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SOME PHYSIOLOGICAL VARIATIONS OF AGROPYRON SMITHII RYDB.
(WESTERN WHEATGRASS) AT DIFFERENT SALINITY LEVELS

Rengen Ueng¹,², Ivo E. Lindauer¹,³, and Warren R. Buss¹

ABSTRACT.—The purpose of this study was to determine the physiological responses of Agropyron smithii Rydb. to various saline environments as evaluated in the laboratory. Agropyron smithii Rydb. (Rosana) seeds were germinated, transplanted into nutrient solutions with NaCl concentrations of 0, 50, 100, 150, and 200 mM, and grown for 80 days in a growth chamber. Results indicated that leaf water potential, relative water content of leaf tissue, and concentrations of Na, K, and Cl in plant tissue were significantly affected by increasing NaCl concentration. However, leaf chlorophyll concentration and concentrations of Ca and Mg in plant tissue were not significantly affected by the presence of NaCl.

Key words: Agropyron smithii, salinity, physiology, chlorophyll, sodium, potassium, calcium, magnesium, water potential, chloride.

Agropyron smithii Rydb. (western wheatgrass) is a strongly rhizomatous, glaucous, often glabrous, long-lived perennial grass. It is palatable, cures well on the ground, and is a native grass of the northern Great Plains (Judd 1962, Schultz and Kinch 1976, Stubbenieck et al. 1986). Because A. smithii is a valuable grazing species in the arid West, it is often sought out for revegetation of these soils.

Research has shown that salt stress has an impact on chlorophyll concentrations of leaves, leaf osmotic potentials, and mineral uptake and transport in many plants. Seemann and Critchley (1985) reported that chlorophyll concentration per unit area of Phaseolus vulgaris L. was reduced considerably by NaCl stress. Macler (1988) also reported that in Gelidium coulteri (red alga) the content of chlorophyll was altered with a change in NaCl concentration in the growth media. However, Antflinger (1981) found that concentrations of chlorophyll a, chlorophyll b, and total chlorophyll of Borrichia frutescens were not significantly influenced by salinity.

Clipson et al. (1985) used a dew-point hydrometer to measure leaf osmotic potential of Suaeda maritima L. Dum. seedlings grown under different salinities. They found that leaf osmotic potentials were lower (more negative) for those grown under higher salt concentrations. Black (1960) reported that, under saline conditions, the osmotic potential of Atriplex vesicaria leaves changed in the direction that maintained a constant water potential gradient between leaf and soil.

Evidence is growing that salt stress inhibits the uptake and transport of mineral nutrients in some plants. In Hordeum vulgare L. (barley) seedlings, the uptake and transport of nitrogen (Aslam et al. 1984), phosphate (Maas et al. 1979), K (Lynch and Läuchli 1984), and Ca (Lynch and Läuchli 1985) were reduced by salinity. The transport of K and Ca in Gossypium hirsutum L. was also disrupted by high Na+ concentrations (Cramer et al. 1987). In Salicornia europaea the uptake of K+, Mg2+, and Ca2+ was also reduced by Na+ (Austenfeld 1974).

A study of the physiological responses of A. smithii in saline environments may determine how this species adapts to saline soils and provide additional information for breeding salt-tolerant species. The purpose of this study was to measure leaf chlorophyll concentration, leaf water potential, relative water content of leaf tissue, and mineral content of A. smithii grown under different saline water culture conditions.

MATERIALS AND METHODS

Agropyron smithii Rydb. seeds (Rosana) were germinated in moist vermiculite (temperature alternated between 15°C for 20 h...
and 30°C for 4 h) in complete darkness for 5 days. Thereafter, the seeds were held in darkness (15°C for 3 days), then at an alternating temperature (28 ± 2°C for a 12-h day and 15°C for the 12-h night) as recommended by Toole (1976).

A nutrient solution modified from Arnon and Hoagland (1940) was used in this study (Table 1). One-liter plastic containers were used to hold the experimental plants and nutrient solutions. Plants were supported in square cardboard covers impregnated with paraffin. Both covers and containers were sterilized with a 5% Clorox solution before use.

