Selection for salt tolerance in *Lesquerella fendleri* (Gray) S. Wats.

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Abstract

In a study conducted in 1997–1998 to determine the salt tolerance of *Lesquerella fendleri* (Gray) S. Wats., saline irrigation waters greater than 21 dS m^{-1} electrical conductivity were found to cause high plant mortality. Surviving plants were inter-mated under controlled conditions and seeds were collected from these plants. The following season, seeds of the selected salt tolerant full-sibs, designated WCL-SL1, were direct-seeded into 21 outdoor sand tanks, along with two other lines. One line was the original seed planted the previous year called '1986 bulk', and the other was an unselected line, designated 'line 54'. In an effort to determine whether salt tolerance is a heritable trait in lesquerella, this study compared the salinity response of WCL-SL1 with the two other lines. After seeding into the sand tanks, they were irrigated daily with complete nutrient solutions. Seven salinity treatments were imposed by stepwise additions of mixed salt salinity composed of Na^+, SO_4^{2-}, Cl^-, Mg^{2+} and Ca^{2+}. Final electrical conductivities (EC_i) of the irrigation waters were 3, 7, 11, 15, 18, 21 and 24 dS m^{-1}. Two weeks after salinization, significant treatment differences in both plant height and survival were observed among lines. The parental line '1986 bulk' was most sensitive, WCL-SL1 line most tolerant, and 'line 54' intermediate. Shoot weights in all lines decreased as a function of increasing salinity and ranked mean differences within lines were consistent across all salinity levels. At 7, 15 and 18 dS m^{-1} salinity levels, the shoot weights of the WCL-SL1 line were significantly greater than the parental line. Total seed oil and lesquerolic acid contents increased significantly as irrigation water salinity increased from 3 to 18 dS m^{-1}. Improved salt tolerance of line WCL-SL1 was not attributed to differences in mineral ion uptake and accumulation in leaf tissue. Our results indicate that a single cycle of selection with lesquerella in salinized sand cultures resulted in an improved lesquerella line that had higher absolute and relative salt tolerance based on seedling survival, plant height, shoot biomass production, and seed yield than the original line.
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1. Introduction

Workers have screened various crops for salt tolerance. Some of these crop selections have been directly used on salt-affected soils, whereas others

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needed further selection. Shannon (1978) screened crested wheatgrass (*Agropyron elongatum* L.) and identified salt tolerant accessions. In a less heterogeneous crop, such as maize (*Zea mays* L.), Ashraf and McNeilly (1990) produced a highly salt tolerant line after only two cycles of selection. Jain et al. (1990) selected salt tolerant canola (*Brassica napus* L.) through a one-step in vitro selection process.

Limited information is available about the variability in salt tolerance of *Lesquerella fendleri* (desert mustard—Brassicaceae), a native species of semiarid regions of Southwestern United States (Grieve et al., 1997). Lesquerella is a promising new crop with seed oil that contains lesquerolic acid, a hydroxy fatty acid of great value in the production of biobased lubricants, greases, nylon-11, plastics, and protective coatings (Dierig et al., 2002). The current source of hydroxy fatty acids is from castor (*Ricinus communis* L.). The hydroxy triglyceride can be converted to estolides for use as dehydrating oils and thickeners for lubricants (Isbell and Cermak, 2002). Coproducts useful as food thickeners and coatings include a water soluble seed coat gum, which is similar to xanthan found in guar [*Cyanopsis tetragonoloba* (L.) Taub]. Antioxidants and unique glucosinolates can also be recovered from the seed meal fraction (Dierig et al., 2002).

Lesquerella growing in its native habitat occurs mainly on calcareous soils, and frequently with limestone outcroppings (Dierig et al., 1996). Native populations are found in Mexico as far south as San Luis Potosi and north into Texas, New Mexico, and Arizona. A few collections have been made in Utah and Colorado, and one reported from Kansas (Rollins and Shaw, 1973). Lesquerella is a short-lived perennial, but cultivated as a winter annual.

Lesquerella plants produce yellow flowers along a racemose inflorescence. First flowering commences in February and continues until June when irrigation is discontinued for seed harvest. Seeds are formed in protective pods or siliques. A silique may contain up to 32 seeds. Seed yield is determined by the number of siliques, the number of seeds per silique, and the individual seed mass. The desert mustard is open-pollinated by insects such as honey bees and flies (Dierig et al., 2001b). Rollins and Shaw (1973) describe this species of *Lesquerella* as having an unusually large amount of variability.

