Halophytes for Food Security in Dry Lands

Edited by
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CHARACTERIZATION AND FUNCTION OF SODIUM EXCHANGER GENES IN AELUROPUS LAGOPOIDES UNDER NaCL STRESS

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1.1 Introduction

Soil salinization is the key issue in irrigated arid and semi-arid areas that have substantial impact on plant productivity. To cope with salinity, plants have developed several adaptive mechanisms including altered growth pattern, osmotic adjustment, and ion homeostasis (Flowers and Colmer, 2008). These complex traits are extensively reported in both salt-sensitive (glycophytes) and salt-resistant (halophytes) plants (Zhu, 2001; Tester and Davenport, 2003). Moreover, recent molecular studies indicate that halophytes have better ability to alter the expression of genes linked with a wide array of plant processes which support them in surviving in saline areas (Maathuis and Amtmann, 1999; Zhu, 2001). In this scenario, there is a need to enhance knowledge about the multi-genic response of halophytes in NaCl to improve the salt tolerance of conventional crops.

Halophytes can reduce Na⁺ toxicity in cytoplasm, minimize water deficit, manage essential mineral deficiency and reactive species damage when grown under salinity-affected soil in various ways (Blumwald, 2000; Chen et al., 2007; Cosentino et al.,
The Na$^+$ partitioning between the below- and above-ground biomass of plants is an important aspect for salinity resistance (Flowers and Colmer, 2008). Grasses accumulate lower amounts of Na$^+$ in shoots compared to dicots (Marcum, 2008). Plants exclude Na$^+$ from root to soil solution, regulate its loading in vascular tissues, compartmentalize in the vacuole/apoplast and excrete it from above ground epidermal bladder cells to reduce its negative effect on metabolic processes (Tester and Davenport, 2003; Flowers and Colmer, 2008; Shabala, 2013).

The movement of Na$^+$ into the vacuoles or toward apoplast is enabled by the action of tonoplast and plasma membrane-bound Na$^+$/H$^+$ antiporters, respectively, that use the electrochemical gradient of H$^+$ generated by H$^+$ ATPases and H$^+$ PPase. Knowledge is available about the sequence of Na$^+$/H$^+$ antiporters (NHX) (Apse et al., 1999; Shi et al., 2000; Tao et al., 2002; Zhang et al., 2008), expression and function of NHX genes when plants are exposed to salinity (Gaxiola et al., 1999; Oh et al., 2009). Some reports highlight the improvement in the salt tolerance of many crop plants by overexpressing NHX genes (He et al., 2005; Xu et al., 2010).

Poaceae is the most economically important plant family because 70% of all crops are salt-sensitive grasses. About 3.6 billion ha from 5.2 billion ha of the world’s agricultural land is already salt-affected and not suitable for conventional crop farming. In contrast, the demand for food is continuously increasing and we expect to need to feed around nine billion by the end of 2050 (Millar and Roots, 2012). However, extensive efforts are underway to improve the salinity tolerance of conventional crops either through breeding or modern molecular techniques, but still no crop can tolerate half the level of salinity of seawater. In such a scenario, a major breakthrough in crop breeding for salinity tolerance is needed. Regulation of the number, size, and shape of the salt-excreting structure—trichome could be one such possibility. About 15% of halophytic grasses excrete Na$^+$ and Cl$^-$ through bicellular microhairs, which are present on the leaf surface (Adams et al., 1998). Aeluropus lagopoides (Linn.) Trin. Ex Thw. is a salt-excreting, salinity- (1000 mmol L$^{-1}$ NaCl; Gulzar et al., 2003) and drought-tolerant (Mohsenzadeh et al., 2006) grass. Therefore, it could be used as a model plant to improve the salinity tolerance of crops like rice, wheat, and maize (Flowers and Colmer, 2008). Detailed ecological and physiological studies on A. lagopoides have been carried out (Waghmode and Joshi, 1982; Sher et al., 1994; Abarsaji, 2000; Gulzar et al.,
However, information related to the function of its Na\(^+\) transport genes in salinity is lacking. Therefore, the goals of this study were: (i) to isolate the cDNA sequences of \(VNHX\) and \(PMNHX\) from \(A.\ lagopoides\); (ii) to observe the change in the expression of both genes under saline condition; and (iii) to explore the role of both genes in the salt tolerance of \(A.\ lagopoides\).