When A. smithii seedlings were 15 days old (2-3 cm long), they were placed through holes in the cover and held in place with loose wads of cotton wrapped around each stem. Four plants were placed in each container. Each container was aerated for 30 min each 24-h period. For the first 9 days after transplanting, damaged or infected seedlings were replaced with fresh ones.

Salinization of the medium began 9 days after seedlings had been transferred to the nutrient solution. This was done by increasing NaCl concentration in the culture solutions at the rate of 25 mM every 4 days to the final concentrations of 50, 100, 150, and 200 mM. Plants grown in unsalted cultures were used as controls. The nutrient solution was changed every 12 days during the experiment.

Experiments were carried out in a Sherer Gillette Plant Growth Chamber (Model 512 CEL) set for a 12-h day at 28 ± 2°C with a humidity of 40 ± 5% and a 12-h night at 15°C with a humidity of 60 ± 5%. Light was supplied by 12 cool white VHO fluorescent bulbs. Eighty plants (20 containers) were used for each treatment, and all plants in each treatment were numbered. From these 80 plants, 10 were randomly selected for measuring for each treatment. If a plant had more than one culm, the longest was chosen for the data measurement of leaf chlorophyll, leaf water potential, and relative water content of leaf tissue.

Leaf chlorophyll concentration was determined by extracting the chlorophyll in acetone (80% v/v in water) from the second and third leaves collected from a randomly selected plant when the plants were 93 days old. Absorbance of chlorophyll was measured at 645 and 663 nm following the procedure of Witham et al. (1986).

Leaf water potential was evaluated by Chardokov’s procedure as described by Witham et al. (1986) when the plants were 89 days old. All leaves collected from a randomly chosen plant were cut into pieces, mixed, and then equally divided into 17 test tubes containing sucrose solutions with concentrations ranging from 0.1 M to 0.5 M with increments of 0.025 molarity. The relationship between sucrose concentration and leaf water potential is shown in Witham et al. (1986).

Relative water content (RWC) of leaf tissue was determined by using the following relationship modified from Vassey and Sharkey (1989) when the plants were 84 days old:

\[
\% \text{ RWC} = \left( \frac{\text{FW} - \text{DW}}{\text{SAT} - \text{DW}} \right) \times 100
\]

where:

- \(\text{FW}\) = leaf fresh weight in grams,
- \(\text{DW}\) = oven-dried (at 105°C for 72 h) weight in grams,
- \(\text{SAT}\) = weight (in grams) of the tissue after soaking in water for 3 h.

All leaf tissue from a randomly selected plant was used for determining relative water content (RWC).

Plants selected for mineral analysis (K, Na, Ca, Mg, and Cl) were harvested when they were 80 days old. Ten randomly selected plants were washed with distilled water and then placed in an oven at 105°C for 72 h. Dry material from each plant was weighed, ground, and then transferred into a crucible that was ashed in a muffle furnace at 500°C for 4.5 h. The ash was dissolved in 20% HNO₃ and filtered for the determination of K, Na,

<table>
<thead>
<tr>
<th>Salt</th>
<th>g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNO₃</td>
<td>1.02</td>
</tr>
<tr>
<td>Ca(NO₃)₂ • 4 H₂O</td>
<td>0.71</td>
</tr>
<tr>
<td>NH₄H₂PO₄</td>
<td>0.23</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>0.24</td>
</tr>
<tr>
<td>H₂BO₃</td>
<td>2.86</td>
</tr>
<tr>
<td>MnCl₂ • 4 H₂O</td>
<td>1.81</td>
</tr>
<tr>
<td>CuSO₄ • 5 H₂O</td>
<td>0.08</td>
</tr>
<tr>
<td>ZnSO₄ • 7 H₂O</td>
<td>0.22</td>
</tr>
<tr>
<td>H₃MoO₄ • H₂O</td>
<td>0.09</td>
</tr>
<tr>
<td>FeSO₄ • 7 H₂O 5 g/L</td>
<td>0.6 mL/L</td>
</tr>
</tbody>
</table>

& tartaric acid 4 g/L (every 4 days)

### Table 1. Composition of the nutrient solution modified from Arnon and Hoagland (1940).
Ca, and Mg concentrations. Concentrations of K and Na were determined with a flame emission spectrophotometer (Perkin-Elmer, Model 403), and concentrations of Ca, and Mg were determined with an atomic absorption spectrophotometer (also Perkin-Elmer, Model 403).

