IDENTIFYING AND CHARACTERIZING SALT-TOLERANT ALFALFA (*MEDICAGO SATIVA* SUBSP. *FALCATA*) GERMPLASM

Katelin E. Frerichs and Lan Xu*
Department of Natural Resource Management
South Dakota State University
Brookings, SD 57007
*Corresponding author email: Lan.Xu@sdstate.edu

ABSTRACT

Soil salinity limits plant growth and crop production. More than 20% of cultivated land worldwide is affected by salinity. The situation is becoming more severe due to shifts in precipitation and evaporation patterns and improper irrigation. There is an urgent need to develop salt-tolerant, economically valuable plants to minimize the loss of and to sustain agricultural production. Alfalfa is one of the most extensively cultivated forage crops. Some yellow-flowered alfalfa (*Medicago sativa* subsp. *falcata*) have exhibited morphological and physiological drought tolerance. Since soil salinity is associated with physiological drought, it is reasonable to expect that these drought tolerant *falcata* populations could be used for selecting potential parent materials for breeding salt-tolerant cultivars. Uniform seeds from eight alfalfa populations were selected, scarified, and inoculated with *rhizobium* before being seeded in pre-mixed salt-affected soil. Populations consisted of three *falcata* plant introductions (PIs), four predominately *falcata*, and one conventional-hay type as a control. Thirty-six seeds of each population were seeded in six rows per tray with four replicates. Emergence rate, survival, and growth stage were measured after 60 days. The results showed that relative emergence decreased as soil salinity levels increased. Relative emergence increased sharply then plateaued in low saline soil. In medium and high saline soils, relative emergence increased gradually and then also plateaued. PI631678 and PI502441 appeared to show characteristics in line with those of drought-tolerant alfalfa under drought stress, having the most promise as potential parent materials. Most populations consisted primarily of growth stage classes one and two except for ‘Persist II’ and ‘Wind River’, which had a significant amount of advanced development at growth stage class three. Persist II and Wind River populations also had the highest relative emergence despite not being regarded as drought-tolerant populations. These unexpected results could be due to seed size rather than other genetic characteristics.

Keywords

Lucerne, yellow-flowered alfalfa, saline soil, salinity, growth stage, emergence, seed germination, electrical conductivity
INTRODUCTION

Soil salinity is a major limiting factor affecting plant growth and crop production. More than 20% of cultivated land worldwide is affected by excessive soil salinity. This is primarily due to irrigation techniques on cropland which is continuing to increase in severity because of poor quality water and poor drainage (Chinnusamy et al. 2015). When poor quality water that contains salt is used, the soil pores become increasingly blocked over time as salt accumulates; this eventually results in decreased soil permeability. The combination of high salinity and decreased permeability leads to decreased water flow and nutrient availability, slowing or preventing plant growth (Cox et al. 2018). Salinity has been known to delay and reduce seedling emergence, survival, and establishment in many forage crops of various types (Forsberg 1953). In addition to the continuing use of poor irrigation techniques, a shift in precipitation and evaporation patterns is causing the problem of saline soils to increase in severity, particularly in arid to semi-arid regions around the world (Kumar et al. 2017). Because of these factors, the need for the development of economically valuable, salt-tolerant forage crops is becoming increasingly imperative to farmers and ranchers in mitigating losses in agricultural production (Munns and Gilliham 2015).

One such forage crop of economic and ecological importance is alfalfa, a species regarded for its high levels of protein, energy, vitamins, and minerals. As a high-quality forage (high in protein and low in fiber), it is also one of the cheapest forage options for producers when considering its price per pound of protein (Balliette and Torell 1998). It has also been observed to increase carbon and nitrogen levels in rangeland soils, making it useful for enriching overall soil quality and forage production (Mortenson et al. 2004). In this study, yellow-flowered alfalfa (*Medicago falcata*) was of particular interest because it is considered to be more tolerant of drought, cold, and grazing compared to purple-flowered alfalfa (*Medicago sativa* subsp. *sativa*) (Garver 1922). Since drought has similar physiological effects on plants as does salinity (Kumar et al. 2017), it was reasonable to suspect that drought-tolerant populations of alfalfa would have the potential to withstand higher levels of salinity in soil.

