Salt stress tolerance in native Alberta populations of slender wheatgrass and alpine bluegrass

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Acharya, S. N., Darroch, B. A., Hermesh, R. and Woosaree, J. 1992. **Salt stress tolerance in native Alberta populations of slender wheatgrass and alpine bluegrass.** Can. J. Plant Sci. **72**: 785–792. Alpine bluegrass [*Poa alpina* L.] and slender wheatgrass [*Elymus trachycaulus* (Link.) Gould ex Shinners] accessions from alpine and subalpine regions of the Canadian Rocky Mountains and eastern foothills were tested for tolerance to salinity stress. Accessions with higher emergence (%) than salt-tolerant Orbit tall wheatgrass [*Thinopyrum elongatum* (Host) D. R. Dewey, comb. nov.], after 21 d in vermiculite saturated with a NaCl-salinized half-Hoagland solution (electrical conductivity 15 dS m^{-1}) and nurtured in growth cabinets set to repeat 20/15°C day (16-h)/night temperatures, were considered tolerant of salt-stress. This test identified 72 alpine bluegrass and 11 slender wheatgrass salt-tolerant accessions. Most of these accessions originated from two specific sites near the Alberta-British Columbia border. Slender wheatgrass accessions tolerant to NaCl were also tolerant to the other salts commonly found in Alberta soils. In slender wheatgrass, the ability to emerge in a salinized nutrient solution had moderate heritability (61–68%), suggesting the possibility of genetic improvement through selection.

Key words: Salt tolerance, selection, heritability, alpine bluegrass, slender wheatgrass

Acharya, S. N., Darroch, B. A., Hermesh, R. et Woosaree, J. 1992. **Tolérance à la salinité parmi les populations naturelles d'agropyre élancé et de pâturin alpin en Alberta.** Can. J. Plant Sci. **72**: 785–792. Des obtentions de pâturin alpin (*Poa Alpina* L.) et d'agropyre élancé (*Elymus trachycaulus* (Link.) Gould ex Shinners) provenant des zones alpines et subalpines des montagnes Rocheuses canadiennes et de leurs avant-monts orientaux ont été testées sur la tolérance à la salinité. Les plantes donnant un taux de levée plus élevé que la variété tolérante au sel d'agropyre élevé Orbit (*Thinopyrum elangatum* (Host) D. R. Dewey, comb. nov.) après un séjour de 21 jours dans la vermiculite saturée de demi-solution Hoagland salée au NaCl (conductivité électrique 15 dS -m^{-1} et élevées en chambre de croissance réglé par un cycle de température jour (16 heures/nuit) de 20/15°C étaint considérés tolérantes au sel. Ce test a permis d'identifier 72 obtentions de pâturin alpin et 11 obtentions d'agropyre élevé comme tolérantes au sel. La plupart provenaient de deux stations situées à proximité de la frontière Alberta-Colombie-Britannique. Les obtentions d'agropyre tolérantes au NaCl possédaient aussi une tolérance envers les autres sels qu'on retrouve couramment dans les sols de l'Alberta. Chez l'agropyre élancé l'aptitude à la levée dans une solution nutritive saline révélait une héritabilité moyenne (61–68%) ce que ouvre la porte aux possibilités d'amélioration génétique par la sélection.

Mots clés: Tolérance au sel, sélection, héritabilité, pâturin alpin, agropyre élancé

Soil salinity has removed several thousand hectares of agricultural land from production in the southern Canadian prairies (Majerus 1990). Increased salinization of productive range and wildland has become a major concern in the Rocky Mountains and foothills of southern Alberta (Leskiw 1988). Thus concern for the problem of soil salinity is increasing under agricultural and non-agricultural situations. Research on salt stress tolerance in plants, including identification of

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salt-tolerant species and cultivars, has become increasingly important for maintaining agricultural production and in some cases, vegetation cover in saline areas.

