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Screening for Salinity Tolerance in Alfalfa: A Repeatable Method

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ABSTRACT

A lack of salt-tolerant cultivars may be due in part to inadequate evaluation protocols used for selection. Our objective was to develop a greenhouse protocol that is simple and consistently separates genotypes for their relative ability to survive under saline conditions. In 2000 and 2001, 12 alfalfa (*Medicago sativa* L.) cultivars were seeded in 3.8- by 21-cm cone-shaped containers plugged with capillary matting and filled with silica sand. Six-week-old seedlings were submersed in a NaCl-nutrient solution starting at an electrical conductivity (EC) of 3.0 dS m^{-1} and increased 3.0 dS m^{-1} every 1 to 2 wk for 10 to 13 wk. Probit analysis was used to estimate the time and salt dose to reach 50 (LD_{50}) and 75% (LD_{75}) mortality. Probit results were compared with cultivar ranking for mean percentage plant mortality when overall trial mortality reached approximately 50 and 75%. Pearson's rank correlations between 2000 and 2001 at the LD_{50} and LD_{75} levels were $r = 0.90$ ($P < 0.001$) and $r = 0.88$ ($P < 0.001$), respectively. Rank correlations between 2000 and 2001 based on means when overall trial mortality levels were approximately 50 and 75% were $r = 0.92$ ($P < 0.001$) and $r = 0.85$ ($P < 0.001$), respectively. The correlations between mean percentage cultivar mortality rankings and the probit-based rankings were above $r = 0.90$ ($P < 0.001$) in both years. The high correlations verify that this protocol produces repeatable results and provides a method to effectively screen large numbers of plants for survival under saline conditions.

In semiarid regions, limited water and hot dry climates frequently cause salinity concentrations that limit or prevent crop production. At low concentrations salt suppresses plant growth, and at higher concentrations can cause death (Shannon, 1984). In some areas under intensive crop management, it has been economically possible to desalinze the soil (Kelley et al., 1979). For many agricultural purposes and in locations such as arid range lands, the cost and lack of water make reclamation of saline soils prohibitive. The alternative reclamation procedure is to grow salt-tolerant species and cultivars in the soils with salt problems. Unfortunately, the most salt-tolerant species are generally not the most productive or desirable. Improving salt tolerance does show promise in desirable range land species, including alfalfa (Noble et al., 1984; Allen et al., 1985, 1986; Mohammad et al., 1989; Rumbaugh and Pendery, 1990; Smith et al., 1994), tall wheatgrass [*Elytrigia pontica* (Podp.) Holub] (Shannon, 1978), slender wheatgrass [*Elymus trachycaulus* (Link) Gould ex Shinners], alpine bluegrass (*Poa alpina* L.) (Acharya et al., 1992), and crested wheatgrass [*Agropyron cristatum* (L.) Gaertn.] (Dewey, 1960, 1962).

Even though the literature contains numerous reports indicating variability for tolerance to salinity in many crops, few salt-tolerant cultivars have been released (Flowers and Yeo, 1995). Sruvastave and Jana (1984) and Shannon (1984) attribute the lack of salt-tolerant cultivars to multiple factors, including inadequate means of detecting and measuring plant response to salinity and ineffective selection methods. Selection of salt-tolerant plants from saline fields or plots seems a logical step for most plant breeders; however, this procedure has not produced consistent results (Shannon, 1984). Selection in the field is not efficient because soil salinity varies substantially with time, location, soil type, and depth. Furthermore, it has been reported that little relationship exists between tolerance at germination and later growth stages in crops, such as alfalfa (Al-Niemi et al., 1992; Johnson et al., 1992), soybean [*Glycine max* (L.) Merr.] (Abel and MacKenzie, 1964), and rice (*Oryza sativa* L.) (Pearson and Bernstein, 1959; Pearson et al., 1966).