### 1.2 Materials and Methods

#### 1.2.1 Plant Material

Tillers of \(A.\ lagopoides\) were collected from a population located in coastal areas of Karachi, Pakistan and used for the growth of new seedlings.

#### 1.2.2 Isolation of the cDNA and Sequence Analysis of \(VNHX\) and \(PMNHX\)

One-month-old plants were treated with half-strength Hoagland culture solution containing 400 mmol L\(^{-1}\) NaCl for 2 days. Total RNA was extracted using an RNAqueous Kit (Ambion). The first strand of cDNA was synthesized from 1 \(\mu\)g RNA (DNA free) with the help of protocol provided with cDNA Takara RNA-PCR Kit (AMV; Ver 3.0). Polymerase chain reaction (PCR) were performed using with a pair of primers: (P\(_1\): 5’TTC ATC TAC CTG CTC CCG CCC ATC AT\(3\)’; P\(_2\): 5’CCA CAG AAG AAC ACG GTT AGA ATA CC\(3\)’) for \(VNHX\) and (P\(_3\): 5’TTC ATC TAC CTG CTC CCG CCS ATC AT\(3\)’; P\(_4\): 5’CCA CAG AAG AAC ACG GTT AGA ATR CC\(3\)’) for \(PMNHX\), which were designed based on the conserved regions of previously reported Na\(^+\)/H\(^+\) antiporter from other plants. PCR product was cloned through TA cloning kit (Takara) and pGEM-T vector. After cloning, plasmid was extracted and used for sequencing. The sequencing of 5’ and 3’ un-translated regions of \(VNHX\) was performed using P\(_5\): 5’GT TGT GTG AAT GAT GCC ACG TC\(3\)’; P\(_6\): 5’GAG AGC AGG AGA TCC CAA TC3’; P\(_7\): 5’CCA CAG AAG AAC ACG GTT AGA ATA CC3’ and M\(_{13}\)-primer: 5’GT TGT TTC CCA GTC ACG AC3’. After amplification of the 3’ and 5’ regions, fragments were sequenced and assembled to provide the full-length cDNA of \(VNHX\). The analysis of the \(VNHX\) and \(PMNHX\) sequences was performed by DNA-Dynamo software and NCBI program.
1.2.3 Growth Conditions and Harvest

Tillers of *A. lagopoides* were potted in plastic pots (26 cm high × 20 cm diameter) in prewashed field collected sand culture and sub-irrigated with half-strength Hoagland nutrient solution (*Hoagland and Arnon, 1950*) to establish for 1 month. Equal-sized plantlets were treated with different concentrations (0, 150, 300, and 600 mmol L\(^{-1}\)) of NaCl. The concentration of test solution was maintained every alternate day by distilled water to compensate for evaporation; whereas all test solutions were completely replaced after every fifth day.

Growth parameters (length of shoot and leaf, number of total and senesced leaves) were recorded initially and at the end of the experiment. Each plantlet was carefully removed from the soil after 15 days of experiment and washed thoroughly. Roots and shoots were washed and separated from each other before treating with liquid N\(_2\). All samples were stored at \(-80^\circ\text{C}\).

