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Original Research

# Trait Response and Change in Genetic Variation upon Selection for Spike Number in Salina Wildrye

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ABSTRACT

Salina wildrye (*Leymus salinus* [M.E. Jones] Á. Löve) is a perennial cool-season grass that potentially could become an important restoration species in the Colorado Plateau. However, its seed production has never been commercially viable due to sparse heading. We compared a 4*x* ssp. *salmonis* population, Lakeside $C_3$, to an 8*x* ssp. *salinus* population, 9043501, for seed production—related traits and measured the response of 9043501 to 2 cycles of selection for increased spike number over a 4-yr period at Millville, Utah. Seed yield of Lakeside and 9043501 was similar ($P > 0.10$) in 2013, but seed yield of 9043501 was 81% greater ($P < 0.10$) than Lakeside in 2014 and 191% greater ($P < 0.01$) in 2015. Lakeside spike number was 99% greater ($P < 0.0001$) than 9043501 in 2013, but they were similar ($P > 0.10$) in 2014 and 2015. Seeds per spike of 9043501 were 71% ($P < 0.05$), 80% ($P < 0.05$), and 209% ($P < 0.01$) greater than Lakeside in 2013, 2014, and 2015, respectively. Selection in 9043501 increased ($P < 0.05$) spike number by 4.3 spikes per plant (19.8%) per cycle of selection in the first seed-production yr (2013), but no change was seen in 2014 or 2015 ($P > 0.10$). Selection did not change ($P > 0.10$) seeds per spike or individual seed mass. Consequently, seed yield increased ($P < 0.05$) 0.32 g per plant per cycle (36.8%) in 2013, with no increase ($P > 0.10$) in 2014 or 2015. Dry matter per plant across the 4 yr increased ($P < 0.01$) 10.3 g per plant per cycle (9.3%), and canopy height increased ($P < 0.01$) 3.9 cm per cycle (6.6%) in 2013. AFLP DNA primers detected a 1.7% loss of genetic variation per cycle, presumably due to a combination of selection and genetic drift, but no plant traits were diminished as a result.



## Introduction

Salina wildrye (*Leymus salinus* [M.E. Jones] Á. Löve = *Elymus salinus* M. E. Jones) is a moderately rhizomatous, cool-season perennial grass native to the Intermountain West (Monsen et al., 2004, p. 381—382). This grass could be used for restoration of harsh, arid, disturbed sites such as those that have been developed for oil or gas production. It may grow on a variety of soil textures ranging from rocky to loams and clays, occupying hillsides, alluvial fans, plateaus, bluffs, canyons, and montane areas as part of salt desert shrubland, sagebrush-grass, mountain mahogany, aspen, and conifer plant communities (Baker and Kennedy, 1985; Vallentine, 1989; Monsen et al., 2004, p. 381—382). *Leymus salinus* ssp. *salinus* is prevalent east of the Wasatch Mountains in eastern Utah, western Colorado, and southwestern Wyoming (Barkworth and Atkins, 1984, Fig. 6) and may be found in association with the saltbush shrub species *Atriplex confertifolia* and *A. gardneri* (Baker and Kennedy, 1985). A second subspecies of *L. salinus,* ssp. *salmonis,* occurs west of the Wasatch Mountains in eastern Idaho, western Utah, and Nevada. While ssp. *salinus* is relatively frequent within its range, the distribution of ssp. *salmonis* is much spottier (Atkins et al., 1984). As far as is known, ssp. *salmonis* is tetraploid ($2n = 28$), while ssp. *salinus* may be tetraploid, hexaploid ($2n = 42$), or octoploid ($2n = 56$) (Atkins et al., 1984). A third subspecies, ssp. *mojavensis,* occurs in southern California and Arizona (Barkworth and Atkins, 1984). *Leymus salinus* and *L. cinereus* have been shown to be the parents of *L. ambiguus* (Culumber et al., 2011, Fig. 2), which occurs on the eastern slopes of the Rocky Mountains in either tetraploid or octoploid states (Atkins et al., 1984).

