Testing Salt Tolerance Variability Among Tall Wheatgrass Lines[1]

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ABSTRACT

The vague symptoms of moderate salt stress on plants and the lack of understanding about the mechanisms that confer salt tolerance have hampered efforts to develop reliable screening procedures for this trait. In the present study a screening procedure was evaluated to determine its usefulness in detecting variation among introductions of tall wheatgrass [*Agropyron elongatum* (Host) Beauv.]. In greenhouse sand flats, 32 lines of tall wheatgrass were established and subjected to stepwise increases in salinity up to 765 meq/liter or until severe leaf damage resulted. The lines were classified into five groups based on relative leaf damage and recovery rates from salt treatment. Repeating the screening procedure on seven each of the most tolerant and most sensitive lines reaffirmed the results of the first screening. Mineral analyses indicated that tolerance was associated with restricted accumulation of Na, Ca, and Cl in the shoots. Proline and soluble sugars contributed to osmotic adjustment at high salinities, but sensitive and tolerant lines did not differ in proline content. This screening technique appears to discriminate between lines with different ion transport properties and different salt tolerances. The sensitive and tolerant lines identified may be beneficial in future breeding and physiological studies.

Additional index words: **Plant selection, Varietal differences, *Agropyron elongatum* (Host) Beauv.**

THE key to improving salt tolerance in plants and studying its inheritance lies in finding sufficient variation within breeding populations and devising a screening procedure capable of identifying resistant or tolerant genotypes. Once genotypes are identified, the breeder or geneticist can use the advanced screening procedures either to improve salt tolerance or to study its heritability.

Tall wheatgrass (*Agropyron elongatum* (Host) Beauv.) is a perennial bunchgrass used as forage on western rangelands. This grass is relatively high yielding under adverse conditions caused by low rainfall, lack of adequate irrigation water, and saline and alkaline soils. It is distinctly superior in salt tolerance to other *Agropyron* species (5). Although techniques for breeding for increased tolerance have been suggested (5, 6) no attempt has recently been made either to improve the salt tolerance of tall wheatgrass or to use the noted variability to study the inheritance of this trait.

In this study, a program was initiated to 1) develop a technique whereby lines could be rapidly and effectively screened for salt tolerance, 2) evaluate the genetic variation extant among *A. elongatum* introductions, and 3) describe factors of plant growth under salinity stress that may prove useful for determining heritable mechanisms that might contribute to tolerance.

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MATERIALS AND METHODS

Plant Establishment. Thirty-two accessions of tall wheatgrass that had been collected from various locations in Europe, Asia, Australia, and South America, were obtained from the USDA Plant Introduction Station in Pullman, Wash. Plant introduction numbers are listed in Table 1. Seeds were germinated on filter paper blotters at 21 C until the plumules had emerged from their coleoptile sheaths. Twenty-five plants of each accession were planted in plastic-lined wooden boxes (36 × 36 × 15 cm) containing 26 kg of fine silica sand. Holes in the plastic allowed adequate drainage. Each box was flushed twice daily with excess amounts of nutrient solution containing 2.5 m*M* Ca $(NO_3)_2$, 3 m*M* KNO_3, 1.5 m*M* $MgSO_4$, 0.17 m*M* KH_2PO_4, 0.05 m*M* Fe as EDTA-complex, and 0.5 ml/liter of micronutrient solution (11). The pH of the solution was 6.0 ± 0.2. Seedlings were kept in a greenhouse and grown for 7 weeks before salination.

Screening Procedure. To prevent phosphate toxicity, a nutrient medium that contained low phosphate concentration was used (15, 16). Salts were added in equal equivalents of NaCl and $CaCl_2$ to prevent Ca deficiencies and Na toxicities. The experiment was repeated during different times of the year on the most tolerant and sensitive accessions to confirm the results and to detect changes in response that might be attributed to different regimes of irradiation and temperature.