1.2.4 Quantification of Gene Expression by qRT-PCR

For quantitative real-time PCR (qRT-PCR), a pair of primers were designed for *PMNHX* (PMN-F: 5’TAT CGA ATG GTG CTC GGA AGA3’; PMN-R: 5’AGC CCA GCC ACA GTA CCG ATA3’) and for *VNHX* (VNHX-F: 5’GCA GGT CCT CAA TCA GGA TG3’; VNHX-R: 5’ACT CCA AGG AAG GTG CTT GA3’) by using the gene sequence information of *A. lagopoides*. Expression of *Actin* gene was used to normalize the data. The quantitative expression data of both genes was recorded on a Light Cycler-Carousel-based System (ROCHE), while the analysis of data was performed by software 4.0. All standard curves had \(R^2 \geq 0.99\).

1.2.5 Measurement of Na\(^+\) in Plant Sap

The press sap method was used (*Cuin et al., 2009*) to determine the soluble fraction of Na\(^+\) in leaves and roots of NaCl-treated plants. Sap was mixed thoroughly before preparing dilutions and used for the determination of Na\(^+\) on atomic absorption spectrometer (AA-700; Perkin Elmer, Santa Clara, CA, USA).

1.2.6 Secretion of Na\(^+\)

Fully expanded young leaves of three plantlets were tagged from each NaCl treatment (0, 150, 300, and 600 mmol L\(^{-1}\)). All tagged leaves were prewashed 72 h before the final data
collection. Leaves were rinsed with 2 mL deionized water and collected in Eppendorf tubes and the rate of Na\(^+\) excretion was determined by atomic absorption spectrometry. The area of rinsed leaves was calculated by Image-J software version 1.45 (http://rsb.info.nih.gov/ij/) and data were expressed in \(\mu\)mol Na\(^+\) cm\(^{-2}\) per day.

1.2.7 Malondialdehyde Content

Malondialdehyde (MDA) content was determined in leaf samples as an indicator of lipid peroxidation (Heath and Packer, 1986). An extinction coefficient of 155 mM\(^{-1}\) cm\(^{-1}\) was used to calculate the MDA content in the supernatant while absorbance was recorded at 532 and 600 nm wavelengths. The result of MDA was expressed as \(\mu\)g mg\(^{-1}\) FW.

1.2.8 Statistical Analyses

Statistical analysis was done by SPSS version 11.0 for Windows (SPSS, 2001). Two-way analysis of variance (ANOVA) was used to test for a significant \((P<0.05)\) effect of NaCl on growth, MDA, Na\(^+\) concentration, and expression data. A post-hoc Bonferroni test was used to test for significant differences between means. Correlation analysis was performed between different parameters of *A. lagopoides* through SPSS. Graphs were constructed with the help of SigmaPlot (11.0).

1.3 Results

1.3.1 Molecular Characterization of *VNHX* and *PMNHX*

The full-length cDNA of *VNHX* contained 2353 bp including a putative poly (A) addition signal site in the end of sequence. Whereas, the un-translated region (UTR) of 5’ and 3’ consisted of 337 and 393 bp respectively, the open reading frame (ORF) of 1623 bp encoded a protein of 540 amino acids with a theoretical molecular mass of 59.36 kDa (Figure 1.1A). The cDNA sequence of *VNHX* has been deposited at GenBank with the name *AlaNHX* under accession number GU199336.1. Sequence homology revealed a high degree of homology sequences of *AlNHX* (*VNHX*) and putative vacuolar Na\(^+\)/H\(^+\) antiporter of other higher plants.
Figure 1.1 Information from two isolated genes from *Aeluropus lagopoides*. (A) The cDNA and deduced amino acid sequence of *VNHX* (*AlaNHX*), and (B) cDNA sequence of *PMNHX*.

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The “expressed sequence tag” (EST) of PMNHX contained 204 bp and showed a high degree of homology with previously reported Na\(^+\)/H\(^+\) antiporter located on the plasma membrane of higher plants (Figure 1.1B). The cDNA sequence of PMNHX has been deposited at GenBank under accession number GW796824.1.

1.3.2 Growth
The number of leaves and plant height decreased significantly (\(P<0.01\)) with the increases in salinity. In addition, a substantial (\(P<0.0001\)) increase in leaf senescence was observed at 300 and 600 mmol L\(^{-1}\) NaCl (Table 1.1; Figure 1.2).