Smith (1993) identified three stages at which alfalfa plants may be affected by salinity: germination, seedling growth, and mature plant growth. Evaluation and selection for salt tolerance (survival) in alfalfa (Allen et al., 1985; Ashraf et al., 1987; Mohammad et al., 1989; Rumbaugh and Pendery, 1990; Al-Niemi et al., 1992; Rumbaugh et al., 1993) and other crops (Dewey, 1960, 1962; Norlyn and Epstein, 1984) at germination is replete in the literature. These are relatively simple procedures and have been used with success. Examples of screening for tolerance during seedling growth and development are fewer and involve growing plants directly in nutrient solution (Ashraf et al., 1987; Almansouri et al., 1999; Zhu et al., 2001) or growing plants in sand or artificial soil (Sacher et al., 1983; Richards, 1992; Shannon, 1978; Steppuhn and Wall, 1999). As suggested by Richards (1992), growing plants in a hydroponic solution to screen for salinity tolerance may be appropriate for marsh plants or for understanding the effects of salt on plant growth, but a hydroponic solution is not representative of the natural habitat of most agricultural plants. In more appropriate systems that use sand as the growth medium, Richards (1992) used 11- by 50-cm tubes, Shannon (1978) used 36- by 36- by 15-cm wooden boxes, and Steppuhn and Wall (1999) used pots, and each involved complex watering systems to control salt concentration.

Our objective was to develop a greenhouse protocol to screen large numbers of plants that is characterized by simplicity, ease of use, and consistent separation of genotypes based on their relative ability to survive under

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Abbreviations: EC, electrical conductivity; r, Pearson's rank correlation; r_s, Spearman's rank correlation; SAR, sodium adsorption ratio.

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saline conditions. A second objective was to determine the variability for salt tolerance among 12 alfalfa cultivars.

MATERIALS AND METHODS

Plant Materials

The study was conducted in a greenhouse in Logan, UT, in 2000 and 2001. Alfalfa cultivars were Alfagraze, Drylander, Forager, Nomad, Rangelander, Ranger, Riggs, Salado, Spreader 3, Travois, Vernal, and Wrangler. Seeds, from the same seed lot in both years, were planted 1.5 cm deep in 3.8- by 21-cm Ray Leach Cone-tainers (Stuewe and Sons, Corvallis, OR) filled with 70-grit silica sand and misted twice daily with tap water until seedlings emerged (7 to 10 d). Silica sand was used because it is an inert media that will minimize the accumulation of salt across time. The 70-grit particle size was used because courser grits did not hold sufficient moisture. The bottom opening of the Cone-tainers was plugged with a 10- by 10-cm square of capillary matting. The matting confined sand to the cones and slowed the flow of water into the cones when placed in the salt solution. Without matting, the nutrient solution moved into the cones quickly, and the sand caused abrasions on roots of the young seedlings, resulting in rapid death of all plants, and differences among cultivars could not be measured.

When seedlings reached the first trifoliate leaf stage, all water applications were made by immersing flats containing 98 cones into a complete nutrient solution (Table 1). The precise mixing time and order was critical to prevent chemical precipitation of nutrients and salts. The storage tank was covered with a heavy black fabric to prevent light from reaching the solution, thereby limiting algae growth. The stock solution was diluted 5:1 and transferred from the storage tank into custom-built rectangular dipping tanks designed to hold four 98-cone flats. Dipping tanks were constructed of 14-gauge galvanized sheet metal and measured 36 cm high by 76 cm wide by 142 cm long. A 2.5-cm lip around the top edge of the tank provided rigidity. Flats were left in the solution for 2 min, the time required for the sand to reach water levels that exceeded field capacity. Plants were dipped on Monday and Thursday mornings every week for the duration of the study. Sand remained moist between each application of the salt-nutrient solution, and water stress did not occur.

Salt Screening

Salt imbalance in nutrient solutions is a frequent deficiency in screening studies and cultivar assessments (Shannon, 1984). To avoid an imbalance in the salt-nutrient solution, NaCl and $CaCl_2$ were used in proportions to maintain a sodium adsorption ratio (SAR) of 3.5. The amounts of NaCl and $CaCl_2 \cdot H_2O$ required in solution to obtain the desired level of salinity, measured by EC, while maintaining a SAR of 3.5 were determined by solving equations for SAR and EC as functions of Ca and Na concentrations as follows:

Richards (1954) gives an approximate relationship between the solution EC and total soluble salt (TSS) as

$$\mathrm{TSS} = 10 \times \mathrm{EC} \qquad [1]$$

where units for TSS are $\mathrm{mmol_c\ L^{-1}}$ and EC is $\mathrm{dS\ m^{-1}}$. The SAR is given by Richards (1954) as

$$\mathrm{SAR} = \mathrm{Na}/\sqrt{\mathrm{Ca} + \mathrm{Mg}} \qquad [2]$$

where SAR is $(\mathrm{mmol\ L^{-1}})^{-1/2}$ and ion concentrations are mmol $\mathrm{L^{-1}}$. For a system containing only NaCl and $CaCl_2$, the EC and SAR are given by

$$2 \times \mathrm{Ca} + \mathrm{Na} = 10 \times \mathrm{EC} \qquad [3]$$

Table 1. List of nutrients used in making an original complete stock nutrient solution used for salt screening solution.†