The initial growing region in the US for cultivation of this potential new crop is expected to be Arizona, New Mexico and Texas. Irrigation costs, saline water and soil conditions, and changing government programs limit the areas where crops may be grown. Marginal lands in many of these areas may be the only cost effective site to grow lesquerella. Therefore, it is necessary to develop stress tolerant germplasm adapted to these areas.

In 1997–1998, screening for salt tolerance of lesquerella germplasm designated '1986 Bulk' was conducted in 24 outdoor sand tanks at the USDA-ARS George E. Brown Jr. Salinity Laboratory, Riverside, CA (Grieve et al., 1999). Briefly, eight salinity treatments were imposed with irrigation waters designed to simulate saline drainage effluent commonly present in the San Joaquin Valley of California (Suarez and Šimůnek, 1997). Electrical conductivities (EC_i) of the solutions were 3 (nutrient solution only), 6, 9, 12, 15, 18, 21 and 24 dS m^{-1}. Plant survival decreased as salinity increased. Replicate plots at the two highest salinity levels had a combined initial population of 216 plants of which only five plants survived at 24 dS m^{-1} and 13 at 21 dS m^{-1}. Surviving plants were rescued, combined, and inter-mated during senescence, and grown under nonsaline conditions. Seeds were collected from these single cycle selections and designated as line WCL-SL1 (Dierig et al., 2001a).

In an effort to determine whether salt tolerance is a heritable trait in lesquerella, a study was conducted to compare the salinity response of a selected line WCL-SL1 with the parent line and another unselected line.

2. Materials and methods

On 28 October 1998, seeds of the selected salt tolerant line, designated as WCL-SL1, were direct-seeded into 21 tanks along with two other lines in a replicated randomized block salinity trial. Other

Table 1
Electrical conductivity and composition of salinizing salts used to irrigate lesquerella in outdoor sand cultures

EC_i (dS m^{-1})	Ca (mol m^{-3})	Mg (mol m^{-3})	Na (mol m^{-3})	SO$_4$ (mol m^{-3})	Cl (mol m^{-3})
3	3.5	2.5	21.5	10.9	10.6
7	7.3	5.8	50.9	25.9	24.7
11	10.2	9.8	87.0	42.0	42.2
15	13.0	13.9	123.0	58.0	59.6
18	13.5	17.8	158.0	71.5	76.4
21	13.6	22.2	196.0	85.5	90.8
24	13.7	26.5	234.0	100.0	105.0

lines used in this study included the parental line '1986 bulk' from which WCL-SL1 was selected in the previous year, and another unselected line '54' as a check (Dierig et al., 2001a).

The tanks ($1.5 \times 3.0 \times 2.0$ m deep) contained washed sand as a growing media, having an average bulk density of 1.4 mg m^{-3}. At saturation, the sand had an average volumetric water content of 0.34 m^3 m^{-3} and 0.1 m^3 m^{-3} after drainage. After seeding, the tanks were irrigated daily with a nutrient solution containing (in mol m^{-3}): 3.5 Ca^{2+}, 2.5 Mg^{2+}, 21.5 K$^+$, 6.0 K$^+$, 10.9 SO$_4^{2-}$, 10.6 Cl$^-$, 5.0 NO$_3^-$, 0.17 KH$_2$PO$_4$, 0.050 Fe (as sodium ferric diethyleneamine pentaacetate), 0.023 H$_3$BO$_3$, 0.005 MnSO$_4$, 0.0004 ZnSO$_4$, 0.0002 CuSO$_4$ and 0.0001 H$_2$MoO$_4$ made up with Riverside municipal water. This solution ($EC_i = 3$ dS m^{-1}) served as the control treatment. Irrigation waters were pumped into the tanks from 3700 l reservoirs and returned by gravity through a subsurface drainage system to maintain a uniform and constant profile of salinity in the sand. Evapotranspiration water loss was replenished automatically each day to maintain constant EC_i in the reservoirs.

Plants were thinned to 24 plants per line in each plot (tank) prior to salinity treatments. Commencing on 21 January 1999, treatments were imposed by stepwise additions of mixed salt salinity (Table 1). Over a period of 1 week, salinity levels in the tanks were increased to 3, 7, 11, 15, 18, 21 and 24 dS m^{-1}. Solution pH ranged between 7.8 and 8.2. The irrigation waters were formulated to simulate the saline-sodic drainage waters typical of those in the San Joaquin Valley of California (Suarez and Šimůnek, 1997). The completely randomized split-plot experimental design consisted of seven irrigation water qualities, three lesquerella genotypes, and three replications.