Salinity treatment was begun on the 8th week. Salt was increased every 2 days by additions of equal equivalent amounts of NaCl and $CaCl_2$ at a total rate of 16.8 meq mixed salt per liter. Weekly notes were made on the comparative appearance and growth of the accessions. When treatment symptoms of leaf burn and wilting had progressed to the point at which 90% or more of the plants appeared near death and all growth had stopped, the plants of that accession were revived by leaching the sand culture repeatedly with distilled water and finally with nonsalinized nutrient solution. In the initial screening, salinity was increased for 3 months (21 March to 19 June 1975) for the most tolerant accessions.

The accessions were categorized into five classes, based upon 1) the concentration of salt withstood before severe leaf damage resulted and 2) the percentage of plant recovery after leaching (Table 1). Seven each of the most tolerant and most sensitive accessions were selected for a secondary screening. These plants were established in sand cultures, as described previouly, using a randomized design with two replications. Salin-

Table 1. Classification of 32 wheatgrass accessions based on their relative salt tolerance.

Class	Final salinity	Recovery	P.I. numbers
	meq/liter	%	
I	765	95–100	142012, 205279 276399, 276709 283164, 297871 315352
II	765	70–94	098526, 179162 179169, 206622 206624, 251443 255146, 255149 308592, 340062 340063, 368850 368851
III	535	<70	234708, 283163 308591, 380626 383545
IV	380	30–80	204383, 222958 238222, 249144 255148
V	380	<10	206623, 222959