Nutrient	Amount	Mixing time
	mg L^{-1}	h
Macronutrients		
$Ca(NO_3) \cdot 4H_2O$	4723	2
KCl, anhydrous	956	2
KH_2PO_4, anhydrous	676	2
KNO_3, anhydrous	1516	2
K_2SO_4, anhydrous	1233	12
$MgSO_4 \cdot 7H_2O$	4314	2
$NH_4H_2PO_4$, anhydrous	1437	2
Micronutrients		
H_3BO_3, anhydrous	4.24	0.5
$CuSO_4 \cdot 5H_2O$	0.20	0.5
$MnSO_4 \cdot 7H_2O$	3.80	0.5
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.43	0.5
$ZnSO_4 \cdot 7H_2O$	0.49	0.5
Fe chelate‡	299.57	0.5

† Macronutrients were added directly to distilled water in a large tank with constant water agitation and air percolation. Micronutrients were mixed individually in a beaker with a stir bar for 1/2 h each, then added to the main tank 5 min apart with agitation. Once the stock solution was mixed, it was transferred to a covered storage tank in the greenhouse and diluted 5:1 with tap water.

‡ As Sequestrene 330 (Ciba-Geigy Corp., Greensboro, NC) 10% Fe concentration. The iron chelate must be added to the main tank last. Adding it earlier limits visibility through the solution. As a final step, the complete solution should be aerated for 12 to 24 h.

$$\mathrm{SAR} = \mathrm{Na}/\sqrt{\mathrm{Ca}} \qquad [4]$$

where all concentrations are in units of mmol $\mathrm{L^{-1}}$. The amount of NaCl and $CaCl_2$ required to create a treatment of specified EC and SAR can be computed from Eq. [3] and [4] as follows. Eq. [4] is solved for Ca, $\mathrm{Ca} = (\mathrm{Na/SAR})^2$, and the result substituted into Eq. [3], yielding the quadratic equation

$$2/\mathrm{SAR}^2 \times \mathrm{Na}^2 + \mathrm{Na} - 10 \times \mathrm{EC} = 0 \qquad [5]$$

The concentration of Na (mmol $\mathrm{L^{-1}}$) is obtained by solving the quadratic equation using the quadratic formula

$$\mathrm{Na} = \frac{-1 + \sqrt{1 + 4\dfrac{2}{\mathrm{SAR}^2}10 \times \mathrm{EC}}}{\left(\dfrac{4}{\mathrm{SAR}^2}\right)} \qquad [6]$$

In turn, the concentration of Ca (mmol $\mathrm{L^{-1}}$) is obtained by solving Eq. [3] for Ca,

$$\mathrm{Ca} = 5 \times \mathrm{EC} - \mathrm{Na}/2 \qquad [7]$$

and substituting the Na concentration computed from Eq. [6]. The mass of salt (mg $\mathrm{L^{-1}}$) is obtained by multiplying Na (mmol $\mathrm{L^{-1}}$) by the molecular weight of NaCl (mg $\mathrm{mmol^{-1}}$) and Ca (mmol $\mathrm{L^{-1}}$) by the molecular weight of the $CaCl_2$ salt ($CaCl_2 \cdot 2H_2O$ or $CaCl_2 \cdot 6H_2O$).

The actual EC was measured with an Orion Model 120 conductivity meter (Thermo Electron, Inc., Beverly, MA) (Table 2). To avoid precipitation during mixing, salts were dissolved into solution separately using a stir plate at 20% concentrations. They were then added to the stock nutrient solution 24 h before use and kept under constant agitation with air.

After 6 wk of growth when roots were well developed, plants were subjected to salt concentrations starting at an EC of 3.0 dS $\mathrm{m^{-1}}$ and increased in 3 dS $\mathrm{m^{-1}}$ increments every 1 to 2 wk until an EC level of 21 dS $\mathrm{m^{-1}}$ was reached in 2000 and an EC of 18 dS $\mathrm{m^{-1}}$ was reached in 2001 (Table 2). The incremental increase in salt concentration is critical to avoid the physiological shock to the plants and rapid death described by Richards (1954) that results in loss of differential response

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Table 2. Calculated and measured electrical conductivity (EC) values of salt nutrient solution used to screen alfalfa plants for salinity tolerance in 2000 and 2001.