Soil salinity in excess of the electrical conductivity (EC) 4 dS m^{-1} affects most plant growth. Above EC 16 dS m^{-1}, there is little crop growth (Alberta Soils Advisory Committee 1987). Salt-tolerant, perennial forage crops capable of establishing on saline soils will provide protection against erosion, compete with weeds, utilize excess soil moisture, and in some situations provide forage for animals. However, most forage cultivars were not selected for their salt tolerance but rather for optimum performance on non-saline soils. Consequently, many forage cultivars fail to produce adequate stand and biomass even under moderate saline conditions.

Among registered forage grasses grown in the northern Great Plains region, tall wheatgrass [*Thinopyrum elongatum* (Host) D. R. Dewey, comb. nov.], Russian wildrye, slender wheatgrass [*Elymus trachycaulus* (Link) Gould ex Shinners], alkali sacaton, Altai wildrye and mammoth wildrye were considered the most salt tolerant (McElgunn and Lawrence 1973; Majerus 1990). Salt-tolerant grasses that are not registered include beardless wildrye, plains bluegrass, alkali bluegrass, Nuttall alkali grass, lemon alkali grass and weeping alkali grass (Greub et al. 1985; Majerus 1990). It was suggested that native plant material collected from salt-affected areas may have tolerance to high levels of salinity (Rana 1985) and should prove effective in the reclamation of such sites. Alpine bluegrass (ABG) [*Poa alpina* L.] and slender wheatgrass (SWG) native to the Rocky Mountains in Canada are considered suitable for reclamation of high-stress environments (Hardy BBT Ltd. 1989). Collections at the Alberta Environmental Centre (AEC) of alpine and subalpine accessions of these species may include some salt-tolerant types. Such accessions might be useful in the development of lines for revegetating disturbed areas affected by high salinity.

Systems for selection and evaluation of salt tolerance at various growth stages have focused on the use of salinized nutrient solutions under controlled environments (Jana et al. 1980; McKimmie and Dobranz 1987). Isolation of salt-tolerant genotypes at the germination and seedling stages is considered practical and effective (Blum 1985) because high salt concentrations delay and decrease germination and emergence (Mueller and Bowman 1989). Response of seedlings to salinity is considered predictive of adult plant response to salinity (Greenway 1965; Blum 1985). Ashraf et al. (1986) reported the development of salt-tolerant lines in four grass species through a limited number of selections at the seedling stage. Although such response to selection usually indicates high heritability, there is little information on heritability estimates for salt tolerance in perennial grasses.

MATERIALS AND METHODS

In a series of growth cabinet experiments, a method modified from Jana et al. (1980) and McKimmie and Dobranz (1987) was used to evaluate the response of grass cultivars and native grass accessions to salt stress tolerance. For all experiments, 16 Ferdinand (six cells/book) root-trainers (1.8 × 1.8 × 10 cm) were arranged on a layer of cheesecloth and placed in holding trays. Medium-textured sterile vermiculite was placed 9 cm deep in each cell. The assembled root-trainers were then placed in plastic trays (30 × 40 × 10 cm). Freshly prepared half-Hoagland solution salinized with analar salts provided the salt stress at emergence. The vermiculite in each germination tray was saturated with 4.5 L of salinized nutrient solution which occupied about half the tray. Salinity of the solution was measured in dS m^{-1} using a YSI Model 32 conductivity meter. Since EC is directly related to the concentration of soluble salts in the solution the method provided an index of salt concentration (Maas and Hoffman 1977). After seeding, the seeds were covered with 0.5 cm of fine vermiculite. The seeded trays were placed in growth cabinets set to 22/15°C temperature regimes and 16-h photoperiods with 285 μmol m^{-2} s^{-1} light intensity. About 300 mL of distilled water was added daily to each tray to keep the salt concentration constant and maintain the solution level. Salt build-up at the surface of the vermiculite was washed down twice a week with the tray solution. Emergence was counted weekly for 3 wk. Percent emergence data was arcsine-square root transformed for statistical analyses.

The present experiments were conducted to (1) develop a rapid and effective selection method, (2) identify salt-tolerant alpine bluegrass and slender wheatgrass accessions from collections maintained at AEC, (3) relate tolerance to NaCl with that of other salts, and (4) estimate heritability for salt tolerance in slender wheatgrass.

