The Mo-hydroxylases xanthine dehydrogenase and aldehyde oxidase in ryegrass as affected by nitrogen and salinity

Moshe Sagi *, Rustem T. Omarov, S. Herman Lips

Biostress Research Laboratory, The Jacob Blaustein Institute for Desert Research and Department of Life Sciences, Ben-Gurion University of the Negev, Sede Boqer 84990, Israel

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Abstract

The influence of salinity and nitrogen source on xanthine dehydrogenase (XDH; EC 1.2.1.37) and aldehyde oxidase (AO; EC 1.2.3.1) was studied in annual rye grass (Lolium multiflorum cv. Westerwoldicum). The activities of AO and XDH in the roots and shoots of rye grass plants increased with salinity and NH₄⁺ concentration. The salinity-enhanced activities of XDH and AO were more pronounced in the roots than in the shoots. Roots of NH₄⁺-grown plants had higher AO and XDH activities than plants grown in NO₃⁻. Immunoblotting revealed a higher level of AO protein in roots than in shoots. Root AO protein increased with salinity and was the highest in roots of NH₄⁺-grown plants. The assays of the molybdenum cofactor (MoCo) hydroxylases (XDH and AO) showed a similar response to salinity and nitrogen, and differed in molecular weight and substrate specificity. The concentration of ureides (allantoic acid and allantoin) increased with salinity and NH₄⁺, especially in the roots. The ureide contents of plants grown on NH₄⁺ were higher than in plants receiving NO₃⁻. The increase in Mo-hydroxylases with salinity and NH₄⁺ may constitute part of the mechanisms of plant adaptation to stress by (1) enhancing the activity of AO, which catalyzes the final step in biosynthesis of phytohormones such as abscisic acid (ABA), and (2) increased XDH activity and the subsequent production of ureides allowing transport of organic nitrogen compounds with a low C/N ratio.

Keywords: Aldehyde oxidase; Ammonium; Rye grass; Salinity; Ure ide; Xanthine dehydrogenase

1. Introduction

Molybdenum cofactor (MoCo) is a component of molybdoenzymes with the exception of nitrogenase [1,2], as indicated by the pleiotropism of MoCo mutations, which affected the expression
of nitrate reductase (NR; EC 1.6.6.1), xanthine dehydrogenase (XDH; EC 1.2.1.37) and aldehyde oxidase (AO; EC 1.2.3.1) [2,3]. AO and XDH, contrary to NR, require an additional sulfur ligand as part of the catalytically active Mo-center [4]. AO activities are widely distributed among eukaryotes and bacteria. However, little is known about its regulation and activities in higher plants, especially in response to environmental factors. Plant AO enzymes have a relatively broad substrate specificity, showing affinity for indole-3-aldehyde, indole-3-acetaldehyde and benzaldehyde [5]. AO may have an important role in plant development and adaptation to environmental stresses since members of the AO multigene family [6] catalyze the final step in the biosynthesis of phytohormones. In plants, AO oxidizes abscisic aldehyde to abscisic acid (ABA) [2,7] and indole-3-acetaldehyde to indole-3-acetic acid (IAA) [5,8]. AO can also oxidize indole-3-aldehyde to indole-3-carboxylic acid in the peroxidative decarboxylation pathway of IAA [9,10], causing an irreversible loss of IAA [8]. Flacca and sitiens, wilting mutants of tomato (Lycopersicum esculentum), with reduced capacity to produce ABA, have been shown to lack the ability to oxidize abscisic aldehyde [11]. Recently it has been reported that the flacca mutant lacks AO and XDH but not NR [12], discarding the possibility of a mutation in genes regulating the production of MoCo in this mutant. The aba3 mutant of Arabidopsis (Arabidopsis thaliana) seems to lack the ability to introduce the additional inorganic sulfur into AO and XDH [13] required for their activity. ABA is involved in the response of plants to environmental stresses such as freezing, cold and salinity [14]. The possible involvement of IAA in the response of plants to stresses such as water deficit and salinity has been suggested recently [15].

Xanthine oxidoreductase has been characterized in higher plants as a dehydrogenase [16] which is NAD⁺ dependent and catalyzes the first oxidative step in purine catabolism. This enzyme is necessary for ureide biosynthesis in higher plants [16] which may take place through de novo synthesis of purines from glutamine [17].