The Cl concentration was determined indirectly by adding a known excess of silver to the sample solutions, which resulted in precipitation of the Cl as AgCl (Perkin-Elmer Corporation 1971). After the separation of AgCl, the concentration of unreacted silver was determined by atomic absorption. Concentrations of Cl were calculated using the formula listed at the end of this paragraph. Samples were prepared by placing the oven-dried plants in a muffle furnace at 500°C for 10 h. The ash was dissolved in distilled water (24-67 mL) to obtain a solution with a Cl concentration of 0-1000 μg/mL Cl and then filtered. Ten mL of sample, 2 mL of concentrated nitric acid, and 10 mL of silver nitrate solution (5000 μg/mL Ag) were transferred into a volumetric flask and diluted with distilled water to a volume of 100 mL. Thirty (30) mL of the mixed solution was centrifuged for 10 min at 2500 rpm. The supernatant was diluted with water 1:100 (v/v). Concentrations of Ag in the diluted supernatant were measured by atomic absorption (Perkin-Elmer, Model 403). Concentrations of Cl in the samples were calculated as follows (Perkin-Elmer Corporation 1971):

\[
\text{Chloride (mg/mL)} = \left(500 - 100 \times \frac{\text{mg/mL Ag in supernatant}}{3.29}\right) \times \text{DF where:}
\]

\[
\text{DF} = \text{dilution factor(s)}.
\]

One-factor ANOVA, following procedures outlined by Kleinbaum et al. (1988), was used to determine statistically significant differences (α = 0.05) among treatments.

**Results**

Leaf water potential, relative water content of leaf tissue (RWC), and concentrations of Na, K, and Cl in plant tissue were significantly affected by the presence of NaCl, whereas concentrations of leaf chlorophyll, Ca, and Mg in the plant tissue were not (Table 2).

Mean values of leaf water potential decreased (were more negative) as external NaCl concentrations increased. The range was -0.34 MPa to -0.96 MPa for plants grown in nutrient solutions with 0 mM and 200 mM NaCl, respectively. RWC decreased as NaCl concentration in nutrient solutions increased and varied from a maximum of 88% to a minimum of 79% for the plants grown in solutions with 0 mM and 200 mM NaCl, respectively (Table 2).

Concentrations of Na, Cl, K, and Ca in A. smithii tissue increased with an increase in NaCl concentration. Mean values (n = 10) of Na concentration varied from a minimum of 1.6 mg/g to a maximum of 58 mg/g (dry weight) for plants grown in solutions with 0 mM and 200 mM NaCl, respectively (Table 2).

Plant Cl concentration increased from 2.7 mg/g to 35 mg/g (dry weight) for plants grown in solutions with 0 mM NaCl and 200 mM NaCl, respectively. Potassium concentration increased from 47 mg/g to 59 mg/g (dry weight). Calcium concentration varied from a minimum of 4.5 mg/g to a maximum of 5.1 mg/g (dry weight). No appreciable change in Mg concentrations was observed, although they decreased from a maximum of 2.7 in control plants to 2.4 mg/g (dry weight) in plants grown in solutions with 200 mM NaCl (Table 2).