Experiment I

With the objective of validating the screening method and determining the most suitable EC level, seedling emergence of 12 registered forage grass cultivars was compared using half-Hoagland solutions salinized with NaCl to 10, 15 and 20 dS m^{-1}. Each replication comprised 12 seeds (two seeds/cell) of a cultivar and the treatments were arranged in a split-plot design with two replicates (blocks). Salt concentrations were main plots and the cultivars were subplots.

Experiment II

As part of a native grass breeding program, 406 slender wheatgrass and 1441 alpine bluegrass tussocks originating from 315 sites in western Alberta and eastern British Columbia (Fig. 1) were grown in a nursery at AEC. Sites ranged in elevation from 1100 to 2750 m and included a variety of slopes and aspects. To avoid any confounding effect of the collection sites, seeds harvested from AEC nursery were tested for emergence using nutrient solutions salinized to 15 dS m^{-1}. Orbit tall wheatgrass, a Canadian cultivar recommended for saline areas of southern Alberta (Alberta Agriculture 1990), was used as the control in each tray. To ensure rapid germination, all wheatgrass seeds, including Orbit, were dehulled before seeding. Bluegrass seeds were not dehulled. In each tray, five cells in each book were seeded with the test accession (two caryopses/cell) while the middle cell of 10 books (one caryopsis/cell) was seeded with the control. The accessions were tested in groups of 48 using a randomized complete block design with three blocks. After 21 d, accessions with emergence equal to or better than the control were selected. Vigorous seedlings of the selected accessions were then transplanted into 15-cm pots containing a soil mix and nurtured in a greenhouse until they produced mature seeds.

Experiment III

Salinity in Alberta soils generally results from mixtures of $MgSO_4$, $CaCl_2$ and Na_2SO_4 at various concentrations (Alberta Agriculture 1985). To determine the relationship between tolerance to NaCl and to other salts, half-Hoagland solution was salinized with analar NaCl, Na_2SO_4, $MgSO_4$ and a mixture of salts made up of a ratio by weight of 5 Na_2SO_4 : 2.5 $MgSO_4$: 1 $CaCl_2$, to yield an EC of 15 dS m^{-1}.

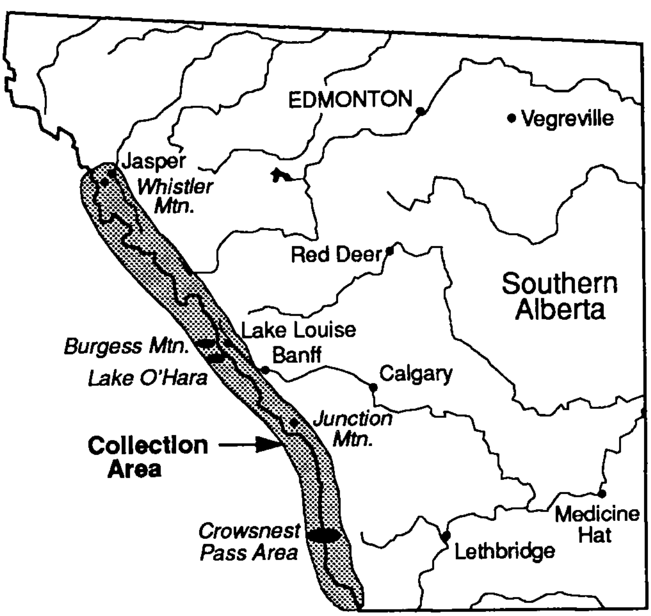

Fig. 1. Geographic origin of Alberta Environmental Centre collections.

Six selected SWG accessions (exp. II), eight random non-selected SWG accessions, and two controls, Orbit tall wheatgrass and Revenue slender wheatgrass, were included in this experiment. A split-plot design with six blocks was used. Each line (subplot) was represented by 10 seeds placed two per cell in five cell books. All 16 lines fitted in one tray and each tray (main plot) was placed in a different salt solution. The data on emergence after 21 d were analyzed using a fixed effects analysis of variance (ANOVA). Mean emergence of seedlings in the four salts were compared using Tukey's studentized range test at $\alpha = 0.05$ (Statistical Analysis System Institute, Inc. (SAS) 1985). Single degree of freedom contrasts were used to compare emergence of the accessions.