AO and XDH have several common characteristics. Both enzymes are mono-oxohydroxylases [13,18], whereas NR requires a di-oxomolybdenum center [18]. XDH and AO of various organisms have a high degree of homology in their amino acid sequence and contain binding sites for two iron–sulfur centers and a MoCo-binding region [6,19].

AO activity increased with salinity in roots of barley [20]. Increased salinity and nitrogen concentration in the medium resulted in enhanced NR activity in vitro as well as in MoCo content of ryegrass, although the MoCo increase was more pronounced than that of NR, especially in the roots [21,22]. Nitrate and ammonium may affect MoCo allocation to different Mo-enzymes [22] and constitute key factors in the adaptation of the growth rate of plants to environmental changes which trigger the changes of hormonal balance in the xylem sap in which cytokinin decrease and ABA increases [23,24] under stress. Considering that MoCo is an essential component not only of NR but of other Mo-enzymes as well, the present study was carried out to follow the effect of salinity and nitrogen ions on XDH and AO in roots and shoots of annual ryegrass.

2. Materials and methods

2.1. Plant material

Ryegrass seeds (Lolium multiflorum var. Westerwoldicum) were germinated and grown as previously described [21]. Experiments were conducted with (1) nutrient solutions containing 0.5, 4.5, or 9.0 mM NH₄⁺ as (NH₄)₂SO₄, and (2) nutrient solution containing two levels of salinity and 4.5 mM NH₄NO₃, NaNO₃ or NH₄⁺ as (NH₄)₂SO₄. Salinity consisted of a mixture of NaCl and CaCl₂·2H₂O (4.5:1, w/w) in half-strength Hoagland nutrient solution [25] to yield an electrical conductivity (EC) of 11.2 dS m⁻¹. Half-strength Hoagland nutrient solution (EC = 2 dS m⁻¹) served as a control. The pH of the nutrient solutions was adjusted to 6.0–6.5 with H₂SO₄. Nitrapyrin (N-serve 24E; Dow Chemical, King’s Lynn, UK) 4 µl l⁻¹ and 7.5 mg l⁻¹ dicyandi-
amide (Sigma, St Louis, MO) were added to the nutrient solutions to prevent nitrification. The plants were irrigated twice daily with identical volumes of nutrient solution to prevent an increase in EC of more than 10% in the leachate as compared with the input solution.

Plants were harvested 14 days after the beginning of the treatments at which time plants parts were weighed and samples taken for enzymes assays. The rest of the fresh plant material was oven-dried at 65°C for 72 h to determine dry mass.

2.2. Tissue extraction

Shoot and root samples were extracted immediately after harvesting. Crude extracts used for the assays of XDH and AO in native gel electrophoresis (PAGE) and AO Western blot analysis were prepared by tissue maceration with acid-washed sand in ice-cold extraction medium containing 250 mM Tris–HCl (pH 8.5), 1 mM EDTA, 1 mM dithiothreitol (DTT), 5 mM cysteine, 5 mM Na₂MoO₄, 10 mM antipain, 0.1 mM phenylmethylsulfonyl fluoride, 10 mM reduced glutathione and 0.03 mM flavine adenine dinucleotide. Samples of 1 g fresh weight of shoot or root tissue were extracted in 3 ml and 1 ml of buffer (1:3 or 1:1, w/v), respectively. The homogenized plant material was centrifuged at 30,000 × g in a Sorvall RC-5 refrigerated centrifuge at 3–5°C for 15 min. The resulting supernatant was used for subsequent assays.
Table 1
XDH activity in polyacrylamide gel or in vitro and aldehyde oxidase activity in ryegrass shoots as affected by N source and salinity in the nutrient solution

<table>
<thead>
<tr>
<th>N source</th>
<th>Salinity (dS m(^{-1}))</th>
<th>XDH activity</th>
<th>AO activity (density as % of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>In gel (10(^{-3}) units mg(^{-1}) h(^{-1}))</td>
<td>In vitro (nmol NADH mg(^{-1}) min(^{-1}))</td>
</tr>
<tr>
<td>NO(_3^−)</td>
<td>2.0</td>
<td>0.045 c</td>
<td>5.1 a</td>
</tr>
<tr>
<td></td>
<td>11.2</td>
<td>0.075 a</td>
<td>5.0 a</td>
</tr>
<tr>
<td>NH(_4)NO(_3)</td>
<td>2.0</td>
<td>0.052 c</td>
<td>1.7 d</td>
</tr>
<tr>
<td></td>
<td>11.2</td>
<td>0.052 c</td>
<td>2.5 bc</td>
</tr>
<tr>
<td>NH(_4^+)</td>
<td>2.0</td>
<td>0.048 c</td>
<td>2.0 e</td>
</tr>
<tr>
<td></td>
<td>11.2</td>
<td>0.065 b</td>
<td>3.0 b</td>
</tr>
</tbody>
</table>

XDH was assayed with hypoxanthine, and its standard curve was calculated from the band density obtained with commercial xanthine oxidoreductase in the same gel and assay. AO was assayed with heptaldehyde.

