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MORPHOLOGY AND GROWTH VARIATIONS OF AGROPYRON SMITII RYDB. (WESTERN WHEATGRASS) AT DIFFERENT SALINITY LEVELS

Rengen Ueng1,2 and Ivo E. Lindauer1,3

ABSTRACT.—The purpose of this study was to determine the morphology and growth responses of Agropyron smithii Rydb. to various saline environments as evaluated in the laboratory. Agropyron smithii Rydb. (B. smithii) seeds were germinated, transplanted into nutrient solutions with NaCl concentrations of 0, 50, 100, 150, and 200 mM, and grown for 80 days. Plant height, length of culm, and number of culms per plant were significantly reduced by the presence of NaCl in the nutrient solutions. As the external NaCl concentrations increased, values of root and plant ratio and leaf dry/fresh mass ratio increased significantly; biomass decreased significantly. However, the stomatal index, number of leaves per culm, and ratio of leaf length to leaf width were not significantly affected by the presence of NaCl.

Key words: Agropyron smithii, western wheatgrass, salinity, salt, morphology, sodium chloride, biomass, stomata, culm, leaf, root.

Agropyron smithii Rydb. (western wheatgrass or bluestem wheatgrass) is a native grass of the northern Great Plains (Schultz and Kinch 1976). It is common in dry prairies, dry sagebrush deserts, foothills, and along ditch banks and roadsides in sandy to heavy soils (Cronquist et al. 1977, Great Plains Flora Association 1986). Agropyron smithii is a valuable forage crop because of its high soil-stabilizing potential, rapid and vigorous seedling development, high nutritive value, and ability to withstand grazing (Knipe 1973, Cronquist et al. 1977).

Agropyron smithii Rydb. has been examined widely for several decades primarily in studies concerning germination and soil-related growth factors. Environmental factors (light and temperature) influencing germination have been investigated by Plummer (1943), Delouche and Bass (1954), Delouche (1956), Knipe (1973), Bokhari et al. (1975), Schultz and Kinch (1976), Toole (1976), and Sabo et al. (1979). The effects of day length and temperature on flowering and growth have been studied by Benedict (1940). The effects of topography, soil texture, and soil moisture on the distribution of A. smithii in the Arapaho Prairie (Arthur County, Nebraska) have been studied by Barnes and Harrison (1982). However, research concerning morphology and growth responses of this plant to salinity is very limited. Since A. smithii is a valuable grazing species in the arid West, it is often sought out for revegetation of these soils. A study of the morphology and growth responses of this species in saline environments may serve to help determine how this species adapts to saline soils.

The purpose of this study was to characterize the morphology and growth variations of A. smithii grown under different saline water culture conditions. Morphological variations that were examined include number of stomata per unit area, culm length, number of culms per plant, plant height, ratio of leaf length to width, and number of leaves per culm. Growth variations include fresh mass and dry mass of the whole plant, ratio of root to plant, and ratio of leaf dry mass to fresh mass.

MATERIALS AND METHODS

Thirty grams of A. smithii seeds (Rosana, lot number WR-1059), obtained from Sharp Bros. Seed Co. (101 E. 4th St. Rd., Greeley, Colorado) was germinated in moist vermiculite (15°C C for 20 h and 30°C for 4 h per day) in complete darkness for 5 days. The seeds were held in darkness (15°C C for 3 days) and then placed at alternating temperatures (28 ± 2°C for a 12-h day and at 15°C 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 the nutrient solutions. Square cardboard covers to support the plants were prepared with five equally spaced holes and impregnated with paraffin. Both covers and beakers were sterilized with a 5% Clorox solution before use.

When the A. smithii seedlings were 15 days old (2–3 cm long), they were placed through holes in the cover. Roots of the seedlings were bathed in the nutrient solution while shoot portions were supported in the cover holes with loose wads of cotton wrapped around each stem. Four plants were placed in each container. The hole in the center of the cardboard was for aeration. 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.