1.3.3 Peroxidation of Lipid Membrane
MDA content was unchanged at up to 300 mmol L\(^{-1}\) NaCl treatment, whereas around a 40% increase was found when plants were treated with 600 mmol L\(^{-1}\) NaCl compared to non-saline controls (Figure 1.1).

### Table 1.1 Results of One-Way ANOVA Showed the Effect of NaCl on Different Parameters of *Aeluropus lagopoides*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>df</th>
<th>Mean Square</th>
<th>(F^{\text{significance}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height of shoot</td>
<td>3</td>
<td>1480.183</td>
<td>15.911*</td>
</tr>
<tr>
<td># of leaves</td>
<td>3</td>
<td>1318.175</td>
<td>8.901*</td>
</tr>
<tr>
<td># of yellow leaves</td>
<td>3</td>
<td>32.458</td>
<td>86.555***</td>
</tr>
<tr>
<td>MDA</td>
<td>3</td>
<td>2025.764</td>
<td>138.764***</td>
</tr>
<tr>
<td>Na(^+)—secretion</td>
<td>3</td>
<td>102.231</td>
<td>3.032*</td>
</tr>
<tr>
<td>Leaf—Na(^+)</td>
<td>3</td>
<td>321064.380</td>
<td>158.165***</td>
</tr>
<tr>
<td>Leaf—VNHX</td>
<td>3</td>
<td>55926.904</td>
<td>95.712***</td>
</tr>
<tr>
<td>Leaf—PMNHX</td>
<td>3</td>
<td>83948.736</td>
<td>76.645**</td>
</tr>
<tr>
<td>Root—Na(^+)</td>
<td>3</td>
<td>407017.080</td>
<td>293.535***</td>
</tr>
<tr>
<td>Root—VNHX</td>
<td>3</td>
<td>838020.333</td>
<td>67.553**</td>
</tr>
<tr>
<td>Root—PMNHX</td>
<td>3</td>
<td>9805.678</td>
<td>22.467**</td>
</tr>
</tbody>
</table>

\(* P<0.05; ** P<0.001; *** P<0.0001.\)
Values of correlation are provided with degree of significance.
1.3.4 Flux in Na$^+$

Na$^+$ content increased significantly ($P<0.0001$) in both leaves and roots of A. lagopoides under NaCl treatment (Table 1.1; Figures 1.3 and 1.4). Moreover, this increase was approximately tenfold higher in plants treated with 600 mmol L$^{-1}$ NaCl than in nonsaline controls (Figures 1.3 and 1.4). In general, the amount of Na$^+$ was similar in both parts of plants except at 300 mmol L$^{-1}$ NaCl where roots accumulated a higher amount of Na$^+$ than leaves (Figures 1.3 and 1.4).

1.3.5 Secretion of Na$^+$

Sodium excretion from the leaf surface increased significantly ($P<0.01$) with increase in NaCl concentrations up to 300 mmol L$^{-1}$ NaCl, however, no difference was noted in the Na$^+$ secretion rate of plants exposed to 300 and 600 mmol L$^{-1}$ NaCl (Figure 1.5).

**Figure 1.2** Change in the growth and biochemical parameters [filled circles: increase in number of leaves plant$^{-1}$; empty circles: increase in height of shoot plant$^{-1}$; filled squares: malondialdehyde (MDA) content; empty squares: number of senescent leaves plant$^{-1}$] of Aeluropus lagopoides treated with different NaCl concentrations (0–600 mmol L$^{-1}$) for 15 days ($n = 3$). Values with at least one Bonferroni letter the same were not significantly different at $P<0.05$. 