The irrigation waters were analyzed bimonthly by inductively coupled plasma optical emission spectrometry (ICPOES) to confirm that target concentrations of Ca^{2+}, Mg^{2+}, Na$^+$, K$^+$ and S were being maintained. Chloride was determined by coulometric–amperometric titration (Cotlove, 1963).

The number of surviving plants and plant heights were measured weekly. Leaf samples were taken for ion analysis on 26 May 1999. Plant tissue was washed with deionized water, dried at 70 °C, and ground to pass a 60-mesh screen. Total S, Ca^{2+}, Mg^{2+}, Na$^+$, K$^+$ were determined on the nitric–perchloric acid digests of the tissue using ICPOES. Chloride was determined on nitric–acetic acid extracts of plant material by coulometric–amperometric titration (Cotlove, 1963).

Irrigation was discontinued and plants were sprayed with Cyclone/Starfire (post emergence application as a crop desiccant) on 8 June 1999 and harvest on 15 June 1999. Individual dry shoots were weighed. Seeds from each plant were collected, weighed, and retained for oil analysis. Seed oil quantity and composition were determined by the analytical procedures described in Dierig et al. (1996). Oil content was measured by a Pulsed NMR analyzer and oil composition on a gas chromatograph. Statistical analyses were performed by ANOVA with mean comparisons at $P < 0.05$ based on Tukey's Studentized Range Test (SAS release version 6.12, SAS Institute Inc., 1997).
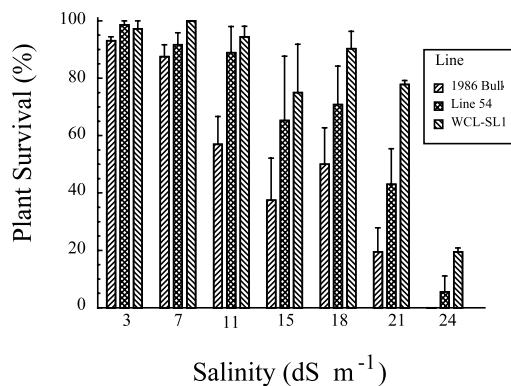
Fig. 1. Survival of three lesquerella lines 6 weeks after imposing salinity (standard error bar represents only the upper limit).

3. Results and discussion

3.1. Survival, plant height and biomass production

At the beginning of salinization, WCL-SL1 shoots were significantly taller than plants of the '1986 bulk' and line '54' in all plots, with mean heights of 11.7, 6.62 and 4.09 cm, respectively. Within 2 weeks after salinization, striking differences in both plant height and survival were observed among the lines due to salinity stress. Plant survival decreased with time, salinity concentration and line. WCL-SL1 was the most tolerant to salinity, line '54' intermediate, and the parental line '1986 bulk' the most sensitive. After 6 weeks of treatment, WCL-SL1 had significantly higher rates of survival than the parental line '1986

Table 2
Effect of salinity on plant height of lesquerella lines measured 134 days after planting

Irrigation water salinity (dS m^{-1})	Plant height (m)		
	Line '1986 bulk'	Line '54'	WCL-SL1
3	0.79 a[a]	1.03 a	1.70 b
7	0.72 a	0.90 a	1.47 b
11	0.61 a	0.69 a	1.19 b
15	0.47 a	0.52 a	0.87 a
18	0.61 a	0.51 a	1.03 b
21	0.38 a	0.58 a	0.93 b

[a] Within rows, means followed by a different letter are significantly different at the 0.05 probability level according to Tukey's Studentized Range test.

Table 3
Effect of salinity levels on shoot dry weight of lesquerella lines

Irrigation water salinity (dS m^{-1})	Shoot dry weight (g per shoot)		
	Line '1986 bulk'	Line '54'	WCL-SL1
3	20.5 a[a]	32.7 a	41.5 a
7	17.0 a	24.8 ab	36.9 a
11	11.5 ab	13.8 bc	25.0 ab
15	2.2 b	8.9 c	16.1 b
18	1.6 b	3.9 c	6.6 b

[a] Within columns, means followed by a different letter are significantly different at the 0.05 probability level according to Tukey's Studentized Range test.