To allow the plants to acclimate to the saline culture media without excessive mortality, salinization of the medium began 9 days after the seedlings had been transferred to the nutrient solution. This was done by increasing the NaCl concentration in the culture solutions at the rate of 25 mM every four 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.

The experiments were carried out in a Sherer Gillette plant growth chamber (Model 512 CEL). Plants were grown under a 12-h day at 28 ± 2°C with humidity of 40 ± 5% and a 12-h night at 15°C with humidity of 60 ± 5%. Light was supplied by 12 cool white VHO fluorescent bulbs. Containers were placed randomly in the growth chamber. There were 80 plants (20 containers) for each treatment. All plants in each treatment were numbered. Data collection began when the plants were 80 days old. A subsample of 10 plants was measured for each treatment. Plants were selected for measurement without regard to container by picking numbers from a table of random numbers. It was possible that more than one plant per container was sampled for a treatment. This may have biased the results if differences in plant responses existed among containers.

### Table 1. Composition of the nutrient solution modified from Arnon and Hoagland (1940).

<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>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Salt</th>
<th>mg/L</th>
</tr>
</thead>
<tbody>
<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>H₃BO₃·4 H₂O</td>
<td>0.09</td>
</tr>
<tr>
<td>FeSO₄·7 H₂O·5 g/L</td>
<td>0.6 mLL (every 4 days)</td>
</tr>
<tr>
<td>tartaric acid 4 g/L</td>
<td></td>
</tr>
</tbody>
</table>

**Morphological Measurements**

The number of stomata per mm² was determined by obtaining stomatal peels made by applying a thin layer of AB Dick mimeograph correction fluid on the center (abaxial surface) of a leaf and allowing it to dry. Using double-sided Scotch tape, we removed the peel and placed it on a microscope slide. Counts were made at a magnification of 200X by using a calibrated ocular grid (Antlfinger 1981). The second leaf from the top of a randomly selected plant was used for this count.

Culm length was determined by measuring from the cardboard to the top node. Plant height was determined by measuring the full length of the plant above the cardboard (including leaf). Leaves longer than 3 cm were counted to determine the number of leaves per culm. The third leaf from the top was chosen for leaf length and width ratio measurement. The reading for leaf width was taken from the widest part of the leaf.

If a plant had more than one culm, the longest one was chosen for all of the above measurements.

**Growth Measurements**

Plants were harvested when they were 80 days old. Growth of the plants was determined by first measuring fresh mass and then dry mass following a drying period of 72 h at 105°C. The aboveground and belowground (above cardboard and below cardboard) dry masses were measured separately for determining root/plant ratios. For measurements of leaf dry and fresh mass ratios, all fresh leaves collected from a randomly selected plant were
TABLE 2. Morphological variation of *A. smithii* grown in nutrient solutions with five different NaCl concentrations. Values represent mean (n = 10) ± one standard deviation. Significance levels are for the F-tests from the ANOVAs.

<table>
<thead>
<tr>
<th>Character</th>
<th>0</th>
<th>50</th>
<th>100</th>
<th>150</th>
<th>200</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stoma #/mm² leaf</td>
<td>56 ± 9.5</td>
<td>56 ± 8.2</td>
<td>58 ± 9.8</td>
<td>59 ± 9.8</td>
<td>61 ± 5.2</td>
<td>.619</td>
</tr>
<tr>
<td>Culm #/plant</td>
<td>14 ± 4.7</td>
<td>5 ± 1.2</td>
<td>4 ± 1.0</td>
<td>2 ± 0.7</td>
<td>2 ± 0.7</td>
<td>.0001</td>
</tr>
<tr>
<td>Leaf #/plant</td>
<td>5 ± 0.7</td>
<td>5 ± 0.7</td>
<td>5 ± 0.63</td>
<td>4 ± 0.52</td>
<td>4 ± 0.52</td>
<td>.575</td>
</tr>
<tr>
<td>Culm length (cm)</td>
<td>19 ± 4.9</td>
<td>13 ± 2.8</td>
<td>14 ± 4.2</td>
<td>10 ± 2.0</td>
<td>8 ± 1.6</td>
<td>.0001</td>
</tr>
<tr>
<td>Plant height (cm)</td>
<td>52 ± 5.0</td>
<td>40 ± 6.9</td>
<td>37 ± 4.8</td>
<td>31 ± 6.5</td>
<td>27 ± 6.1</td>
<td>.0001</td>
</tr>
<tr>
<td>Leaf length (cm)</td>
<td>31 ± 3.8</td>
<td>26 ± 5.9</td>
<td>23 ± 5.1</td>
<td>21 ± 5.5</td>
<td>19 ± 4.7</td>
<td>.0001</td>
</tr>
<tr>
<td>Leaf width (cm)</td>
<td>0.5 ± 0.06</td>
<td>0.47 ± 0.06</td>
<td>0.44 ± 0.05</td>
<td>0.37 ± 0.05</td>
<td>0.32 ± 0.06</td>
<td>.0001</td>
</tr>
<tr>
<td>Leaf length/width</td>
<td>63 ± 9.9</td>
<td>50 ± 13</td>
<td>51 ± 7.7</td>
<td>60 ± 13</td>
<td>60 ± 12</td>
<td>.386</td>
</tr>
</tbody>
</table>