<table>
<thead>
<tr>
<th>NaCl (mmol L$^{-1}$)</th>
<th>Increase in number of leaves plant$^{-1}$ (%)</th>
<th>Increase in height of shoot plant$^{-1}$ (%)</th>
<th>MDA (µmol g$^{-1}$ FW)</th>
<th>Number of senescent leaves plant$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>70</td>
<td>70</td>
<td>0</td>
<td>0</td>
</tr>
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<td>150</td>
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<td>600</td>
<td>40</td>
<td>40</td>
<td>21</td>
<td>21</td>
</tr>
</tbody>
</table>

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Figure 1.3 Bars represent the concentration of Na\(^+\) in leaf of *Aeluropus lagopoides* treated with different NaCl concentrations (0–600 mmol L\(^{-1}\)) for 15 days (*n* = 3). Change in the expression of genes in leaves was shown by line graph (square and circle symbols were used for *VNHX* and *PMNHX* gene, respectively). Values with at least one Bonferroni letter the same were not significantly different at *P*<0.05.

Figure 1.4 Bars represent the concentration of Na\(^+\) in roots of *Aeluropus lagopoides* treated with different NaCl concentrations (0–600 mmol L\(^{-1}\)) for 15 days (*n* = 3). Change in the expression of genes in roots was shown by line graph (square and circle symbols were used for the *VNHX* and *PMNHX* genes, respectively). Values with at least one Bonferroni letter the same were not significantly different at *P*<0.05.
1.3.6 Gene Expression

The expression of \textit{AlaNHX} (\textit{VNHX}) gene was significantly up-regulated in both leaves \((P<0.0001; \text{Table 1.1; Figure 1.3})\) and roots \((P<0.001; \text{Table 1.1; Figure 1.4})\) of plants when treated with NaCl. However, higher gene expression was observed in roots than leaves, especially in plants treated with 300 and 600 mmol L\(^{-1}\) NaCl \((\text{Figures 1.3 and 1.4})\). The expression of \textit{AlaNHX} gene was similar at 300 and 600 mmol L\(^{-1}\) NaCl, where it was approximately tenfold (root) and fourfold (leaf) greater than the respective nonsaline controls \((\text{Figures 1.3 and 1.4})\). A negative correlation \((r^2 = 0.79; P<0.05)\) was found between the expression of \textit{AlaNHX} and \textit{PMNHX} genes in leaves \((\text{Table 1.2})\). Expression of \textit{PMNHX} gene increased significantly \((P<0.001)\) under NaCl treatment \((\text{Table 1.1; Figures 1.3 and 1.4})\). \textit{PMNHX} gene showed approximately threefold higher expression in leaves than roots \((\text{Figures 1.3 and 1.4})\). Gene expression did not change in leaves under salinity except at 150 mmol L\(^{-1}\) NaCl where substantial up-regulation was found \((\text{Figure 1.3})\). In contrast to leaves, the maximum expression of \textit{PMNHX} gene was found in roots treated with 600 mM NaCl \((\text{Figure 1.4})\).
Table 1.2 Pearson Correlation Analysis Between Changes in Different Parameters of *Aeluropus lagopoides* Under NaCl