Yellow-flowered alfalfa was first introduced to the United States by Niels Ebbesen Hansen, around the 1920s after Hansen, while on an expedition in Russia and Siberia, made observations that yellow-flowered alfalfa in its native land had drought-resistant characteristics. This ultimately sparked his inquiries of its ability to be introduced to the United States and its potential usefulness in agricultural forage production (Rumbaugh 1979).

Our objective was to identify potential parent materials from the USDA Plant Introduction (PI) Collection and naturalized populations for breeding salt-tolerant alfalfa cultivars. This was achieved by characterizing variations in their emergence rate, growth, and development. In this study, we tested the hypothesis that drought-tolerant traits in alfalfa will allow it to grow and persist in highly saline soils, as Kumar et al. (2017) suggested.
METHODS

**Seed Source.** The eight alfalfa populations used in this study included three commercial cultivars (Persist II, Sholty, Wind River), two experimental populations (SD202, SD203), and three plant introductions (PIs) from the National Plant Germplasm System (PI502441, PI538984, PI631678) (Table 1).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Description</th>
<th>Developer/Marketer/Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI502441</td>
<td><em>M. sativa</em> subsp. <em>falcata</em></td>
<td>Russian Federation, Lat. 46° 11‘24” N, Long. 43° 53‘24” E, Elev. 550 m</td>
</tr>
<tr>
<td>NPGS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI538984</td>
<td><em>M. sativa</em> subsp. <em>falcata</em></td>
<td>Kazakhstan, Lat. 49° 12‘36” N, Long. 55° 50‘24” E</td>
</tr>
<tr>
<td>NPGS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI631678</td>
<td><em>M. sativa</em> subsp. <em>falcata</em></td>
<td>Mongolia, Lat. 49° 46‘40” N, Long. 91° 53‘52” E, Elev. 1463 m</td>
</tr>
<tr>
<td>NPGS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sholty</td>
<td>Predominantly <em>M. sativa</em> subsp.*falcata</td>
<td>SDSU, Brookings, South Dakota</td>
</tr>
<tr>
<td>SD 202</td>
<td>Predominantly <em>M. sativa</em> subsp.*falcata</td>
<td>SDSU experimental with coil shaped seed pods collected from a feral population in native rangeland in northwest SD</td>
</tr>
<tr>
<td>(Coiled)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD 203</td>
<td>Predominantly <em>M. sativa</em> subsp.*falcata</td>
<td>SDSU experimental with sickle shaped seed pods collected from a feral population in native rangeland in northwest SD</td>
</tr>
<tr>
<td>(Sickle)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Persist II</td>
<td><em>M. sativa</em>, Cultivar,     Conventional Hay-Type</td>
<td>Millborn Seeds Inc.</td>
</tr>
<tr>
<td>Wind River</td>
<td><em>M. sativa</em> subsp. <em>falcata</em></td>
<td>Wind River Seed, Manderson, Wyoming, developed by Norman G. Smith, Lodgepole, South Dakota</td>
</tr>
</tbody>
</table>

**Soil Preparation and Treatments.** Soil from a field affected by high salinity levels near Clark, South Dakota, and non-saline soil were thoroughly and equally mixed with a soil mixer on a volume to volume basis to create four salinity levels (Control, Low, Medium, and High) based on saturated paste electrical conductivity (EC); soil chemical properties were measured by Ward Laboratories (Table 2).