Time in salt solution	2000 Calculated EC	2000 Measured EC	2001 Calculated EC	2001 Measured EC
wk	dS m^{-1} †			
1	3	4.43	3	4.69
2	6	7.12	6	7.23
3	9	8.97	9	9.47
4	12	11.04	9	9.47
5	15	14.62	12	11.23
6	15	15.72	15	15.1
7	18	17.16	18	17.56
8	18	18.33	18	18.72
9	18	17.11	18	19.04
10	21	21.2	18	18.92
11	–	–	18	17.78
12	–	–	18	18.32
13	–	–	18	17.86

† Electrical conductivity at 25°C.

among plants. The salt screening was started during the first week of February each year. Greenhouse temperature ranged from 20°C on cold nights to 28°C on sunny days. Supplemental lighting was not used. Photoperiod was approximately 10 h when the studies were initiated in February and 14 h upon completion in late April to early May.

Design and Analysis

A randomized complete block design was used with four replications. Each cultivar was represented by 30 plants per replication. In the 2000 screening, Replication 4 was located near an exhaust fan. These plants died in approximately one half the time as those in the other three replicates and were not included in the analysis. Each 98-cone flat contained two cultivars, each in a seven-by-seven plant configuration. To avoid a border effect, the outside row of cones from each flat was not evaluated, resulting in a five-by-six plant configuration of evaluated plants. Outside cones were often bumped during the dipping process, and preliminary tests (data not presented) indicated that plants in these cones died at an accelerated rate.

Starting 4 wk after the first exposure to salt, the number of dead plants was recorded each time plants were dipped in the salt solution. Salt concentration was increased incrementally across time; thus, plant death was a dose–time response. To account for both relative time and salt concentration, a cumulative linear value was calculated that accounted for salt concentration as measured by EC of the solution and the number of days at each EC concentration. This value, termed *ECdays*, was calculated by multiplying the EC concentration by the number of days at that concentration and summed across time, as shown below:

$$\text{ECdays}_i = \Sigma(\text{EC}_1 \times \text{D}_{\text{EC}_1} + \text{EC}_2 \times \text{D}_{\text{EC}_2} + \ldots \text{EC}_i \times \text{D}_{\text{EC}_i})$$

where EC_i = the ith electrical conductivity concentration, and D_{EC_i} = the number of days at the ith electrical conductivity concentration.

Probit analysis (SAS Institute, 1999) was used to estimate the lethal dose in terms of ECdays to kill 50% (LD_{50}) and 75% (LD_{75}) of plants for each cultivar. To facilitate separation of cultivar means, probit analysis was also used to estimate the ECdays to reach LD_{50} and LD_{75} on individual replications, and the results were then subjected to an ANOVA. Plant mortality data at a specific point in time were subjected to an ANOVA when approximate overall mortality had reached 50 and 75% in each of the 2 yr. These points were selected in time to compare means calculated for each cultivar in an ANOVA to the respective LD_{50} or LD_{75} calculated for each cultivar in a probit analysis. The closest evaluation dates to the 50 and 75% mortality target had an average 55.5 and 72.4% mortality in 2000 and 55.4 and 73.6% mortality in 2001. To simplify discussion, these data from both years are referred to as 55 and 73% mortality (as opposed to LD_{50} and LD_{75}). A protected LSD ($P = 0.05$) was used to separate cultivar means. Spearman rank and Pearson correlation coefficients were calculated between years (runs) to determine the repeatability of the protocol and to compare the two methods of evaluating the data.

RESULTS AND DISCUSSION

Significant differences ($P < 0.01$) were observed among cultivars both years at both the LD_{50} and LD_{75} levels. Comparison of results from the probit analysis between the 2 yr showed a high level of repeatability (Tables 3 and 4). The Pearson correlation coefficient between 2000 and 2001 LD_{50} values was $r = 0.83$ ($P < 0.001$). Similarly, the correlation between the 2 yr for the LD_{75} values was $r = 0.86$ ($P < 0.001$). Rank correlations were slightly higher, with a Spearman's rank correlation of $r_s = 0.90$ ($P < 0.001$) between the 2 yr at the LD_{50} level, and $r_s = 0.88$ ($P < 0.001$) at the LD_{75} level. As indicated by high correlations, rank changes tended to be small between years (Tables 3 and 4). A notable difference between the 2 yr was the increased number of ECdays estimated to reach the LD_{50} and LD_{75} levels. For instance, in 2000 the average requirement to reach

Table 3. Correlation matrix (r values) for response to salinity of 12 alfalfa cultivars: Spearman rank correlations (above the diagonal) and Pearson correlations (below the diagonal). Response measured as the required time-dose (in ECdays†) to kill 50 and 75% of plants (LD_{50} and LD_{75}), and percentge cultivar plant mortality when overall plant mortality reached 55 and 73% mortality.