The emergence data of slender wheatgrass, excluding the tall wheatgrass control, were subjected to the VARCOMP procedure of SAS (1985) software to estimate variance components for accessions, salts × accession interaction and error. The variance for accessions represented the genetic variance (σ^2_g) and environmental variance (σ^2_e) was estimated by the sum of the variances for salts, accession by salt interaction, and error. Broad sense heritability (h^2) was calculated as $\sigma^2_g/(\sigma^2_g) + \sigma^2_e)$ (Allen et al. 1985).

Experiment IV

Seeds from the 11 selected slender wheatgrass accessions with better emergence than Orbit tall wheatgrass (exp. II) were planted in greenhouse soil-beds for seed production. Mature seeds harvested from these plants were subjected to the same test described in exp. II with Orbit as control. A randomized complete block design with six replicates was used. Emergence of first and second generation seeds was expressed as percentage of Orbit emergence to standardize the two experiments.

Realized heritability was estimated as $h^2 = R/S$, where R = response to selection and S = selection differential (Falconer 1981). The selection differential was the difference between the mean emergence of the selected accessions and the mean emergence of all slender wheatgrass accessions in the initial generation. The response to selection was calculated the same way, but was based on the second generation. The mean emergence of the slender wheatgrass accessions was assumed to be constant in both generations. The standard error for the realized heritability was estimated as $SE(h^2) = SE(R)/S$ (Wong and Baker 1986).

RESULTS

Experiment I

The forage grass cultivars differed significantly in their ability to emerge and survive for 21 d under saline conditions (Table 1). Orbit tall wheatgrass emerged about 20% better than the Cabree Russian wildrye and 45% better than the Prairieland altai wildrye, the other two grasses recommended for saline areas. At 15 dS m^{-1} the salt-tolerant Orbit emerged >90% but its emergence was only 75% at 20 dS m^{-1}.

Mean seedling emergence for all cultivars decreased as the salt concentration (EC) increased. However, the effect of salt concentration or the salt concentration × cultivar interaction were not significant in this experiment.

Table 1. Mean emergence of 12 grass cultivars after 21 d of incubation at three NaCl concentrations

Common, scientific and cultivar name	Mean emergence (%)
Tall wheatgrass [*Thinopyrum elongatum* (Host) Dewey, comb. nov.] cv. Orbit	85
Russian wildrye [*Psathyrostachys juncea* (Fisher) Nevski] cv. Cabree	65
Northern wheatgrass [*Elymus lanceolatus* (Scribn. & Smith) Gould] cv. Elbee	56
Crested wheatgrass (*Agropyron cristatum*) (L.) Gaertner) cv. Parkway	46
Timothy [*Phleum pratense* L.] cv. Climax	44
Brome (*Bromus inermis* Leyss.) cv. Carlton	44
Creeping red fescue (*Festuca rubra* L.) cv. Boreal	44
Altai wildrye (*Leymus angustus* (Trin.) Pilger) cv. Prairieland	40
Intermediate wheatgrass [Thinopyrum intermedium (Host) Barkworth & Dewey] cv. Clarke	40
Western wheatgrass [*Pascopyrum smithii* (Rybd.) Love] cv. Walsh	40
Slender wheatgrass [*Elymus trachycaulus* (Link) Gould ex. Shinners] cv. Revenue	33
Kentucky bluegrass [*Poa pratensis* L.] cv. Nugget	32
SE	7

Experiment II

Seventy-two of the 1441 (5%) alpine accessions emerged better than the Orbit tall wheatgrass control, at 15 dS m^{-1} NaCl solution (Fig. 2). Eleven of the 406 (3%) slender wheatgrass accessions emerged better than the control, at the same salinity level (Fig. 3). These accessions were selected for further testing.

Ten of the slender wheatgrass selections and 29 of the alpine bluegrass selections originated from the Crowsnest Pass area of Alberta (Fig. 1). Alpine bluegrass selections also originated from Burgess Mountain (29 accessions), Lake Louise (2), Lake O'Hara (11) and Junction Mountain (1) (Fig. 1). The site of origin for the selected accessions varied with respect to their elevation, aspect and slope.