Each 25-cm X 25-cm seed flat with holes in the bottom was filled with equal amounts of designated salinity level soil; seed flats were then placed in 50-cm X 25-cm plastic trays with two same type soil flats per tray. This was done so that after planting, the populations could be watered by filling the bottom tray with deionized (DI) water, allowing the alfalfa to have access to water without leaching the salt out of the soil, thus maintaining the experimental salinity levels. Soils were saturated with DI water and drained overnight. Soil moisture readings were taken before planting at field capacity around 23% and then maintained at those levels throughout the experiment.
Table 2. Soil salinity treatment and soil chemical properties. Soil chemical properties were measured by Ward Laboratories, Inc., Kearney, Nebraska.

<table>
<thead>
<tr>
<th>Soil Salinity Treatment</th>
<th>Paste pH</th>
<th>Paste EC (mmho/cm)</th>
<th>Paste Ca (ppm)</th>
<th>Paste Mg (ppm)</th>
<th>Paste Na (ppm)</th>
<th>Paste SAR</th>
<th>Available N (ppm)</th>
<th>Total P (ppm)</th>
<th>Total K (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Saline:Saline Ratio (V:V)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (4:0)</td>
<td>7.4</td>
<td>1.76</td>
<td>252</td>
<td>59</td>
<td>15</td>
<td>0.2</td>
<td>41.9</td>
<td>19</td>
<td>116</td>
</tr>
<tr>
<td>Low (3:1)</td>
<td>7.3</td>
<td>6.01</td>
<td>537</td>
<td>245</td>
<td>941</td>
<td>8.4</td>
<td>56.3</td>
<td>25</td>
<td>174</td>
</tr>
<tr>
<td>Medium (2:2)</td>
<td>7.3</td>
<td>10.95</td>
<td>524</td>
<td>469</td>
<td>2082</td>
<td>15.8</td>
<td>80.2</td>
<td>32</td>
<td>214</td>
</tr>
<tr>
<td>High (1:3)</td>
<td>7.3</td>
<td>14.64</td>
<td>505</td>
<td>714</td>
<td>3092</td>
<td>20.7</td>
<td>88.0</td>
<td>31</td>
<td>221</td>
</tr>
</tbody>
</table>

1EC = Electrical Conductivity, 2SAR = Sodium Absorption Rate

Experimental Design and Procedure. A two-way factorial (8 × 4) experimental design consisted of the combination of eight alfalfa entries and four salinity levels (Control, Low Salinity, Medium Salinity, and High Salinity) with four replicates.

Before planting, we took initial field EC readings using a Field Scout Direct Soil EC Probe (Spectrum Technologies, Inc.); field EC readings were also taken every two weeks throughout experimentation to ensure saline levels remained at desired levels.

Intact seeds from each of the eight populations were selected and scarified with 320 sandpaper (Narem 2009) then inoculated with *rhizobium* prior to planting. Thirty-six seeds (six seeds/row X six rows) were planted approximately one centimeter below the soil surface in each of the 25-cm X 25-cm trays filled with the designated salinity level of field soil.

Adequate moisture levels were maintained by watering equal amounts of deionized (DI) water for each tray by filling the bottom flats and misting the tops for an equal amount of time with a sprayer.

Growth Conditions. Plants were maintained in a greenhouse at 24 ± 3 °C and a 16 hours light/eight hours dark photoperiod cycle. Trays were rotated every two weeks throughout the experiment to compensate for the potential effects of microclimate conditions in the greenhouse.

Data Collection. The number of seedlings that emerged were recorded every other day for 60 days. Electrical conductivity (EC) readings were taken and recorded every two weeks throughout the experiment to ensure proper saline levels were maintained. After 60 days, the individual plant growth stage for each plant was determined and recorded as cotyledon, unifoliolate, first trifoliolate, second trifoliolate, third trifoliolate, and so on. The height of each plant was also measured from the base to the apex of the plant (Figure 1).
Data Analysis. Relative emergence for each population was calculated with the following equation (Kim et al. 2012):

\[
\text{Relative emergence (\%)} = \frac{\text{No. of seedlings in the saline soil treatment}}{\text{No. of seedlings in the control}} \times 100
\]

The growth stage and development (GS) of each alfalfa plant was classified into one of three GS classes, then grouped for each population within each saline soil treatment.