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Leaf—Na⁺</th>
<th>Leaf—VNHX</th>
<th>Leaf—PMNHX</th>
<th>Root—Na⁺</th>
<th>Root—VNHX</th>
<th>Root—PMNHX</th>
<th>Na⁺—Secretion</th>
<th>MDA</th>
<th>Total leaves</th>
<th>Yellow Leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf—Na⁺</td>
<td>—</td>
<td>0.84</td>
<td>—</td>
<td>0.96</td>
<td>0.95</td>
<td>0.94</td>
<td>0.76</td>
<td>0.78</td>
<td>—</td>
<td>0.86</td>
</tr>
<tr>
<td>(Significance)</td>
<td>**</td>
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<td>**</td>
</tr>
<tr>
<td>Leaf—VNHX</td>
<td>0.84</td>
<td>—</td>
<td>—0.79</td>
<td>0.88</td>
<td>0.89</td>
<td>0.75</td>
<td>0.82</td>
<td>0.55</td>
<td>—0.93</td>
<td>0.77</td>
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<tr>
<td>(Significance)</td>
<td>**</td>
<td>ns</td>
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<td>ns</td>
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<tr>
<td>Leaf—PMNHX</td>
<td>—0.40</td>
<td>—0.79</td>
<td>—</td>
<td>—0.44</td>
<td>—0.48</td>
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<tr>
<td>Root—Na⁺</td>
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<td>—</td>
<td>0.98</td>
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<td>Root—VNHX</td>
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<td>—0.48</td>
<td>0.98</td>
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<tr>
<td>Root—PMNHX</td>
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<td>Na⁺—Secretion</td>
<td>0.76</td>
<td>0.82</td>
<td>—0.60</td>
<td>0.80</td>
<td>0.71</td>
<td>0.77</td>
<td>—</td>
<td>0.57</td>
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<td>0.78</td>
<td>0.55</td>
<td>—0.26</td>
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<td>0.91</td>
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<tr>
<td>Total leaves</td>
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<td>—0.85</td>
<td>—0.88</td>
<td>—0.74</td>
<td>—0.72</td>
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<td>*</td>
<td>*</td>
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<tr>
<td>Yellow leaves</td>
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<td>0.78</td>
<td>0.94</td>
<td>0.64</td>
<td>0.90</td>
<td>—0.81</td>
<td>—</td>
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<tr>
<td>(Significance)</td>
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<td>*</td>
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<td>ns</td>
<td>*</td>
<td>ns</td>
<td>*</td>
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</tr>
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</table>

* *P* < 0.05; ** *P* < 0.001; ns, nonsignificant.

Values of correlation are provided with degree of significance.
1.4 Discussion

Survival of salt-excreting grasses under saline conditions depends on the extent of Na\(^+\) accumulation in cytoplasm which is the function of increase in the ability of Na\(^+\) exclusion, excretion and sequestration into vacuoles (Ahmed et al., 2013). Sodium/hydrogen antiporter genes are considered to play an important role in controlling Na\(^+\) flux, cytoplasmic pH and cell volume (Mahnensmith and Aronson, 1985). To better understand salt-tolerance mechanisms in *A. lagopoides* that survives successfully under highly saline conditions we cloned and characterized the cDNA of salt stress-related genes (*PMNHX* and *VNHX* (*AlaNHX*). A full-length cDNA was isolated from *A. lagopoides* grown under saline conditions which was 2353 bp long including the predicted ORF of 1623 bp long (338–1960 bp of full-length cDNA) which encodes protein consisting of 540 amino acids. Comparison of both cDNA sequences with other proteins indicates that *AlaNHX* shares a higher identity with *AlNHX* isolated from *Aeluropus littoralis* (Zhang et al., 2008). Similarly, the EST of *PMNHX* had shown greater homology with the *SOS1* gene of *Phragmites australis* (Takahashi et al., 2009). These data allowed us to classify *PMNHX* and *AlaNHX* as new members of the plasma membrane and vacuole Na\(^+\)/H\(^+\) antiporter family and to suggest that they might be involved in Na\(^+\) regulation.