		LD_{50}		LD_{75}		55% mortality		73% mortality	
		2000	2001	2000	2001	2000	2001	2000	2001
LD_{50}	2000		0.90***	0.94***	0.91***	0.97***	0.91***	0.97***	0.86***
	2001	0.83***		0.92***	0.94***	0.92***	0.92***	0.92***	0.84***
LD_{75}	2000	0.98***	0.86***		0.88***	0.91***	0.85***	0.96***	0.79**
	2001	0.85***	0.97***	0.86***		0.91***	0.92***	0.90***	0.96***
55% mortality	2000	−0.95***	−0.90***	−0.93***	−0.92***		0.92***	0.96***	0.85***
	2001	−0.84***	−0.96***	−0.84***	−0.93***	0.93***		0.90***	0.90***
73% mortality	2000	−0.90***	−0.86***	−0.91***	−0.85***	0.93***	0.90***		0.85***
	2001	−0.75**	−0.92***	−0.78**	−0.96***	0.83***	0.87***	0.81**	

**$P \leq 0.01$.
***$P \leq 0.001$.
† ECdays = electrical conductivity of salt solution multiplied by the number of days at a given electrical conductivity and summed across time.

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Table 4. Salt tolerance of 12 alfalfa cultivars based on the number of ECdays required to reach 50 (LD_{50}) and 75% (LD_{75}) mortality.

	LD_{50}				LD_{75}			
	ECday‡		Rank		ECday		Rank	
Cultivar†	2000	2001	2000	2001	2000	2001	2000	2001
Wrangler	652	982	2	1	761	1096	2	1
Alfagraze	678	952	1	2	771	1073	1	4
Nomad	651	940	3	3	740	1090	4	2
Salado	620	930	5	4	719	1075	5	3
Ranger	649	871	4	6	753	997	3	5
Forager	610	869	6	7	683	992	8	6
Vernal	596	873	9	5	703	975	6	7
Travois	602	865	7	9	697	948	7	9
Spredor 3	601	867	8	8	669	975	9	8
Rangelander	561	826	10	10	622	911	10	11
Riggs	508	811	11	12	597	915	11	10
Drylander	473	819	12	11	554	896	12	12
Mean	600	884			689	995		
LSD (0.05)	82	70			103	77		

† Cultivars arranged by average rank.
‡ ECdays = electrical conductivity of salt solution multiplied by the number of days at a given electrical conductivity and summed across time.

the LD_{50} was 600 ECdays compared with 884 in 2001 (Table 4). The difference between the 2 yr may reflect the rate at which the salt concentration was increased (Table 2). Factors not measured, such as higher solar radiation during 2000, might have resulted in increased greenhouse temperature, causing the cooling fans to operate more frequently. The net effect of this would have been increased transpiration and/or evaporative loss with an associated increased salt concentration in the relatively small rooting area of the cone-tainers. Regardless of the cause, the relative ranking for salt tolerance among the cultivars was consistent between years.

Differences observed among cultivars were significant at the 55 and 73% mortality levels ($P = 0.01$ and $P = 0.05$, respectively) in 2000, and cultivars were highly significant ($P < 0.001$) at both the 55 and 73% mortality levels in 2001. The Pearson correlation of cultivar mean percentage dead plants between the 2 yr at the 55% mortality level was $r = 0.93$ ($P < 0.001$), with a corresponding rank correlation of $r_s = 0.92$ ($P < 0.001$) (Tables 3 and 5). At the 73% mortality level, the Pearson correlation between cultivar mean percentage dead plants was $r = 0.81$ ($P = 0.001$) and the corresponding rank correlation was $r_s = 0.85$ ($P < 0.001$). While the correlations between years from the probit analysis results were similar at the LD_{50} and LD_{75} levels, the correlation between cultivar mean percentage dead plants at the 55% mortality level was somewhat better than that at the 73% mortality level. This is not surprising since the separation between cultivar means at the 55% mortality level was larger, with a difference of 35% points between the highest and lowest cultivar, compared with just over a 20 percentage point difference at the 73% mortality level (Table 5). Even though there was a larger separation at the lower level, the rank correlations between the two mortality levels was high, with $r_s = 0.96$ ($P < 0.001$) in 2000 and $r_s = 0.90$ ($P < 0.001$) in 2001 (Table 3). Similarly, high rank correlations were observed between the LD_{50} and LD_{75} from the probit analysis with $r_s = 0.94$ ($P < 0.001$) in both 2000 and 2001.