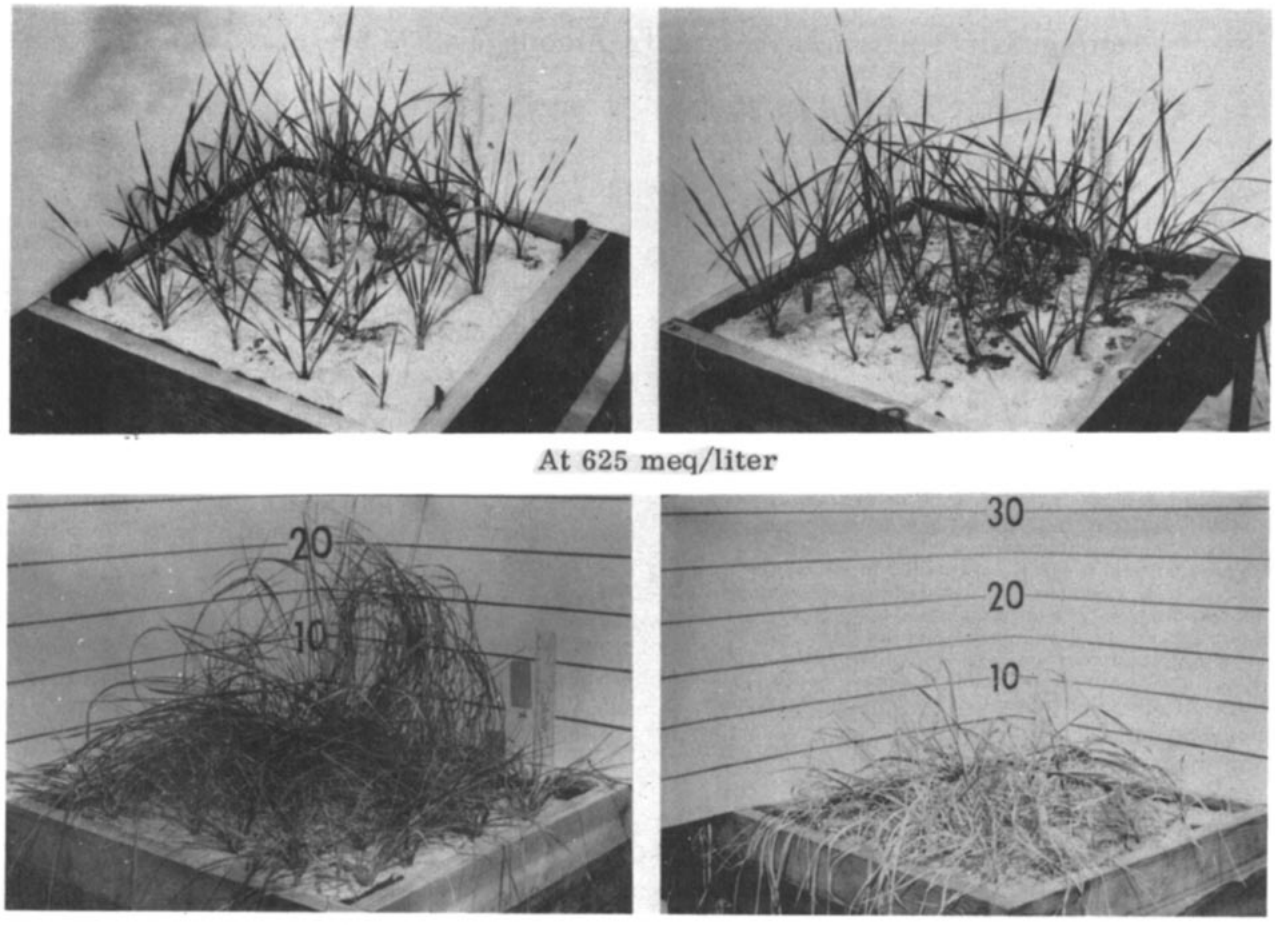

Fig. 1. Effect of salinity treatment on tolerant and sensitive accessions of tall wheatgrass. Salinity increases are described in Materials and Methods.

ity was increased from 28 Aug. to 8 Dec. 1975, during which daytime high (monthly average) in the greenhouse decreased from 33 ± 3 to 28 ± 3 C. In contrast, in the preliminary screening, temperatures increased from 26 ± 3 to 33 ± 4 C as salinity increased.

When salinity reached a concentration at which the sensitive selections exhibited severe leaf burn and nominal growth, both replicates of the sensitive accessions and one replicate of the tolerant accessions were harvested. Salinity treatment was continued on the remaining replications of the tolerant accessions until those too showed symptoms of severe damage due to salt. Leaf and culm materials were weighed and rinsed for 10 sec in distilled water to remove surface salt. Roots were separated from the sand by shaking on a screen (9 apertures/cm²) and rinsed for 60 sec with a fine hose spray. Shoot and root materials were oven-dried at 70 C and finely ground for further analysis.

Plant Analysis. Following digestion in nitric-perchloric acid, plant material was analyzed for cations by atomic absorption spectrophotometry and for P by molydovanadate reagent (22). Total N was determined by Kjeldahl digestion and titration, and chloride by potentiometric titration (4).

Soluble carbohydrates were extracted from the dried grass samples with 90% ethanol and were assayed using anthrone (19). Free proline was estimated by an in vitro technique developed from the procedures of Stewart and Boggess (20). Leaf samples (0.5 g) were cut from plants growing in nonsalinized soil and sections (approx. 5 to 7 mm²) they were placed in cotton-stoppered, 50-ml Erlenmeyer flasks containing 25 ml of nutrient solution or nutrient solution and salt. Samples were incubated in a light chamber for 12 hours, and proline was determined (1).

RESULTS

Tall wheatgrass developed burn at the leaf tips as the salt concentration of irrigation solution increased. Burn symptoms increased until entire leaves and, finally, whole plants were affected. When salinity treatment was stopped and residual salts were leached from the root zone, some plants recovered. Based on these observations, the 32 entries were placed in five classes ranging from tolerant to sensitive (Table 1). Class I consisted of tolerant entries in which 95 to 100% of the individuals lived and continued to grow under the highest salinity treatment. Classes IV and V consisted of sensitive entries that showed the first symptoms of salt damage and ion toxicities. After leaching, Class IV entries showed moderate (30 to 80%) recovery from this treatment, but in Class V less than 10% of the individuals survived. The sharp contrast between Class I and V populations is evidenced in Fig. 1 by examples of entries before and after salination. The photographs were made during the second screening and are representative of the differences noted between sensitive and tolerant lines in both experiments.

Average daytime temperatures increased during the first experiment, whereas they were high at the begin-

Table 2. Mineral content of leaf and root tissue of the sensitive and tolerant selections of tall wheatgrass.