Table 4
Statistical analysis of dry weight data

Dependent variable: dry weight per plant

Source	DF	SS	MS	F value	Pr > F	R-square	C.V.	Root MSE	DWT mean
Model	14	6328.327	452	10.99	0.0001	0.850736	35.34	6.413	18.147
Error	27	1110.326	41.1						
Total	41	7438.653							

Source	DF	Type I SS	MS	F Value	Pr > F
EC_i	4	4512.426	1128	27.43	0.0001
Line	2	1528.400	764.2	18.58	0.0001
$EC_i \times$ Line	8	287.499	35.94	0.87	0.5501

Source	DF	Type III SS	MS	F Value	Pr > F
EC_i	4	4512.426	1128	27.43	0.0001
Line	2	1476.587	738.3	17.95	0.0001
$EC_i \times$ Line	8	287.499	35.94	0.87	0.5501

bulk' and line '54' (Fig. 1). Plant survival of WCL-SL1 was unaffected by salinity treatments ranging from 3 to 11 dS m^{-1} with a slight decrease occuring between 11 and 21 dS m^{-1}. However, a dramatic drop in the number of surviving plants of WCL-SL1 occurred in the 24 dS m^{-1} treatment after 6 weeks. Plants from line '54' declined more dramatically at 21 dS m^{-1}. The parental 'line 1986 bulk' declined significantly in the 11 dS m^{-1} treatment. None of the parental plants survived the 24 dS m^{-1} salinity level. There were no significant differences between lines at 3 and 7 dS m^{-1} salinity levels. Salinity did not appear to affect plant survival of the three lines until EC_i reached 11 dS m^{-1}.

Six weeks after salinization, WCL-SL1 was significantly taller than plants from the other two lines for each treatment, except the 15 dS m^{-1} treatment (Table 2). In this case, the plant height was greater but not significantly different at the 0.05 probability level from the other lines. There were no significant differences for lines '1986 bulk' and '54' at any treatment level. A sufficient number of plants were not available for making comparison at the 24 dS m^{-1} level.

Salinity reduced vegetative growth more in the unselected lines compared with the WCL-SL1 (Table 3). Analysis of the final shoot dry weights

indicated that salinity and line effects were significant, but no interaction was present (Table 4). Increasing salinity decreased the average shoot weights in all lines and ranked mean differences within lines were consistent across all salinity levels from 3 to 18 dS m^{-1}. At 7, 15 and 18 dS m^{-1} average shoot dry weights of WCL-SL1 were significantly greater than the parental line. The average mean shoot weight of line '54' was intermediate between the other two lines. For all salinity levels, WCL-SL1 had higher average shoot weights (25.9 g per plant) than either line '54' (17.4 g per plant) or the parental line (11.2 g per plant).

3.2. Shoot-ion relations

Patterns of ion accumulation in leaves of all three genotypes were similar to those reported previously (Grieve et al., 1999) for the parental line irrigated with the same high sodium-high sulfate waters as were used in the present study. Leaf-Na and -Cl increased as the concentrations of these ions and salinity increased (Table 5). Leaf-Ca decreased significantly with increasing salinity, despite a 4-fold increase in external Ca^{2+}. This response was undoubtedly the result of increasing concentrations of Na^+ and Mg^{2+} in the irrigation waters as these cations may not only reduce Ca^{2+}

Table 5
Leaf mineral composition of three lesquerella lines grown at five salinity levels 211 days after planting

Irrigation water salinity (dS m^{-1})	Ca (mmol kg^{-1} dry weight)	Mg (mmol kg^{-1} dry weight)	Na (mmol kg^{-1} dry weight)	K (mmol kg^{-1} dry weight)	S (mmol kg^{-1} dry weight)	Cl (mmol kg^{-1} dry weight)
Line '1986 bulk'						
3	1445 a [a]	588 c	50 c	796 a	533 a	461 c
7	1323 a	663 ab	209 b	639 a	552 a	896 b
11	1263 a	755 a	632 a	501 b	596 a	1414 a
15	1051 b	786 a	871 a	355 c	588 a	1425 a
18	–	–	–	–	–	–
Line '54'						
3	1559 a	598 b	62 d	642 a	574 a	566 c
7	1605 a	828 ab	117 c	441 b	604 a	963 b
11	1462 ab	816 ab	593 b	398 b	560 a	1660 ab
15	1231 b	987 a	1205 a	280 c	692 a	2303 a
18	999 b	879 a	1213 a	274 c	547 a	1919 a
Line WCL-SL1						
3	1211 a	490 c	29 d	774 a	562 a	379 c
7	1239 a	614 b	183 c	714 a	703 a	893 b
11	1092 ab	638 b	617 b	606 ab	710 a	1304 ab
15	950 ab	786 a	1142 ab	405 b	704 a	1665 ab
18	776 b	714 a	1397 a	345 b	589 a	1743 a