- GS Class One: From cotyledon to first trifoliolate leaf
- GS Class Two: From second trifoliolate to third trifoliolate leaf
- GS Class Three: ≥ fourth trifoliolate leaf

Frequency of each GS class was analyzed using a Chi-squared ($\chi^2$) analysis (Statistix 9 2008) to detect the differences ($P < 0.05$) among salinity treatments within each population and among the eight populations.

RESULTS

Relative Emergence. Plants in low salinity soil showed rapid relative emergence and then leveled off quickly (<12 days) with Persist II and Wind River having the
highest relative emergence overall at 87 to 80%, followed by PI631678 (71%) and PI538984 (59%). PI502441 showed the lowest relative emergence at 50% (Figure 2).

![Graph showing relative emergence of alfalfa in various soil salinities over 60 days.](image)

**Figure 2. Relative emergence of alfalfa in various soil salinities over 60 days.**

For medium and high salinity soil, the relative emergence increased more gradually over time and then leveled off. In medium saline soil, Persist II showed the highest relative emergence (around 54%), followed by SD 203 (40%), Wind River (33%) and Sholty (23%); PI502441 had the lowest relative emergence, maintaining about 2% until the last week of the experiment when it increased to about 10%. In high saline soil, Persist II had the highest relative emergence (44%) followed by Wind River (32%), Sholty (16%), PI631678 (15%) and PI502441 (15%). Populations of SD 203, SD 202, and PI538984 had the lowest at around 5% relative emergence (Figure 2).
**Growth Stage.** The percentage of each GS showed significant differences among all populations within each treatment and among treatments within each population ($P < 0.05$). Most of the populations consisted primarily of GS classes one and two; PI502441 was nearly 100% GS classes one and two. Persist II, on the other hand, was about 56% GS class three in high saline soil, and the Wind River population was made up of approximately 50% GS class three in high saline soil (Figure 3).

![Figure 3. Percentage of growth stage of populations in different treatments at 60 days.](image-url)
DISCUSSION

Generally, relative emergence decreased as soil salinity levels increased. Most populations displayed a sharp increase then plateaued in low salinity compared to the more gradual increase in the medium and high salinity soils. This pattern is in accordance with delayed emergence, which is characteristic of drought-tolerant alfalfa under drought or harsh environmental conditions (Kim et al. 2012).

Overall, Persist II and Wind River had the highest relative emergence and significantly advanced growth stage and development demonstrated by a large percentage of GS class three. In a previous study, these two populations were not considered to be drought resistant compared to other populations (Hanson et al. 2015). This leads us to believe that this unexpected result could be attributed to the fact that Persist II and Wind River had comparatively larger seeds overall and not to drought resistant or salt-tolerant characteristics (Figure 4, Table 3). A larger seed size would mean that the alfalfa would have had a larger source of nutrients to access during the beginning stages of growth which would allow them not only to emerge quicker but also to grow larger. Steppuhn et al. (2008) observed that larger seed size was related to greater emergence for alfalfa and contributed to taller plants, findings which support the results observed in this experiment.

Figure 4. Seed size of alfalfa populations (except for PI502441) (6.7X).

Table 3. Mean 100-seed weights of seven alfalfa populations with 4 replications

<table>
<thead>
<tr>
<th>Entry</th>
<th>100-seed weight(^1) (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persist II</td>
<td>0.2287 (\text{A})</td>
</tr>
<tr>
<td>Wind River</td>
<td>0.1789 (\text{B})</td>
</tr>
<tr>
<td>SD 202 (Coiled)</td>
<td>0.1744 (\text{B})</td>
</tr>
<tr>
<td>SD 203 (Sickle)</td>
<td>0.1709 (\text{B})</td>
</tr>
<tr>
<td>PI 631678</td>
<td>0.1647 (\text{B})</td>
</tr>
<tr>
<td>Sholty</td>
<td>0.1266 (\text{C})</td>
</tr>
<tr>
<td>PI502441</td>
<td>0.1184 (\text{C})</td>
</tr>
<tr>
<td>PI 538984</td>
<td>0.1052 (\text{D})</td>
</tr>
</tbody>
</table>