Selection	Irrigation solution	Na	Ca	K	Mg	Cl	PO_4	N
	meq/liter				μeq/g dry weight†			
				Shoots				
Sensitive	625	925	1,421	728	240	2,142	67.7	28.2
	±s.d.	115	194	89	23	207	9.4	1.0
Tolerant	625	486	647	835	188	1,133	83.5	32.2
	±s.d.	57	47	77	21	126	5.8	1.9
Tolerant	855	923	1,383	654	176	3,113	74.3	27.6
	±s.d.	94	204	95	25	514	10.8	1.8
				Roots				
Sensitive	625	674	359	213	97.3	1,210	92.9	19.2
	±s.d.	124	96	33	8.2	364	9.1	1.9
Tolerant	625	376	353	425	158.0	1,061	96.3	20.8
	±s.d.	95	56	139	27.0	253	15.7	2.6
Tolerant	855	535	502	325	122.0	1,192	97.2	19.4
	±s.d.	265	123	118	24.0	211	23.9	1.3

† Values are means and associated standard deviations of the pooled sensitive (n = 14) and tolerant lines (n = 7).

ning of the second experiment and declined slightly during its course. In the first experiment sensitive entries showed severe leaf burn by the time salt concentrations reached 380 meq/liter. Tolerant entries showed moderate to severe damage at 765 meq/liter. In the second experiment, leaf burn, corresponding to that noted in the first experiment, did not occur until salt concentrations reached 625 meq/liter for the sensitive accessions and 855 meq/liter for the tolerant accessions. Although differences between sensitive and tolerance entries were still evident at high salt concentrations during periods of lower temperature, sensitive entries apparently benefited more than tolerant ones.

Mineral Content. At 625 meq/liter, concentrations of treatment salts (Na, Ca and Cl) in shoots of sensitive entries were double those in shoots of tolerant entries (Table 2). Tolerant types acquired comparable levels only after the salt content of the irrigation solution reached 855 meq/liter. High salt content of the shoots of the sensitive entries was accompanied by slightly lower N, P, and K contents as compared with the tolerant lines.

Tolerant and sensitive entries did not differ in root concentrations of Ca, N, P, or Cl at 625 meq/liter. Sensitive lines had higher root Na concentrations and lower levels of K and Mg. At 855 meq/liter, tolerant lines showed increases in Na and Ca, as well as decreases in K and Mg. Root Cl concentrations, however, did not vary significantly with treatment or with plant tolerance.

Soluble Sugar and Proline Contents. Although free proline content in leaves increased in response to increased salinity at 350 meq/liter, tolerant lines did not produce significantly higher amounts than sensitive lines (Table 3). On the other hand, at 625 meq salt/liter, the soluble sugar content (Table 4) was substantially higher in the leaf tissues of tolerant lines than in sensitive selections and increased further as salinity increased to 855 meq/liter. Soluble sugar content of the roots of sensitive and tolerant lines did not differ at 625 meq/liter; however, the sugar content in roots of tolerant lines decreased at higher salinity (855 meq/liter).

Table 3. Proline contents of excised leaves of tolerant and sensitive selections of tall wheatgrass incubated in the light in nutrient solution with or without additions of salt.

Salinity treatment	Proline content	
	Tolerant	Sensitive
meq/liter	μmole/g fresh wt	
Control	6 ± 1	5 ± 1
175	18 ± 3	9 ± 3
350	20 ± 3	24 ± 4

Table 4. Soluble sugar contents of sensitive and tolerant lines of tall wheatgrass at different treatment salinities.

Salinity treatment	Selections	Soluble sugar	
		Shoot	Root
meq/liter		mg/g dry wt†	
625	Sensitive	47 ± 10	50 ± 10
625	Tolerant	69 ± 5	53 ± 10
855	Tolerant	142 ± 5	22 ± 5

† Means are pooled results of two replicates each of either seven tolerant or sensitive lines.

DISCUSSION

Salt injury is usually manifested as a general reduction in plant growth and, in more severe cases, as leaf burn and death. But the vagueness of phenotypic symptoms of moderate salt stress, and the lack of a comprehensive understanding of principal physiological mechanisms of salt injury makes it difficult to choose reliable screening criteria (15, 17). Nevertheless, a practical standard for a forage grass is the maintenance of growth and the avoidance of leaf damage under high salinity.