Experiment III

The effects of slender wheatgrass accessions and salt type on seedling emergence were significant, but the interaction effect was not significant (Table 2). The mean emergence of all accessions was highest in the mixture of salts (85%) and in Na_2SO_4 (81%). Emergence in $MgSO_4$ (68%) was significantly lower than that in the salt mixture or Na_2SO_4. Seeds in solutions salinized with NaCl had 54% mean emergence, a value significantly lower than in all other solutions. Mean emergence of selected wheatgrass accessions (86%) was significantly greater than the mean emergence (65%) of non-selected accessions, and the two control cultivars Orbit (69%) and Revenue (45%) (Table 2). The difference between the selected and non-selected groups was greatest when NaCl was used. Emergence of non-selected accessions was not significantly different from Orbit, but both the non-selected accessions and Orbit emerged significantly better than Revenue. Using variance components, broad sense heritability for slender wheatgrass emergence after 3 wk was estimated at 61%.

Experiment IV

The mean emergence (97% of Orbit) of the selected accessions in the second generation was lower than the emergence determined

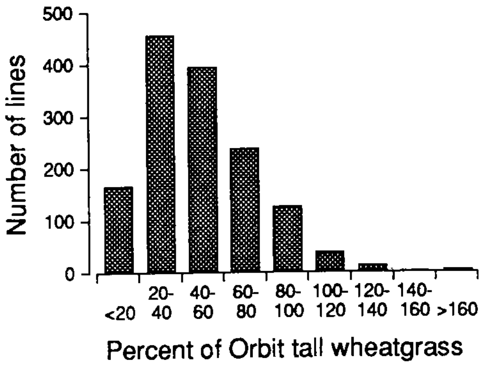

Fig. 2. Emergence of 1441 accessions of alpine bluegrass at 15 dS m^{-1} as a percentage of Orbit tall wheatgrass.

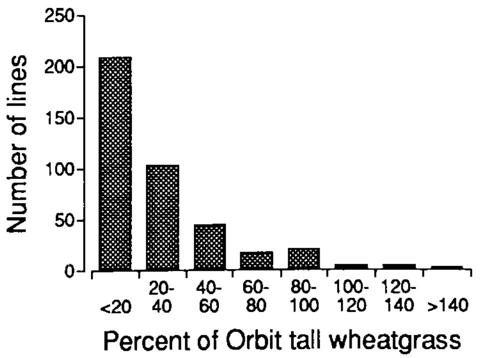

Fig. 3. Emergence of 406 accessions of slender wheatgrass at 15 dS m^{-1} as a percentage of Orbit tall wheatgrass.

(130% of Orbit) for the first generation (Table 3). However, this lower emergence was still much greater than the population mean (28% of Orbit). The realized h^2 was estimated at 68 $\pm$ 48%.

DISCUSSION

Orbit tall wheatgrass emerged better than the other grass cultivars and was chosen as the control for subsequent experiments. Superior salt tolerance of this species under field conditions was observed earlier (McElgunn and Lawrence 1973; Holm 1983; Majerus 1990). A nutrient solution salinized to an EC of 15 dS m^{-1} with NaCl was considered adequate to identify salt-stress-tolerant genotypes.

Table 2. Fixed effects model ANOVA on 21-d emergence data of slender wheatgrass accessions, Revenue slender wheatgrass and Orbit tall wheatgrass in four salt solutions (exp. III)

Source	DF	ANOVA MS	*F* value
Block	5	0.170	1.4NS
Salt	3	4.490	38.7**
Main plot error	15	0.117	
Accession	15	1.514	36.3**
Selected vs. Non-selected	1	6.837	151.2**
Selected vs. Orbit	1	1.743	38.5**
Non-selected vs. Orbit	1	0.002	0.0NS
Selected vs. Revenue	1	6.612	146.2**
Non-selected vs. Revenue	1	1.657	36.6**
Orbit vs. Revenue	1	0.913	20.2**
Residual	9	0.550	
Accession × Salt	4	0.064	1.4NS
Subplot error	300	0.045	