Different letters following the mean values in each column indicate significant differences (P<0.05, Duncan test, n=8 and n=6 different experiments of XDH and AO, respectively).

Crude extracts used for assays of XDH and AO in vitro were prepared according to Triplett et al. [26]. Samples of 1 g fresh weight tissue were ground in liquid nitrogen and the resulting powder was mixed with 0.25 g polyvinylpolypyrrolidone and then extracted in 1 ml of 50 mM potassium phosphate buffer (pH 7.8) containing 1 mM DTT. The homogenized plant material was centrifuged as described above. The resulting supernatant was brought to 60% saturation with solid ammonium sulfate. After stirring for 30 min, the mixture was centrifuged at 40,000 \(\times g\) for 20 min. The pellet was redissolved in 1–2 ml of 50 mM potassium phosphate buffer (pH 7.8) and desalted on a 1 \(\times\) 15 cm Sephadex G-25 (Pharmacia) column equilibrated with 50 mM potassium phosphate buffer (pH 7.8). All steps were carried out at 4°C.

2.3. Xanthine dehydrogenase assay

XDH activity was detected in gels after electrophoresis using hypoxanthine as a substrate [27]. XDH activity was also assayed in vitro according to Triplett et al. [26] by following spectrophotometrically NADH production at 340 nm. The assay included 1 mM hypoxanthine, 2.5 mM NAD\(^+\), 1 mM DTT and 100–200 ml of desalted crude extract containing 300–500 \(\mu\)g mg\(^{-1}\) protein in 1.5 ml of 50 mM potassium phosphate buffer (pH 7.8) all at 25°C.

2.4. Aldehyde oxidase assay

AO activity was assayed monitoring the decrease of absorbance of 2,6-dichloroindophenol (DCIP) as an electron donor at 600 nm [28] in a Genesis-2 (Milton Roy, USA) spectrophotometer. The reaction mixture of 1.5 ml consisted of 100–200 \(\mu\)g protein of the desalted extract, 0.002% DCIP, 0.1 mM phenazine methosulfate, and 2 mM indole-3-aldehyde in 50 mM potassium phosphate buffer (pH 7.4). Specific activity of AO was expressed as nmol reduced DCIP mg\(^{-1}\) protein min\(^{-1}\). AO activity was also detected in gels by staining following native PAGE. The gel was immersed after electrophoresis in 0.2 M phosphate buffer, pH 8.0, for 10 min followed by gentle shaking of the gel in a reaction mixture containing 0.1 mM phenazine methosulfate (PMS), 1 mM 3(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) and 1.5 mM heptaldehyde or 1 mM indole-3-aldehyde or 1 mM acetaldehyde in 0.1 M Tris–HCl buffer, pH 8.0, at 25°C.
2.5. Gel electrophoresis and analysis of enzyme activity

Enzymes separation and staining was carried out using 1.5-mm thick slabs of native 7.0% polyacrylamide gels loaded with 300 mg shoot proteins or 100 mg root proteins. Enzyme activity of the Mo-oxidoreductases was estimated on the basis of MTT or p-nitrobluetetrazolium reductions, which resulted in the development of specific formazan bands. The quantity of formazan was directly proportional to enzyme activity during a given incubation time, with substrate and tetrazolium salt supplied in excess [29]. Quantitative analyses were made by scanning the formazan bands in the gel with a computing laser densitometer (Molecular Dynamic) using Image Quant version 3.19.4 software. Standard curves ($R^2 = 0.998$) for estimation of XDH activity were based on formazan band density in gels loaded with increasing content of commercial xanthine oxidoreductase (1 unit per 1.3 mg protein; Sigma, St Louis, MO). XDH activity in gels was expressed as enzyme units mg$^{-1}$ protein h$^{-1}$. AO activity was estimated in relation to that in plants grown with nitrate in the absence of salinity.

