μM or applied as a weekly foliar spray of 3 μM.

The solutions were renewed every 2 weeks, and every other week an equal amount of solution was added to maintain the initial volume. Each plot comprised three replications within the molybdate treatment and N-source. Seeds of *Salicornia* were sown directly on perlite irrigated with 50% seawater. After emergence, the seedlings were gradually exposed over a month to increase salinity up to full-strength seawater. A uniform plant density of 1000 seedlings per m^2 was established.

Starting at 12 weeks after sowing, when plants were at a height of ca 15 cm, plants were harvested by cutting the young shoots ca 7 cm above the surface. A multiple-harvest system was applied by cropping the plants every 4 weeks after plant re-growth, resulting in a total of 5 harvests. After each harvest the crop was weighed and yields were calculated as fresh biomass accumulation per m^2.

2.2. In vivo NR activity

Fresh shoot samples were collected always after 11:00 a.m. when the NR activity was at its highest (Supplementary Fig. A). The collected shoot tips (up to 2 cm) and roots were cut 2–5 mm long and vacuum infiltrated in a reaction mixture containing 0.1 M KNO_3^- and 0.1% isopropanol in 50 mM potassium phosphate buffer (pH 7.5), in 1:20 (w:v) ratio. NR activity in NH_4^+-fed plants was detected also in the absence of 0.1 M KNO_3^- in the reaction mixture. The reaction was allowed to proceed for 30 min at 30 °C in