**Discussion**

Concentrations of chlorophyll a, chlorophyll b, and total chlorophyll in leaf tissue were not significantly affected by NaCl treatments (Table 2). This finding agrees with the previous study of *Borrichia frutescens* by Antlfinger (1981) but not with the study of Seemann and Critchley (1985) on *Phaseolus vulgaris* L. This discrepancy might be a result of using different methods of expressing chlorophyll concentrations. The unit used in this study was mg chlorophyll/g fresh tissue, while the unit used by Seemann and Critchley (1985) was g chlorophyll/m² leaf area. Since water content of A. smithii leaves decreased significantly as salinity increased (Table 2), this might have caused the values of chlorophyll concentration to be overestimated for plants grown in solutions with high concentrations of NaCl because of reduction of leaf fresh weight. If chlorophyll concentrations of A. smithii were expressed as g/unit leaf area, the treatment difference might have been apparent. Further study is needed to confirm this thesis.
Several environmental factors that influence chlorophyll content in *A. smithii* have been identified. Lauenroth and Dodd (1981) discovered that when *A. smithii* was exposed to SO$_2$, chlorophyll *a* and *b* concentrations were reduced. Chlorophyll *a* was found to be more sensitive to SO$_2$ than was chlorophyll *b*. Bokhari (1976) found that temperature, water stress, and nitrogen fertilizer also influenced the content of chlorophyll *a* and *b*.

There was a significant change in leaf water potential of *A. smithii* along the salinity gradient. Leaf water potential became lower (more negative) in response to increased levels of NaCl (Table 2). The decline of leaf water potential caused by salinity has also been found in other plants such as *Cochleria officinalis*, *Atriplex littoralis*, and *Limonium vulgare* (Stewart and Ahmad 1983).

Reduction of tissue water potential induced by the addition of salt into the nutrient solution has several impacts on plants that are similar to those induced by water stress. Examples of these are the inhibition of cell growth, cell wall synthesis, protein synthesis, carbon assimilation, respiration (Glass 1988), photosynthesis (Black and Bliss 1980), and other enzyme activities (Stewart and Ahmad 1983).

Reduction of leaf water potential was thought to be a strategy to maintain turgor and avoid desiccation in saline environments (Glass 1988). The change of leaf water potential can occur in a variety of ways, such as changing osmotic potential or turgor pressure, or the combination of the two. However, studies of angiosperm halophytes by Stewart and Ahmad (1983) have shown that changes in cell osmotic potential are the major components that effect changes in leaf water potential. In leaf tissue of *Limonium vulgare*, leaf water potential and osmotic potential decreased in a parallel fashion over a change in growth media water potential from near zero to −2.7 MPa. The turgor potential was more or less constant up to −1.8 MPa. When *L. vulgare* was grown in media having salinity greater than −2.7 MPa, turgor pressure often decreased. It is currently believed that the decrease in turgor pressure is the primary event inhibiting growth. Plant cells will grow only when the protoplast exerts a positive pressure on the cell wall. Crop plants were found severely wilted when leaf water potentials were lower than a range of −1.2 to −1.6 MPa (Hanson et al. 1977). Thus, the ability of plants to maintain their leaf water potentials above the turgor loss point in a saline environment may be used as a measure of their salt tolerance. Data from the present study are unable to predict (1) the value of leaf water potential at which *A. smithii* will lose its turgor or (2) how osmotic potential and turgor pressure respond to water stress induced by saline environment.

RWC of leaf tissue is sometimes used to indicate the degree of water deficit. This value in *A. smithii* was significantly reduced by the presence of NaCl in nutrient solutions (Table 2). Although RWC has a positive relationship with tissue turgor pressure, the correlation between these two parameters varies from species to species. For example, at the same

### Table 2. Leaf chlorophyll content (mg/g fresh tissue), leaf water potential (MPa, 20°C), relative water content of leaf tissue (RWC %), and mineral content of a whole plant (mg/g in dry weight) of *A. smithii* grown in nutrient solutions with five NaCl concentrations. Values represent mean (n = 10) ± 1 SD. Significance of the F-test from the ANOVA is also given.