[a] Within columns and lesquerella lines, means followed by a different letter are significantly different at the 0.05 probability level according to Tukey's Studentized Range test.
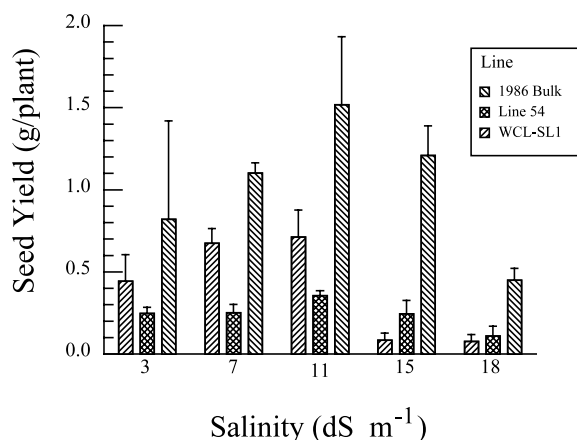
Fig. 2. Seed yield of three lesquerella lines grown at five salinity levels (standard error bar represents only the upper limit).

Table 6
Oil content and lesquerolic acid content of lesquerella seeds grown at five salinity levels

Irrigation water salinity (dS m^{-1})	Oil content (%)	Lesquerolic acid (%)
3	15.0 a [a]	46.4 a
7	16.4 ab	47.9 ab
11	17.6 bc	49.4 b
15	18.3 c	49.8 b
18	18.7 c	49.2 b

[a] Within columns, means followed by a different letter are significantly different at the 0.05 probability level according to Tukey's Studentized Range test.

activity in solution, but also may displace Ca^{2+} from its extracellular binding sites within plant organs and further disrupt Ca^{2+} acquisition (Grattan and Grieve, 1999). External K^+ concentration was constant throughout this study, and leaf-K decreased significantly. This was probably due to increasing concentrations of Na^+ in solution, which altered the selectivity of root membranes for K^+ over Na^+ and inhibited K^+ uptake. Total-S in the leaves was not affected by substantial increases in external SO_4^{2-}.

Leaf-Ca and -Mg in line WCL-SL1 tended to be lower than in the other two lines, but this response was significant only for line '54' (Table 5). There were no significant differences among genotypes for other ions. It is unlikely that the improved salt tolerance of line WCL-SL1 can be attributed to differences in ion accumulation.

3.3. Seed oil and yield

Seed yield (g per plant) increased in all three lines of lesquerella as salinity rose to 11 dS m^{-1} (Fig. 2). This behavior follows the survival data where lesquerella performed better under moderate saline conditions of up to 11 dS m^{-1}. WCL-SL1 seed yield was about two times the parental line '1986 bulk'. At higher salinities, seed yields of WCL-SL1 were greater than both '1986 bulk' and '54' lines combined. Very few plants survived at the 21 and 24 dS m^{-1} levels so comparisons of seed yield would not be meaningful.

Treatments of 11 dS m^{-1} and higher caused an increase in extractable seed oil (Table 6). The overall seed oil content of line'54' (16.0%) was significantly lower than lines '1986 bulk' and WCL-SL1 (17.1 and 17.8%, respectively). The lesquerolic acid content was higher in the three high salinity treatments compared with the low treatment (Table 6). There were no significant differences among the three lines in seed oil quality; however, lesquerolic acid content was significantly higher in the saline treatments above 11 dS m^{-1} than in the nonsaline control treatment.

A single cycle selection of lesquerella in salinized sand cultures resulted in an absolute and relative salt tolerant line as measured by plant survival, shoot dry matter production, plant height, and seed yield. Line WCL-SL1 dramatically out-performed the two non-selected lines for these traits. There were some differences between the unselected lines that were most likely due to the highly polymorphic nature of this species (Rollins and Shaw, 1973).

Plant adaptations to stress tolerances can occur at: (a) the developmental level; (b) the structural level; (c) the physiological level; or (d) the biochemical level (Tal, 1994). We observed no significant differences among lines in leaf ion concentrations in this study other than the leaf-Ca and -Mg of line WCL-SL1 which tended to be lower than the other genotypes. The single cycle selection process that led to the salt tolerance of

WCL-SL1 most likely affected a biochemical process where a single gene product, such as an enzyme change in a metabolic pathway, occurred. This line should be suitable for planting on saline soils of the western US for improved yields.

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