**Significant at $P = 0.01$; NS, not significant at $P = 0.05$.

Table 3. The first and second generation emergence of selected slender wheatgrass accessions under saline conditions expressed as a percentage of Orbit tall wheatgrass emergence after 21 d

	Mean emergence (% of Orbit)	
Accession	First generation	Second generation
23	123	130
43	136	83
46	157	123
81	104	87
267	154	130
268	131	53
272	131	60
275	104	113
313	108	110
315	116	87
588	164	90
Accession mean	130	97
Accession SE	12	16
Population mean	28	28
Population SE	17	17

At this concentration, mean emergence of all cultivars was over 50%, the difference in emergence between the two extreme cultivars was wider than at 10 dS m^{-1} and Orbit emergence was not adversely affected. Also, the tolerance ranking of the grass cultivars was consistent with published reports (Holm 1983; McKenzie 1988; Majerus 1990).

The alpine bluegrass and slender wheatgrass accessions collected from alpine and sub-alpine areas were expected to be adapted to stresses such as cold, short growing season and fluctuating temperature and moisture conditions. Their specific adaptation to salt stress was unknown. In general, bluegrasses are not considered salt-tolerant and were therefore excluded from salt-tolerance tests (Greub et al. 1985). The salt-tolerant alpine bluegrass accessions may provide germplasm for development of salt-tolerant cultivars in this and other bluegrass species. Revenue slender wheatgrass had superior performance on wet saline soils in Montana compared with other registered grass cultivars (Majerus 1990). However, this cultivar performed poorly under artificial saline conditions (exps. I and III) and under saline field conditions in Saskatchewan (Holm 1983). Identification of salt-tolerant slender wheatgrass accessions was therefore encouraging from the cultivar improvement standpoint.

Most salt-tolerant selections originated from the Crowsnest Pass area, a hot, dry region, and from an area of the Alberta-British Columbia border underlain by the Burgess shales, a marine shale formation. This suggests that plants with special characters can be found in specific locations. Collection of diverse native accessions from a large number of locations will increase the probability of obtaining agronomically acceptable stress-tolerant types through selection without hybridization. Such a breeding approach will ensure the preservation of gene combinations that have allowed plants to withstand a variety of stresses.

NaCl-selected accessions, when tested in other salts, emerged better than non-selected accessions (exp. III). Although the maximum difference between the two groups was observed in NaCl solution, the accession × salt interaction effect at emergence was not significant. This indicates that selections made in a medium salinized by one salt will also do well in a medium salinized by a different salt or a combination of salts.

The artificial greenhouse environment under which the second generation seeds of the selected accessions were produced may have caused a decrease in mean emergence in exp. IV. Hermesh and Acharya (1992) reported that maternal environment can have such an effect on native grass seed germination. In our calculations, the population mean from a large number of accessions was assumed to be constant over the two generations and this may have resulted in underestimation of the realized h^2 value. Realized h^2 for salt tolerance at emergence in slender wheatgrass (68%) was higher than the 38% reported for cotton (Ledbetter 1986) and the 50% for alfalfa (Allen et al. 1985). A similar (61%) broad sense h^2 was also estimated from variance components in exp. III after week 3. These high h^2 estimates may be characteristic of this species or due to the method used for estimation of salt-tolerance. Despite high standard errors, these heritability estimates (>60%) indicated that in slender wheatgrass, salt-tolerance at emergence is influenced at least moderately by genotype. It should therefore be possible to improve salt-tolerance in this species through selection.

Salinity damage is always most prevalent during germination and seedling stages (Maas and Hoffman 1977). The selected alpine bluegrass and slender wheatgrass accessions should emerge well and establish better stands than other grass cultivars under saline conditions. Since there are suggestions that salt tolerance of seedlings is highly predictive of the response of adult plants to salinity (Greenway 1965; Blum 1985) and seedling screening of barley (Norlyn 1980) and wheat (Kingsbury and Epstein 1984) resulted in identifying salt-tolerant adult individuals, the selected native grass accessions are expected to show salt-stress tolerance throughout their life cycle.

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