Germination salt resistance of alfalfa (*Medicago sativa* L.) germplasm in relation to subspecies and centers of diversity*

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Received 27 June 1989. Revised December 1989

Key words: alfalfa, breeding, germination, germplasm, *Medicago sativa* L., saline soils, salinity, selection, sodium chloride

Abstract

Saline soils and water severely limit the productivity of crop and pasture lands in semiarid and arid environments. The breeding of salt resistant cultivars of some crops is a partial solution to this problem. To breed for increased salt resistance, scientists must characterize the potentials and limitations of germplasm resources. This study measured the salt resistance of 761 alfalfa (*Medicago sativa* L. Emend. Sensu Lato) plant introduction accessions to NaCl during germination and characterized the resistance by subspecies, country of origin, and center of diversity. Experiments indicated that germplasm from the arid Indian and African centers excelled in NaCl resistance during germination. Germplasm from the Falcata center was least resistant. *M. sativa* L. subsp. *sativa* was more than twice as resistant as *M. sativa* L. subsp. *ambigua* or subsp. *falcata*. Thus, more resistant germplasm potentially adapted to the warm desert regions is available than resistant germplasm better suited to alfalfa production in more temperate regions.

Introduction

Approximately one-third to one-half of all irrigated lands contain undesirably high levels of salt (Yensen, 1988). Salinity also reduces forage production in large areas of nonirrigated pasture and rangelands. Salt resistant grasses and legumes are also needed for roadside conservation plantings (Greub et al., 1985). Breeding and selecting salt resistant cultivars could meet these needs.

Various techniques to screen and select alfalfa (*Medicago sativa* L. Emend. Sensu Lato) germplasm resistant to salt during germination have been developed. Carlson *et al.* (1983) germinated alfalfa seeds in antibiotic agar plates at eight NaCl concentrations. A quadratic response curve was generated for each alfalfa population and the NaCl concentration that inhibited germination of 50% of the viable seeds (IC50) was computed by probit procedures. Smith and Dobrenz (1987) used salt solutions in petri dishes containing only a Whatman1 no. 2 filter paper and no agar. This reduced the cost of the experimentation. Assadian and Miyamoto (1987) moistened sponges with saline solutions, placed seeds on one-half of each sponge, and covered them with the other half.

Although the mechanisms by which salt inhibits seed germination and seedling growth are not fully understood (Bliss *et al.*, 1984; Ramagopal, 1987), two types of resistance were identified in ‘Mesa Sirsa’ alfalfa: resistance to an ion toxicity effect specific to NaCl and a more general resist-

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* Joint contribution of the USDA-Agricultural Research Service and the Utah Agricultural Experimental Station Journal Paper no. 3782.
rance to lowered water potential (Allen et al., 1986). Broad sense heritability for germination NaCl resistance during five cycles of mass selection of Mesa-Sirsa was 50% (Allen et al., 1985). Narrow sense heritability of 'CUF 101' alfalfa seedling resistance to NaCl was 41% (Noble et al., 1984). Ashraf et al. (1987) employed different techniques and computed a realized heritability of 31% for the NaCl resistance of seedling 'Euver' alfalfa. Alfalfa germplasm resistant to salinity during germination and adapted to the warm desert region of the United States has been released (Dobrenz et al., 1983; 1989).

Although genetic resources used in breeding for salt resistance have typically been well adapted cultivars, there may be valuable sources of resistance in wild relatives of crop plants (Shannon and Qualset, 1984). Natural selection has resulted in halophytic maritime populations of at least one legume species (Trifolium repens L.) otherwise considered to be sensitive to NaCl (Ab-Shukor et al., 1988). Gunn et al. (1978) distinguished nine subspecies of M. sativa L. and Barnes et al. (1977) described nine germplasm sources (centers) representing most of the genetic diversity in alfalfa cultivars used in the United States. This study evaluated a portion of the accessions in the USDA-Agricultural Research Service alfalfa germplasm collection for their ability to germinate in NaCl solutions. Responses of these accessions were related to the subspecies and centers of diversity of the crop.

Materials and methods

Experimental entries consisted of 761 Plant Introduction accessions of Medicago sativa L. from the USDA-Agricultural Research Service Regional Plant Introduction Station at Pullman, WA. Seeds were those originally obtained by the collector (50 accessions) or which had been produced in isolation cages at Reno, NV or Prosser, WA during the preceding 9 years. All accessions had been stored at low temperatures at Ames, IA or Pullman, WA prior to evaluation. The Plant Introduction Station identified the subspecies of 517 of the accessions and 116 additional accessions were identified from listings by Gunn et al. (1978). The remaining 128 accessions could not be assigned to a subspecies or were mixtures of subspecies. It was possible to assign 420 accessions to one of the nine centers of diversity based upon the country in which the germplasm had been collected. Incomplete information precluded the assignment of 341 accessions.

The research approach of Carlson et al. (1983) was combined with the method of Smith and Dobrenz (1987). Eight NaCl concentrations were used: 0.0, 85.6, 128.3, 171.1, 213.9, 256.7, 299.4, and 342.2 mM NaCl in deionized water. After the completion of the experiment, 24 accessions with exceptionally low germination in 0.50% NaCl were reevaluated in 0.25% NaCl. Experimental units were 100-mm disposable plastic petri plates containing a single piece of Whatman 1 no. 2 filter paper to which was added 25 scarified alfalfa seeds, 0.75 mL of 0.8% aqueous solution of a fungicide (0.006 g phenyl mercuric ammonium acetate per plate), and 4.5 mL of the appropriate salt solution. The osmotic values of the germination media were measured as mmol kg⁻¹ with a Wescor Model 5500 vapor pressure osmometer and converted to MPa units (Table 1) by means of the following equation obtained from S. E. Smith (1988, personal communication):

\[
\text{Osmotic potential (MPa)} = [0.173 - (0.0269)(\text{mmol/kg})] \times 0.10.
\]

<table>
<thead>
<tr>
<th>NaCl concentration (wt/wt)</th>
<th>(mM)</th>
<th>Osmotic potential (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.0</td>
<td>-0.05234</td>
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<tr>
<td>0.25</td>
<td>42.8</td>
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<tr>
<td>0.50</td>
<td>85.6</td>
<td>-0.33539</td>
</tr>
<tr>
<td>0.75</td>
<td>128.3</td>
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<tr>
<td>1.00</td>
<td>171.1</td>
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<td>1.25</td>
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<tr>
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<td>256.7</td>
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</tr>
<tr>
<td>2.00</td>
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