Growth of grasses was reduced when exposed to salinity, even if they survived in higher NaCl concentrations (Gulzar et al., 2003; Barhoumi et al., 2007; Flowers and Colmer, 2008). Similarly, *A. lagopoides* has the ability to survive in up to 1000 mmol L\(^{-1}\) NaCl but nonsaline conditions appear to be optimal for the production of plant biomass (Gulzar et al., 2003). The negative correlation between total number of leaves and Na\(^+\) content \((r^2 = -0.86; \ P<0.001; \ Table \ 1.2)\), but a greater positive correlation between Na\(^+\) and leaf senescence \((r^2 = 0.90; \ P<0.001; \ Table \ 1.2)\) was found. A decreasing trend in the shoot length, leaf elongation, and leaf emergence in higher salinities could be attributed to minimal Na\(^+\) accumulation in shoots (Torrecillas et al., 2003). A delay in the emergence of new leaves and accelerated shedding of mature leaves at 600 mmol L\(^{-1}\) NaCl could be related to the specific ionic toxicity, particularly Na\(^+\) and Cl\(^-\) (Rudmik, 1983). Halophytic grasses usually employ mature leaf shedding and decreasing leaf elongation rates that could help to reduce the Na\(^+\) transport towards young and active plant tissues, but at the cost of reduced biomass (Munns, 2002; Flowers and Colmer, 2008). In contrast, a rapid growth reduction in 600 mmol L\(^{-1}\) NaCl-treated plants is due to oxidative stress (Sobhanian et al., 2010) indicated by higher
MDA content (40% of respective nonsaline treatment). This was further evident from a positive correlation of MDA with leaf Na ($r^2 = 0.78; P<0.01$; Table 1.2) and leaf yellowing ($r^2 = 0.90; P<0.001$; Table 1.2). MDA did not change in up to 300 mmol L$^{-1}$ NaCl, indicating the efficient removal of toxic ions like Na$^+$ from the metabolically active cytoplasm (Munns and Tester, 2008; Oh et al., 2009), which was made possible through Na$^+$ compartmentalization inside the vacuole by VNHX (Cosentino et al., 2010) as we found a positive correlation between $\text{AlaNHX}$ gene expression and leaf Na ($r^2 = 0.84; P<0.001$; Table 1.2). However, the expression of $\text{SOS1}$ appeared to be unchanged during salinity stress but the higher extent of expression might be sufficient for Na$^+$ transport towards apoplast. The transmembrane transport of Na$^+$ either through $\text{AlaNHX}$ or $\text{SOS1}$ would be dependent on the H$^+$ gradient that was established by the activity of H-ATPase and H-PPase enzyme (Hedrich et al., 1989). The distribution of Na$^+$ varies between below- and above-ground tissues and also depends on plant species (Abogadallah, 2010; Yang et al., 2010). Most halophytic grasses accumulate a higher amount of Na$^+$ in roots (Marcum, 2008). The positive correlation between the Na$^+$ content of leaves and roots ($r^2 = 0.96; P<0.001$; Table 1.2) expressed by the $A. \ lagopoides$ plant accumulated Na in both organs but a higher amount of Na$^+$ was found in roots when the plant was exposed to up to 300 mmol L$^{-1}$ NaCl. This finding also validates the expression of VNHX gene which was threefold higher in roots than leaves. However, the expression of $\text{PMNHX}$ was around twofold higher in leaves than roots. In leaves the higher expression of $\text{PMNHX}$ than VNHX could help in the loading of Na in epidermal bladder cells for secretion through salt glands. In $A. \ lagopoides$, the higher uptake of Na$^+$ directly correlates with the secretion rate ($r^2 = 0.76; P<0.01$; Table 1.2). The expression of both genes ($\text{PMNHX}$ and VNHX) and salt secretion rate were similar in seedlings treated with 300 and 600 mmol L$^{-1}$ NaCl, suggesting Na$^+$ toxicity in plants treated with 600 mmol L$^{-1}$ NaCl.

The expression of both sodium exchanger genes $\text{PMNHX}$ and VNHX depends on tissue type and salt concentration and makes $A. \ lagopoides$ a highly salt-resistant grass. The synchronized alteration in $\text{PMNHX}$ and VNHX expression helps $A. \ lagopoides$ to survive up to 300 mmol L$^{-1}$ NaCl by successfully compartmentalizing Na$^+$ in apoplasts and vacuoles, respectively. The effective Na$^+$ secretion and shift in the biomass allocation toward roots also provide support in reducing the ion toxicity. However, plants were facing ion toxicity at 600 mmol L$^{-1}$ NaCl due to limited Na$^+$ excretion and uncoordinated transcription of Na$^+$ transporter genes. This information
will help to understand the salt-tolerance mechanisms of grasses and its use for better yields of conventional crops in saline land.

References