Because no plant materials of salina wildrye have been released for commercial seed production, only wildland-collected seed has been available for seeding operations, which means that seed quality is often poor, seed prices are high, and seed availability is limited. However, 9043501, an octoploid population collected in northeastern New Mexico (Colfax County), has been identified by the Upper Colorado Environmental Plant Center in Meeker, Colorado as a promising plant material (Monsen et al., 2004). This population keys to *L. salinus* ssp. *salinus* because it possesses neither the pubescent basal leaves of ssp. *salmonis* nor the flat leaf blades of ssp. *mojavensis* (Barkworth and Atkins, 1984); that is, its leaves are glabrous and strongly involute.

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The greatest limiting factor to the adoption of this species in the seed trade is its poor seedling establishment and low seed yield relative to other cool-season perennial rangeland grasses. Low seed yield is primarily due to the production of relatively few inflorescences (spikes) per plant. For this reason, we conducted two cycles of phenotypic recurrent selection for increased spike number in an attempt to increase seed production potential of 9043501. As *L. salinus* is an allogamous species, hybridization occurs between plants each generation, forcing genetic recombination and making it a logical candidate for recurrent selection. Before this selection, we had conducted two cycles of selection for salinity tolerance, but response to that earlier selection is not germane to this study. In an evaluation of response to selection at Millville, Utah, we included the octoploid 9043501, the four cycles (populations) of selection on 9043501, and Lakeside $C_3$, a tetraploid ssp. *salmonis* population used for comparison with 9043501.

We had three objectives in this study. Objective 1 was to compare the *L. salinus* ssp. *salmonis* Lakeside $C_3$ population ($2n = 4x = 28$) to the *L. salinus* ssp. *salinus* 9043501 $C_0$ population ($2n = 8x = 56$) (henceforth 9043501) for the previously mentioned traits. Objective 2a was to evaluate the effect of two cycles of selection on 9043501 for spike number per plant ($C_3$, $C_4$) on spike number per plant, seed yield per plant, and the two other components of seed yield, seeds per spike and individual seed mass. Objective 2b was to evaluate the effects of selection on 9043501 on two vegetative traits, dry matter per plant and canopy height, and two germination traits, percentage and rate. Objective 3 was to measure genetic similarity within 9043501 and each of four cycles of selection in 9043501, two ($C_1$, $C_2$) for salinity tolerance and two ($C_3$, $C_4$) for spike number. This was done to determine whether genetic variation had been lost from the 9043501 base population as a consequence of artificial selection. Genetic variation can be lost from populations subjected to selection primarily due to genetic drift, a process by which a population is genetically narrowed due to a relatively small number of individuals, leading to unavoidable mating of relatives and consequential inbreeding.

## Materials and Methods

### *Selection History*

Four cycles of selection have been practiced on 9043501. Selection was applied for tolerance to salinity in the first two cycles and for spike number (ocular estimate) in the latter two cycles. For $C_1$, seeds were germinated in an EC = 24 solution in the laboratory (Peel et al., 2004). Seeds that germinated were planted individually into 1 020 silica sand-filled cones in a greenhouse and subjected to a salinity protocol (dunked in saline solution 2× per week), escalating from EC = 12 (17 February 1997) to EC = 48 (31 March 1997). In April 1997, 10% of the plants were selected for vigor under these conditions (i.e., salinity tolerance, and transplanted into fresh containers). These selections were transplanted to Greenville Farm (Cache County, Utah) on 30 May 1997, and recombined seed was harvested in 1998 ($C_1$). For $C_2$, $C_1$ seeds were germinated in an EC = 33 solution. Once the seedlings recovered from this stress, they were subjected to a salinity protocol escalating from EC = 6 (1 February 1999) to EC = 42 (11 April 1999). In April 1999, 10% of the plants (1 320 individuals) were selected for salinity tolerance and transplanted into fresh containers before transplanting to North Park Farm (Cache County, Utah) in May. Recombined seed was harvested in 2000 ($C_2$).