Weighed and then placed in an oven for 72 h at 105°C.

One-factor ANOVA was used to determine significant differences (α = .05) among different treatments. The one-factor ANOVA test used procedures outlined by Kleinbaum et al. (1988).

RESULTS

Morphological Measurements

The number of stomata from plants grown in the five different nutrient solutions did not change as the external sodium chloride concentrations increased (Table 2). The number of culms per plant decreased as the external sodium chloride concentrations increased. The number of leaves per culm was not affected by the external sodium chloride concentrations. Length of culms and height of plants decreased as concentrations of sodium chloride in nutrient solutions increased. Generally, plants that had a longer culm also had a greater plant height (Table 2). Leaf length and width decreased as external sodium chloride concentrations increased. However, the ratio of leaf length to leaf width was not affected by external sodium chloride concentrations (Table 2). External sodium chloride reduced leaf size and did not affect leaf shape.

Growth Measurements

Growth of *A. smithii* as measured by dry mass and fresh mass was inhibited by the presence of sodium chloride in nutrient solutions. Size reduction was most obvious when the 0 mM and 50 mM sodium chloride treatments were compared. As salt concentrations increased above 50 mM, dry and fresh mass differences between two adjacent treatments became less obvious (Table 3). Growth of the shoot was more sensitive than the root to sodium chloride. Plants grown in solutions with higher concentrations of sodium chloride had higher root/plant ratios (Table 3). To determine leaf succulence, the ratio of leaf dry mass and fresh mass was measured. Mean dry/fresh mass ratios increased slightly with an increase in external sodium chloride concentrations (Table 3). Thus, external sodium chloride did not stimulate succulence of *A. smithii* leaf tissue.

DISCUSSION

Morphological Measurements

The number of stomata per unit area was not significantly influenced by the presence of NaCl. This finding agrees with a previous study of *Borrichia frutescens* by Antlfinger (1981). It is apparent that *A. smithii* does not reduce transpiration by decreasing stomatal unit area to cope with water stress resulting from the presence of NaCl in nutrient solutions. However, many plants in high-light and low-humidity environments such as deserts reduce transpiration by lowering their stomatal index to cope with water stress (Raven et al. 1981).

The number of leaves per culm of *A. smithii* was not affected by increasing salt concentration. This unexpected result does not agree with findings in *Oryza sativa* (rice) (Yeo and Flowers 1984) and in *Borrichia frutescens* (Antlfinger 1981). Yeo and Flowers (1984) reported that in saline habitats the reduction of leaf number per culm in *O. sativa* was the result of an increase in leaf death rate.
and an unaltered production of new leaves. Yeo and Flowers (1982) also found that leaf sodium content of *O. sativa* increased as leaf age increased when this plant was exposed to saline environments. Early shedding of older leaves and young-to-old leaf gradients of sodium content permit younger leaves to remain at sublethal salt concentrations. So, reduction in the number of leaves per culm in saline environments for some species could be considered a survival factor. *Agropyron smithii* does not reduce the number of leaves per culm in saline environments and apparently does not use this means of survival. In contrast to *Oryza sativa*, Jefferies and Rudmik (1984) reported that in *Triglochin maritima*, which is a perennial species and widespread in saline and calcareous habitats, the death rate of leaves decreased in response to increased salinity. Further studies are needed to clarify the discrepant responses of this character to saline environments.