Cultivar ranking was similar between probit analysis and means at specified mortality levels. The correlation between the cultivar ranking at the 55% mortality level with the LD_{50} was $r_s = 0.97$ ($P < 0.001$) in 2000 and $r_s = 0.92$ ($P < 0.001$) in 2001. The correlation between cultivar ranking of the 73% mortality level and the LD_{75} was $r_s = 0.96$ ($P < 0.001$) in 2000 and 2001 (Table 3).

Plants were observed in all cultivars that showed some level of salt tolerance and were still alive when the study was completed. However, of the cultivars tested,

Table 5. Percentage mortality within 12 individual alfalfa cultivars when overall trial mortality reached 55 and 73%.

	55% Mortality				73% Mortality			
	Dead		Rank		Dead		Rank	
Cultivar†	2000	2001	2000	2001	2000	2001	2000	2001
	%				%			
Wrangler	40.0	35.8	3	1	63.3	62.5	2	1
Alfagraze	37.7	36.7	1	2	58.9	66.7	1	4
Nomad	37.8	42.5	2	3	68.9	65.8	4	3
Salado	52.2	54.2	5	4	71.1	63.3	5	2
Ranger	46.7	58.3	4	7	65.6	71.7	3	5
Forager	53.3	56.7	6	6	73.3	75.8	6	6
Vernal	56.7	55.8	7	5	74.4	76.7	7	7
Travois	57.8	60.0	8	8	74.4	78.3	7	9
Spredor 3	60.0	63.3	9	9	75.6	80.8	8	10
Rangelander	63.3	68.3	10	10	77.8	81.7	9	11
Riggs	73.4	69.2	12	11	80.0	78.3	10	8
Drylander	73.3	70.8	11	12	81.1	82.5	11	12
Mean	55.2	55.4			72.4	73.6		
LSD (0.05)	19.9	14.5			12.4	7.7		

† Cultivars arranged by average rank.

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Wrangler, Alfagraze, Nomad, and Salado had the highest number of plants that were able to withstand the increasing levels of salt (Tables 4 and 5). Spredor 3, Rangelander, Riggs, and Drylander had the least number of plants that showed tolerance to the increasing levels of salt.

CONCLUSIONS

High correlations between years, when comparing both means and results from probit analysis, verify that this protocol produces repeatable results. High rank correlations between the levels of mortality indicate that relative ratings of cultivars remain consistent even as mortality increases to the 75% level. However, a stronger correlation at the midlevel of mortality and a larger range between means indicate that a separation of cultivars would probably best be accomplished near 50% mortality. Ranking entries by estimating LD_{50} values or using means when approximate overall trial mortality has reached 50% appear to be equally efficient. In designing the study to calculate LD_{50} values by using 30-plant replicates and scoring plants dead or alive likely increased repeatability compared with many one-plant replicates visually scored for injury (data not shown).

This protocol provides a method to effectively screen large numbers of plants for their relative ability to survive under saline conditions. Compared with systems described by Shannon (1978), Richards (1992), and Steppuhn and Wall (1999), it is simple and requires relatively little investment. While we used this protocol to evaluate alfalfa, it should be equally effective, with minor modifications, to screen other perennial and annual crops. Furthermore, this protocol will be useful as a selection tool since virtually any level of selection intensity can be obtained and survivors from the best families selected. Clones or progeny from selections can then be tested using the protocol or in the field. While different nutrient solutions were not tested, the one used provided excellent plant growth and ensured that plants were not stressed for nutrients during the screening process.

A limitation of making selections in a greenhouse using this protocol is the potential for associated changes due to plieotropic effects. While final evaluation and selection for salt tolerance in alfalfa and other plant species will require field evaluations, this is a simple and efficient method of screening for tolerance to saline conditions during the first 3 to 4 mo of growth.

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