2.6. AO Western blot analysis

The level of AO protein in roots and shoots extracts was detected by Western blot analysis. Aliquots of the crude extracts containing 300 mg shoot proteins or 100 mg root proteins were subjected to native PAGE. The separated proteins were blotted onto nitrocellulose membrane (0.2 μm pore size; Schleicher and Schüll, Dassel, Germany). Immunodetection of the AO protein was carried out with polyclonal antibodies received by
Table 2
XDH activities and aldehyde oxidase activities in ryegrass roots as affected by N source and salinity in the nutrient solution

<table>
<thead>
<tr>
<th>N source</th>
<th>Salinity (dS m⁻¹)</th>
<th>XDH activity (10⁻³ units mg⁻¹ h⁻¹)</th>
<th>AO activity (density as % of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Heptaldehyde</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>2.0</td>
<td>0.17 d</td>
<td>100 d</td>
</tr>
<tr>
<td></td>
<td>11.2</td>
<td>0.46 d</td>
<td>355 a</td>
</tr>
<tr>
<td>NH₄NO₃</td>
<td>2.0</td>
<td>0.29 c</td>
<td>251 b</td>
</tr>
<tr>
<td></td>
<td>11.2</td>
<td>0.47 b</td>
<td>330 a</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>2.0</td>
<td>0.34 c</td>
<td>175 c</td>
</tr>
<tr>
<td></td>
<td>11.2</td>
<td>0.80 a</td>
<td>346 a</td>
</tr>
</tbody>
</table>

XDH was assayed with hypoxanthine, and its standard curve was calculated from the band density obtained with commercial xanthine oxidoreductase in the same gel and assay. AO was assayed with heptaldehyde or indole-3-aldehyde. Different letters following the mean values in each column indicate significant differences (P<0.05, Duncan test, n=10 to n=6 different experiments).

courtesy of Tomokazu Koshiba (Tokyo, Japan) [5]. Density scanning was done as described above.

Binding of the maize antibodies to ryegrass AO proteins was verified by immunoprecipitation using protein-A–Sepharose CL-4B (Pharmacia). All the activity detected by native PAGE in the extracts was removed after the antibodies and Sepharose were added, while all of the activity remained in the supernatant when control mouse serum was used.

2.7. Separation of XDH and AO

Separation of XDH and AO was performed on native 5.0% polyacrylamide gel using crude extracts containing 300 mg shoot proteins or 100 mg root proteins obtained from plants grown in 4.5 mM NH₄⁺. Shoot crude extract was heated at 60°C for 150 s, and then centrifuged at 30,000 x g in a Sorvall RC-5 refrigerated centrifuge at 3–5°C for 10 min; the resulting supernatant was subjected to the gel. This procedure allowed the activation (data not shown) and separation of XDH and AO in the shoot extracts. Root crude extract was subjected to the gel without the heat treatment.

Strips of the same gel were stained in seven different reaction mixtures containing MTT and PMS with the following substrates: heptaldehyde, heptaldehyde + hypoxanthine, hypoxanthine, hypoxanthine + indole-3-aldehyde, indole-3-aldehyde, hypoxanthine + acetaldehyde or acetaldehyde.

2.8. Determination of ureides and soluble proteins

Ureides were extracted with 80% ethanol and determined according to Vogel and Van Der Drift [30] using allantoic acid and allantoin as references (Sigma, St Louis, MO). Soluble proteins in the assays were measured as described by Bradford [31] using crystalline bovine serum albumin as a reference.
3. Results

3.1. The effect of salinity and nitrogen on XDH and AO

Salinity increased XDH and AO activities in shoots of plants grown with either NH$_4^+$ or NO$_3^-$, using heptaldehyde or indole-3-aldehyde as substrates following gel electrophoresis and in vitro assays (Fig. 1 and Table 1). A significant increase in the activity of Mo-hydroxylases was also observed in roots of plants exposed to salinity (Figs. 1 and 2 and Table 2). XDH and AO activities were highest in roots in which the effect of salinity was considerably larger than in shoots (Fig. 1 and Tables 1 and 2). Roots of plants grown in NH$_4^+$ had a higher XDH and AO activity than plants receiving NO$_3^-$ (Figs. 1 and 2 and Table 2).