\(^1\)Means followed by the same uppercase letter in the column do not differ at \(\alpha = 0.05\) by Tukey test.
We have also considered the possibility that the alfalfa plants may have used an avoidance mechanism in response to the saline soils. We observed that in the higher saline soil trays, plants generally grew better around the edges which is also where the holes in the bottom of the trays were located. It is possible that the plants were able to find these holes which would consequently lead them to the saline free DI water located in the bottom tray. Every tray in the experiment had holes in the same position, but we observed that the larger seeds were still able to emerge sooner and grow taller in comparison to the smaller seeded populations.

Of the smaller seeded populations, Sholty, PI502441, and PI631678 showed the highest relative emergence. This was likely the result of their drought tolerant traits, which would allow them to grow in higher saline soils. Although not conclusive, these results tended to support the original hypothesis. The study by Hanson et al. (2015) on drought-tolerant alfalfa germplasm identified the existence of drought-tolerant traits in these populations, and Kumar et al. (2017) identified similarities between the mechanisms used to combat drought and salt stress on plants. Since Hanson et al. (2015) identified the existence of drought tolerant traits in these three populations of alfalfa, and since Kumar et al. (2017) suggested a connection between drought tolerance and salt tolerance, our results support this connection.

Another factor that may have contributed to variation among treatments for frequencies of different growth stages is the lower concentration in N, P, K, Ca, and Mg levels in the non-saline soil compared to the other soil treatment mixtures (Table 2). Proper levels of these micronutrients in soil are important for growth and development (Chechin and de Fátima Fumis 2004), although these macronutrients met the minimum required for proper alfalfa development (SDSU 2005), some, such as K, may not be sufficient for proper rapid development. Variation among treatments could also be due to inadequate micronutrients, which we did not measure in this study.

Except for Persist II and Wind River, alfalfa populations in this study commonly exhibited GS classes one and two most probably because of the high salt content of the soil, meaning that they were unable to grow past this point into the third GS class, as was observed generally in plants by Bernstein (1975).

Information gained through this preliminary study could be expanded upon in a variety of ways such as experiments that hold nutrient levels and seed size constant, while varying only the salt concentration that the alfalfa is exposed to. Characteristics of the populations observed in this study have the potential to be used for economic and environmental value. Yellow-flowered alfalfa has already been acknowledged for its dual use as wildlife habitat and a forage crop (Boe et al. 1998), and results of this study could be potentially beneficial to increasing improvements in wildlife habitat quality and availability while simultaneously being a productive option for farmers and ranchers.

As has been well-documented previously, seed size appears to promote alfalfa emergence and early growth and development. This seems to be the case for Persist II, a large seeded species with no known *falcata* pedigree. We found that drought tolerant alfalfa populations, particularly PI631678, PI502441, and Sholty, grew well in saline soil; however, our study needs to be repeated to
determine if these populations will yield a productive forage crop. In addition, the effect of seed size on germination and growth of drought tolerant alfalfa populations should be determined.

ACKNOWLEDGEMENTS

We would like to thank Nicholas Asmussen for his continual support throughout this study, for Surendra Bam and Chloe Madsen for their help in the greenhouse and lab, for Christine Morris and Dr. Cheryl Reese for their valuable expertise in soil, and for Drs. Arvid Boe and Shenni Tian for pre-submission review of the manuscript, and providing valuable comments that greatly improved the manuscript. Research was partially funded by the Orville and Enolia Bentley Undergraduate Research Award program of South Dakota State University.

LITERATURE CITED


SDSU CES USDA 2005. South Dakota Fertilizer Recommendations Guild. EC750