Selecting conditions of salinity stress is paramount because temperature, humidity, and fertility have all been shown to interact with salinity in affecting the plant (3, 12, 14). Sand cultures, frequently irrigated with excess amounts of nutrient solutions, allow adequate leaching and prevent accumulation of residual salts. Salinity and fertility can be effectively controlled and gradual increases in salt concentrations can be made with ease. The use of glasshouse flats is a compromise that allows the testing of moderate numbers of entries while maintaining some environmental control.

The screening procedure reported here effectively identified two groups of entries that contrast sharply in salt tolerance. The identification of seven tolerant genotypes provides an initial germplasm base for salt tolerance (Class I, Table 1); and because the selections originate from diverse geographic origins, it is probable that considerable genetic plasticity for other agronomic traits exists in this germplasm pool. Additionally, the identification of sensitive genotypes (Classes IV and V, Table 1) allows crosses to be made with chances of grouping segregates for inheritance studies.

Tolerance in selected lines is associated with restricted accumulation of Ca, Na, and Cl in the plant tops and the maintenance of low Na:K ratios (<1). At 625 meq/liter salinity, concentrations of leaf Ca, Na, and Cl in the tolerant lines were half of those in the sensitive lines. Hannon and Barber (10) found

a similar situation in clones of *Festuca rubra* and *Agrostis stolonifera* subjected to high concentrations of seawater. Tolerant clones of these species reacted similarly to tall wheatgrass by restricting the movement of Na and Cl into shoots while maintaining high K and low Mg concentrations. They referred to this mechanism of tolerance as exclusion. Levitt (13) and Greenway (9) in their dichotomies of salt tolerance or resistance mechanisms have referred to exclusion as an avoidance mechanism in which roots are impermeable to salts up to a point. At some critical threshold, roots become permeable and a burst of salt causes damage and death. This definition, which equates exclusion with ion impermeability, seems limited and contrasts to the definition of Hannon and Barber (10), whose data showed that both sensitive and tolerant clones allowed the movement of salt into roots and shoots. Furthermore, restricted accumulation is not solely responsible for increased salt tolerance. Elzam and Epstein (7, 8) compared the salt tolerance and ion uptake kinetics of *A. intermedium*, Amur, and *A. elongatum* (P.I. 98526). The accession of tall wheatgrass, which is ranked in Class II (Table 1), was more salt tolerant than *A. intermedium* but at high salinity had higher concentrations of Cl, Na, and Ca in the shoots.

Whereas the basic response of glycophytes to salinity is ion exclusion, angiospermous halophytes survive salinity by ion accumulation (9). All ecotypes of the tall wheatgrass tested accumulate ions in both roots and shoots; however, tolerant lines seem to restrict accumulation of Na in the root and Na, Ca, and Cl in the shoot. Because the plant takes up salt as a means of osmotic adjustment and thereby maintains a favorable water balance (2), the deficits in osmotica found in the tolerant lines must be corrected in some way. The synthesis of organic osmoticum would be a likely alternative.

Free proline, a neutral amino acid, has been proposed as a major osmotic agent (18). Proline accounts for more than 30% of the free amino acid pool in some halophytes and increases in response to water deficit. Additionally, high concentrations do not seem detrimental to in vitro enzyme activity (21). In tests with tall wheatgrass, proline increased in response to added salinity or the associated osmotic stress, but these increases were not specific to the tolerant lines (Table 3). Soluble sugar contents, conversely, were significantly lower in sensitive lines than in tolerant lines at 625 meq/liter (Table 4).

Thus, the key to increased salt tolerance in tall wheatgrasses seems to be in total plant response. Mechanisms of glycophytic salt exclusion restrict accumulation of large amounts of salts; but like the halophytes, tall wheatgrasses allow some ion uptake for osmotic adjustment. The ability of *A. elongatum* to grow well at higher concentrations of internal salts than *A. intermedium* (7, 8) may indicate that tall wheatgrasses have true salt tolerance as described by Levitt (13). Salts, proline, and soluble sugars all contribute to osmotic adjustment and salt tolerance, and several other factors are likely involved. Further studies, using milder salt stresses, will be needed to determine more about the physiological differences between these two groups of selected material.

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