The band densities of AO proteins of root extracts cross reacting with the antibodies following native PAGE increased significantly in plants grown under salinity (Fig. 3). AO band density was higher in roots extracts from plants grown with NH$_4^+$ than with NO$_3^-$ . The same treatments did not affect the level of AO proteins in leaves extracts which were significant lower than in the roots (Fig. 3).

XDH and AO activities increased with NH$_4^+$ in shoots and roots of ryegrass plants (Fig. 4 and Table 3), while the level of AO proteins cross reacting with the antibodies following native PAGE were not affected by the increase of NH$_4^+$ concentration in the nutrient solution (data not shown).

Enzyme activities expressed on a dry or fresh weight basis yielded essentially the same results (data not shown).

3.2. Separation between XDH and AO

AO and XDH in ryegrass roots and shoots differed in molecular weight and in substrate specificity as evidenced by their staining after exposure of native PAGE gels to reaction mixtures containing either indole-3-aldehyde, hypoxanthine + indole-3-aldehyde, hypoxanthine, hypoxanthine + acetaldehyde, or acetaldehyde, yielding two distinctive bands in the gels (Fig. 5). Staining AO and XDH after exposure of gels to reaction mixtures containing heptaldehyde or hypoxanthine revealed two distinctive bands as well (data not shown).
Table 3

<table>
<thead>
<tr>
<th>NH$_4^+$ (mM)</th>
<th>XDH (% of control)</th>
<th>AO (% of control)</th>
<th>AO (nmol DCIP mg$^{-1}$ protein min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td>Shoot</td>
</tr>
<tr>
<td>0.5</td>
<td>100 b</td>
<td>100 b</td>
<td>100 b</td>
</tr>
<tr>
<td>4.5</td>
<td>150 a</td>
<td>ND</td>
<td>176 a</td>
</tr>
<tr>
<td>9.0</td>
<td>158 a</td>
<td>263 a</td>
<td>180 a</td>
</tr>
</tbody>
</table>

Enzymes activities in gel included the substrates hypoxanthine and indole-3-aldehyde (density as % of control), respectively. Specific activity of AO in the shoot was assayed with the substrate indole-3-aldehyde (nmol DCIP reduced mg$^{-1}$ protein min$^{-1}$).

Different letters following the mean values in each column indicate significant differences. ($P<0.05$, Duncan test, $n=6$ to $n=5$ and $n=7$ to $n=3$ different experiments of XDH and indole-3-aldehyde oxidase in shoot and root, respectively. In the AO specific activity assay, $n=3$ different experiments).

ND, not determined.

3.3. The effect of salinity and nitrogen on allantoin and allantoic acid

Ureides (allantionic acid and allantoin) are products of purine catabolism by xanthine oxidoreductase. Ureide concentration in ryegrass increased with salinity and NH$_4^+$ and was significantly higher in the roots than in the shoots (Table 4). Plants grown in NH$_4^+$ had a higher ureide content than plants receiving NO$_3^-$ (Table 4).

4. Discussion

MoCo content and NR activity in shoots and roots of ryegrass plants increased with rising salinity [21]. Salinity-enhanced NR activity may be related to increased availability of MoCo. NR overexpression in leaves of a tobacco ABA mutant was assumed to result from increased availability of MoCo [7]. The increase in the pool of MoCo in response to salinity or NH$_4^+$ and the higher MoCo demand in ryegrass roots [21,22] suggest the requirement of additional MoCo under stress to allow the synthesis of AO and XDH. Salinity and NH$_4^+$-enhanced XDH and AO activities in ryegrass in the current study could be attributed to increased activity of the Mo-hydroxylase sulfurylase required for the addition of sulfur which activates these enzymes [13,18]. Alternatively, the higher level of AO protein in roots of salinity and NH$_4^+$-treated plants (Fig. 3) correlated with enzyme activities and may indicate enhanced AO apoprotein biosynthesis in the presence of ammonium and/or salinity. AO activity increased with NH$_4^+$ concentration while the level of enzyme protein was not affected, indicating the possible effect of MoCo content on Mo-hydroxylase activities in view of the MoCo content increase with nitrogen in ryegrass plants [21,22].