<table>
<thead>
<tr>
<th>Character</th>
<th>Treatments (mM NaCl)</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±</td>
</tr>
<tr>
<td>Chlorophyll <em>a</em></td>
<td>1.3 ± 0.24</td>
<td>1.5 ± 0.19</td>
</tr>
<tr>
<td>Chlorophyll <em>b</em></td>
<td>0.9 ± 0.16</td>
<td>1.0 ± 0.17</td>
</tr>
<tr>
<td>Total chlorophyll</td>
<td>2.2 ± 0.39</td>
<td>2.6 ± 0.35</td>
</tr>
<tr>
<td>Leaf water potential</td>
<td>−0.34 ± 0.06</td>
<td>−0.6 ± 0.1</td>
</tr>
<tr>
<td>RWC</td>
<td>88 ± 3.8</td>
<td>87 ± 1.3</td>
</tr>
<tr>
<td>Sodium*</td>
<td>1.6 ± 0.15</td>
<td>37 ± 4.9</td>
</tr>
<tr>
<td>Chloride*</td>
<td>2.7 ± 0.25</td>
<td>7.3 ± 0.76</td>
</tr>
<tr>
<td>Potassium</td>
<td>47 ± 4.4</td>
<td>55 ± 5.3</td>
</tr>
<tr>
<td>Calcium</td>
<td>4.5 ± 0.51</td>
<td>4.9 ± 0.64</td>
</tr>
<tr>
<td>Magnesium</td>
<td>2.7 ± 0.21</td>
<td>2.6 ± 0.43</td>
</tr>
</tbody>
</table>

*Significance of test was performed on the transformed data (common logarithms).*
value of RWC, Dubautia ciliolata is able to maintain a higher value of turgor pressure than D. scabra (Robichaux 1984). The ability to maintain higher turgor pressure is thought to be an adaptation to water stress induced by salt. Further study is needed to explore the relationships of RWC, osmotic potential, and turgor pressure of A. smithii in saline environments.

The concentration of Na ion in A. smithii increased dramatically as the external NaCl increased (Table 2). An increase in Na ion has also been found in leaf tissue of Phaseolus vulgaris L. grown in saline environments (Seemann and Critchley 1985). The accumulation of high concentrations of Na ion was thought to balance the low water potential of the external environment in halophytes (Glass 1988). Data from this study suggest that A. smithii may use the same method as other halophytes to maintain a more negative osmotic potential than that of the external medium.

Accumulation of Na in A. smithii tissue also accounts for the reduced growth of this plant, because enzymes of all eukaryotes are sensitive to high concentrations of NaCl (Kramer 1984) and high concentrations of Na cause a disruption of membrane integrity by displacement of Ca from cell surfaces by Na (Cramer et al. 1985, Lynch and Läuchli 1988). Moreover, Cramer et al. (1987) and Jeschke (1984) suggested that a high level of Na in the apoplast could also inhibit the transport of assimilate and K in the phloem, thus reducing growth.

For most plants to survive in saline environments, Na must be excluded from the bulk cytoplasm. In halophytes it has been demonstrated that Na concentrations are relatively low in the cytoplasm compared to the vacuole. In the root cortical cells of the halophyte Suaeda maritima (L.) Dum., Na was found in the vacuoles at four times the concentration in the cytoplasm or cell walls (Hajibagheri and Flowers 1989). Jeschke (1980) suggests that this kind of Na compartmentalization appears to be brought about by selective K ion influx and Na efflux through the plasmalemma and by Na+/K+ exchange across the tonoplast.

In addition to cellular Na compartmentalization, plants employ other methods to avoid or minimize toxic effects of Na. For example, Distichlis stricta (Torr.) Rydb., Atriplex halimus L. (Mozafar and Goodin 1970, Anderson 1974), and members of the families Plumbaginaceae and Frankeniaceae (Helder 1956) have salt-eliminating glands or hairs that are found on the leaves. In Oryza sativa (rice) the salt is translocated to older leaves that then drop from the plant (Yeo and Flowers 1982). Further study is needed to clarify how A. smithii avoids or minimizes toxic effects of salts.

Sodium is not an essential element for A. smithii but is now considered an essential nutrient for plants capable of fixing CO₂ via C₄ organic acids. This includes C₄ and CAM plants (Glass 1988). It is interesting to note that the C₄ plants Zea mays (corn) and Saccharum officinarum L. (sugar cane) have not been shown to require Na (Hewitt 1983).