In January 2006, 686 $C_2$ seedlings were transplanted to greenhouse flats, and on 21 March 2006 38.0% were selected for high seedling vigor based on high tiller number and size. On 14 April 2006, a 252-plant nursery was established on 0.40-m centers at Blue Creek Farm (Box Elder County, Utah). On 18 May 2007, 40 plants (15.9%) were selected because each displayed a greater number of spikes than its eight nearest neighbors. Spikes were removed from unselected plants, and the selections were intermated to produce seed in 2007 ($C_3$). For $C_4$, 980 $C_3$ seedlings were transplanted to greenhouse flats in January 2008, and on 26 March 2008, 358 seedlings were selected for seedling vigor as described earlier. On 2 May 2008, 336 of these selections were transplanted to Blue Creek Farm on 0.30-m centers. On 16 June 2009, 42 plants (12.5%) were selected for spike number relative to the eight nearest neighbors, as described earlier, but spikes were not removed from unselected plants as in 2007 for the previous cycle. Open-pollinated seed was harvested from these 42 plants later that summer ($C_4$).

Lakeside $C_3$ (henceforth Lakeside), the tetraploid check, resulted from three cycles of selection, each of which included selection for vigor score, seed yield, and emergence from deep seeding, on the Lakeside $C_0$ population. Lakeside $C_0$ is a tetraploid ssp. *salmonis* population generated by bulking collections at > 20 sites in the Stansbury Mountains southwest of the Great Salt Lake in Tooele County, Utah.

### *Millville Evaluation Trial*

A replicated evaluation trial comparing 9043501, four cycles of selection from 9043501, and Lakeside was established at Millville Farm (Cache County, Utah) 16 May 2012. The six populations were planted in 10-plant plots in a 5 × 2 arrangement on 30-cm centers with a 60-cm alleyway between plots in both directions. Plots were arranged in a randomized complete block design with 8 replications. Dry matter production was measured 27 September 2012 (5-cm height), 18 July 2013 (10 cm), 16 September 2014 (10 cm), and 5 August 2015 (10 cm). Canopy height was measured 6 June 2013, but not in the following years. Spikes were counted 6 June 2013, 13 June 2014, and 15 June 2015. Seed was harvested before shattering commenced, and seed mass was determined from four 100-seed samples per plot in all 3 seed-production yr.

Germination percentage and rate (Maguire, 1962) were measured on seed harvested in 2013 and 2014. Germination boxes were lined with blotter paper (Anchor Paper Company, Plymouth, MN), with each containing 100 seeds dusted with thiram fungicide. Four boxes were prepared for each plot in the field design, and water was added to begin the trials 29 August 2014 for 2013-harvested seed and 27 November and 4 December 2015 (four field replications each) for 2014-harvested seed. Tests were conducted at 22°C. Germinated seedlings were tallied daily from d 5 (following watering), the onset of germination, through d 24 (when germination was essentially complete), except for boxes for which counts were terminated earlier because 3 successive d without germination were recorded.

### *DNA Variation*

Young leaf tissues from 192 seedlings, including at least 32 individuals from each cycle (population) of selection, 9043501 and $C_1 - C_4$, were lyophilized and milled using a MM300 (Retsch Inc., Newtown, PA) mixer mill for DNA extraction using the DNeasy 96 Plant Kit (Qiagen, Germantown, MD). We obtained complete DNA profiles (six AFLP primer pairs) for 171 seedlings, including at least 27 individuals from each population, using the AFLP technique (Vos et al., 1995) with modifications for detection using fluorescent labels and capillary electrophoresis. Briefly, the selective *Eco*RI primers were fluorescent labeled with 6-FAM and fractionated by capillary electrophoresis on an ABI3100 instrument (PE Applied Biosystems, Foster City, CA) with internal GS-500 size standards (PE Applied Biosystems) for each sample in each channel. The *Eco*RI and *Mse*I preamplification primers both included a single selective nucleotide, A and C, respectively. The selective amplification primers included two additional selective nucleotides to make six different primer combinations: E.AAG//M.CAA, E.ACA//M.CCC, E.ACA//M.CGG, EACG//M.CAA, E.ACG//M.CTC, and E.ACT//M.CTC. Different AFLP markers were identified and scored for the presence or absence of bands (DNA amplicons) on the basis of the relative