Enhanced XDH activity yielded a higher content of ureides, products of purine catabolism. The role of XDH in ryegrass plants during stress may be related to the need for a more efficient use of available carbon skeletons to synthesize organic nitrogen compounds with a low C/N ratio for their transport through the xylem to the shoot (Table 4). Salinity caused a reduction of shoot photosynthesis in annual ryegrass (data not shown) with a subsequent decreased assimilate supply to the root [32]. This shortage could be alleviated by enhanced activity of XDH and the synthesis of ureides, compounds with a considerable lower C/N ratio than asparagine and glutamine, the most common forms of organic nitrogen transported from roots to shoots. Using ureides instead of amides for nitrogen transport of organic compounds from the root could save six or nine ATP-equivalents for each nitrogen transported [17]. High NH$_4^+$ concentrations in the nutrient medium may become toxic when the supply of carbon skeletons for NH$_4^+$ assimilation in the root is insufficient [33]. Enhanced biosynthesis of ureides as transportable nitrogen com-
pounds from the root (Table 4) minimizes the loss of carbon per assimilated nitrogen due to xylem export of nitrogenous compounds [16]. The increase of ureides in roots of plants exposed to salinity is presumably a consequence of the inhibition of nitrate transport to the shoot [34]. Salinity blocked xylem transport of nitrate while nitrate reduction and ammonium assimilation in the roots were enhanced, with a concomitant increase in the demand for carbon skeletons for ammonium assimilation.

Salinity and other stresses induced the generation of active oxygen species [35,36]. Free radicals in plant tissue may be neutralized by uric acid, a product of XDH and an effective scavenger of active oxygen species in many organisms [37,38].

AO catalyzes the last step of the biosynthesis of abscisic acid (ABA) [2,11]. Aldehyde oxidase activity increased with salinity and NH$_4^+$, using indole-3-aldehyde or heptaldehyde as substrates (Tables 1 and 2). These observations agree with the reports on increased ABA concentration in plant tissue [39] under saline conditions and the NH$_4^+$-enhanced transport of ABA to the shoot [40]. ABA is involved in the reaction of plants to environmental stresses such as freezing, cold and salinity [14]. Salinity and nitrogen source affected more root than leaf AO (Figs. 1–3 and Tables 1 and 2), in agreement with the notion that roots are involved in ABA biosynthesis while the leaf produces little ABA [24]. Maize AO in the coleoptile apex, which oxidizes indole-3-acetaldehyde into IAA, exhibited affinity for a number of aldehydes [5]. Changing levels of ABA and IAA in the plant may interact to signal the need for changes in plant functions required for adaptation to stress conditions [41]. The possible involvement of IAA in the response of plants to stresses like water deficit and salinity has been suggested recently [15]. In the current study XDH and AO, assayed with heptaldehyde or indole-3-aldehyde as substrates, showed a similar response to salinity and NH$_4^+$. XDH and AO belong to a gene family that codes for other XDH- or AO-type proteins with variable substrate specificity and expression patterns in response to environmental stress [6]. Differences in molecular weight and substrate specificity between XDH and AO (acetaldehyde, heptaldehyde or indole-3-aldehyde as substrates), which may reflect either different enzymes or a wide substrate affinity of a single enzyme, were observed in ryegrass plants (Fig. 5).

The selective allocation of MoCo to the three Mo-enzymes studied may constitute part of the
Table 4

<table>
<thead>
<tr>
<th>NO$_3^-$ (mM)</th>
<th>NH$_4^+$ (mM)</th>
<th>Salinity (dS m$^{-1}$)</th>
<th>Allantoic acid (nmol g$^{-1}$ DW)</th>
<th>Allantoin (nmol g$^{-1}$ DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Shoot</td>
<td>Root</td>
</tr>
<tr>
<td>4.5</td>
<td>0</td>
<td>2.0</td>
<td>9 e</td>
<td>219 e</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.2</td>
<td>59 d</td>
<td>542 d</td>
</tr>
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<td>4.5</td>
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<td>58 d</td>
<td>1585 c</td>
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<td>ND</td>
<td>70 f</td>
</tr>
<tr>
<td>0</td>
<td>4.5</td>
<td>2.0</td>
<td>95 e</td>
<td>2813 b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.2</td>
<td>604 a</td>
<td>3684 a</td>
</tr>
</tbody>
</table>

Different letters following the mean values in each column indicate significant differences ($P<0.05$, Duncan test, $n=8$).
ND, not determined.

adaption mechanisms of plants under stress conditions [42] and seem to be affected to a considerable extent by specific inorganic nitrogen ions in the roots.

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