The concentration of Cl in A. smithii increased considerably as the external NaCl concentration increased. But overall concentrations of Cl in A. smithii tissue are lower than those of Na (Table 2). The reason that A. smithii keeps Na concentrations higher than those of Cl in saline environments is unclear. A similar increase in Cl was found in leaf tissue of Phaseolus vulgaris L. grown in saline environments (Seemann and Critchley 1985). The accumulation of this element in halophyte tissue is also thought to influence osmotic regulation. Cellular Cl compartmentalization, like that of Na, has been found in Suaeda maritima (L.) Dum. (Hajibagheri and Flowers 1989).

Chloride basically has the same toxic effects on plants as Na does. In fact some toxic effects of NaCl may result from a combination of the two ions. In citrus and grapes, Cl has been shown to be the damaging ion (Shannon 1984). Levitt (1980) claimed that Cl injury occurred earlier and was more severe than Na injury because Cl was accumulated by plants from NaCl more rapidly than Na was. Since the accumulation of Na and Cl in plant tissue is a common phenomenon found in halophytes, the accumulation of these two ions in A. smithii in this study could indicate this species has some level of adaptation to saline environments.

The concentration of K ion increased in A. smithii as the external NaCl increased. However, the increment was not as great as that of Na and Cl (Table 2). These results are comparable to those found by Antilfinger (1981) in Borrichia frutescens but do not agree with the findings in Gossypium hirsutum L. In G. hir-
sutum L., the transport of potassium was disrupted by high Na⁺ concentrations (Cramer et al. 1987). The discrepancy in potassium content might be because G. hirsutum L. is more sensitive to NaCl than are A. smithii and Borrichia frutescens. The increase of the potassium ion content of A. smithii in this study could be due to an altering of the ionic charges within cells resulting from the rise of Cl concentrations. Potassium is known to generate turgor in many non-halophytes and halophytes. It is also an enzyme activator with at least 60 enzymes known to be activated by this ion (Glass 1988).

Studies of cell cultures of Nicotiana tabacum (Watad et al. 1983), Medicago sativa (Crouthman et al. 1979), and Citrus aurantium (Ben-Hayyim et al. 1985) found that a higher level of internal potassium ion could be correlated with a higher level of salt tolerance. Since A. smithii is able to maintain a high internal potassium ion concentration when grown in saline environments, this may be a sign of salt tolerance.

The concentration of Ca was not significantly changed by increased external NaCl concentrations (Table 2). This finding does not agree with the studies on Hordeum vulgare L. (barley) seedlings by Lynch and Läuchli (1985) and on Salicornia europaea by Austenfeld (1974). The discrepancy in the findings may be due to the possibility that A. smithii is more tolerant to NaCl than Hordeum vulgare L. and Salicornia europaea.

In Zea mays root protoplast, Ca is known to be displaced from associated cell membranes by high concentrations of internal Na. This displacement is correlated with increased leakage of potassium ion (Lynch and Läuchli 1988) because Ca is known to maintain cell membrane integrity for plants in saline environments (Poovaiah and Leopold 1976, Leopold and Willing 1984, Cramer et al. 1987).

Magnesium concentration did not change appreciably with increasing external NaCl concentrations (Table 2). The findings do not agree with those studies on Salicornia europaea conducted by Austenfeld (1974). Magnesium is well known for its participation in the chlorophyll molecule. The unaltered concentration of Mg is consistent with the unaltered concentration of chlorophyll in this study. The role of this element in plants responding to external NaCl is not clear at this point.

Conclusions

The unchanged chlorophyll concentration, reduction of leaf water potential, and accumulation of K and Na of A. smithii in this study are signs of adaptation to saline environments. The biomass study of this species (data are not presented in this article) indicates that Agropyron smithii prefers an environment with a low concentration of NaCl, although it can survive in a habitat with a higher concentration of salt.

Literature Cited


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