The number of culms per plant was significantly different among the five treatments (Table 2). Data obtained in this study support the hypothesis that salinity reduces vegetative organ numbers (Jefferies and Rudmik 1984). It appears that concentrations of NaCl above 50 mM do not have much additional negative impact on culm number in this species.

Sodium chloride had a negative impact on the length of culm and plant height of *A. smithii*. Responses of these two characteristics to salt are generally the same as those in *Borrichia frutescens* (Antlfinger 1981).

Leaf lengths and widths were significantly reduced by the presence of sodium chloride in culture solutions. Reduction of leaf growth by NaCl has also been found in some other plants such as *Phaseolus vulgaris* L. (Niemann and Poulsen 1971, Neuman et al. 1988), *Borrichia frutescens* (Antlfinger 1981), and *Triglochin maritima* (Jefferies and Rudmik 1984). One possible mechanism for NaCl inhibiting leaf growth is that reduction of water potential in the root zone causes reduction of turgor in leaf cells, thus reducing growth of the leaf. NaCl-induced reduction of turgor was found in seedlings of *Phaseolus vulgaris* L. grown in media with NaCl (Neumann et al. 1988).

### Growth Measurements

Fresh mass and dry mass of *A. smithii* were significantly reduced by the presence of sodium chloride in the nutrient solutions. Reduction of plant size by sodium chloride has been found in a variety of plants such as *Cicer arietinum* (Lauter et al. 1981) and *Trifolium repentum* (Smith and McComb 1981). Generally, NaCl reduces plant growth through (1) mineral competition, since it reduces the uptake and transport of nitrogen (Aslam et al. 1984), phosphate (Maas et al. 1979), potassium (Lynch and Läuchli 1984), and calcium (Lynch and Läuchli 1985); (2) toxic effects by reducing the photosynthetic production (Ball and Anderson 1986, Muller and Santarius 1978) and enzyme activity of RuBP carboxylase (Seemann and Critchley 1985); and (3) osmotic effects on water availability, which inhibit cell growth, cell-wall synthesis, protein synthesis, carbon assimilation and allocation (Cheeseman 1988), respiration (Glass 1988), and photosynthesis (Black and Bliss 1980).

Table 3. Size variation of *A. smithii* grown in nutrient solutions with five different NaCl concentrations. Values represent mean (n = 10) ± one standard deviation. Significance levels are for the F-tests from the ANOVAs. FM and DM represent fresh mass and dry mass, respectively.

<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>FM (g/plant)</td>
<td>10 ± 1.1</td>
<td>2.5 ± 0.6</td>
</tr>
<tr>
<td>DM (g/plant)</td>
<td>2 ± 0.23</td>
<td>0.6 ± 0.12</td>
</tr>
<tr>
<td>Root/plant ratio</td>
<td>0.12 ± 0.02</td>
<td>0.13 ± 0.05</td>
</tr>
<tr>
<td>Leaf DM/FM ratio</td>
<td>0.24 ± 0.03</td>
<td>0.26 ± 0.02</td>
</tr>
</tbody>
</table>
excessively high (Lützge and Smith 1984). It appears that *A. smithii* uses some other mechanism to tolerate the high concentration of NaCl within cells. Class (1988) reported that halophytes sequestered NaCl within the vacuoles and that the cytoplasmic phase was maintained isosmotic within the vacuole by means of noninjurious organic solutes such as glycerol, sucrose, amino acids (particularly proline), mannitol, and various other N-containing derivatives. Further study is needed to identify how *A. smithii* tolerates high intracellular NaCl concentrations.

**LITERATURE CITED**


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