Recurrent Selection for Seedling Vigor in Kura Clover

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ABSTRACT

Kura clover (*Trifolium ambiguum* M. Bieb.) is a rhizomatous perennial forage legume that has outstanding persistence once established. Low seedling vigor presents a challenge to kura clover establishment. Kura clover seedlings partition a significant portion of their dry matter to below ground growth. Therefore, seedling vigor could be improved by increasing dry matter allocation to the shoot. Our objective was to study the potential of recurrent phenotypic selection in the greenhouse for reduced root/shoot ratio to increase seedling vigor. Three cycles of divergent selection for root/shoot ratio and a control selection for large plant biomass (LP) were performed from a parent population consisting of 'Rhizo', ARS-2678, KZ1, and Erect Spreader populations. Selections for low root/shoot ratio (LoRS) and high root/shoot ratio (HiRS) were performed by independent culling after first selecting for large total biomass. Parent and selected populations were evaluated in the greenhouse and field. Three cycles of selection for LoRS and LP increased shoot yield in the field 42 d after planting (DAP) by 35 and 34%, respectively. Three cycles of selection for LoRS reduced root/shoot ratio in the field by 16%. Selection for HiRS did not affect seedling size in the field. Population mean shoot and total plant weights were correlated between greenhouse and field environments (average $r = 0.89$). Population mean seed weight was correlated with shoot weight in the field ($r = 0.68$, $P < 0.05$). The results indicate that phenotypic greenhouse selection for seedling size is an effective means of increasing seedling vigor in kura clover.

Kura clover (*Trifolium ambiguum* M. Bieb.) is a rhizomatous perennial forage legume that has demonstrated outstanding persistence (Sheaffer and Marten, 1991). It has potential to enhance the productivity and profitability of pastures by functioning as a permanent legume component without need for reseeding. Unfortunately, low seedling vigor slows or prevents the establishment of kura clover (Hill and Mulcahy, 1995; Taylor and Smith, 1998; Townsend, 1970). Therefore, improving seedling vigor has become a necessary goal of our kura clover breeding program.

Research on birdsfoot trefoil (*Lotus corniculatus* L.) provides an excellent source of information on breeding for seedling vigor in a small seeded legume like kura clover (Twamley, 1970, 1972). Twamley (1967, 1974) proposed that selection for seed size might be a simple and inexpensive method to increase seedling vigor. Although seed size is often highly correlated with seedling vigor, further investigation showed that selection only for seed size is not likely to improve vigor (McLean and Nowak, 1997). Seed size may be a poor indicator of seedling vigor because of highly significant maternal effects (Twamley, 1967). Fresh shoot weights of birdsfoot trefoil seedlings grown in the greenhouse were highly correlated to fresh shoot weights in the field 7 wk after planting and may serve as a more precise indicator of seedling vigor than seed size (Twamley, 1967). Twamley (1972) found that selecting the most vigorous birdsfoot trefoil seedlings from the most vigorous lines (genophenotypic selection) proved to be the most effective method of increasing seedling vigor, although there was also a modest parent-offspring correlation ($r = 0.27$) for phenotypic selection only. After three cycles of genophenotypic selection, Twamley (1974) had increased seedling birdsfoot trefoil fresh weight in the greenhouse by 40% at 6 wk of age.

Smith (1995) used two cycles of phenotypic recurrent selection for seed size with six hexaploid kura clover plant introductions to increase seed size by 27%. In a field study, the large seeded population had an establishment rate two times that of the control population 6 wk after planting, but forage yield in the seeding year was similar for both populations.

Kura clover requires vernalization to flower, so a selection program involving genotypic selection will require three growing seasons to perform one cycle of selection. In contrast, phenotypic selection can be performed at a rate of one cycle per year. Phenotypic selection for seedling vigor in kura clover may be more effective than in birdsfoot trefoil if selection could be targeted at a specific cause for low vigor. Genrich et al. (1998) and Hill and Mulcahy (1995) reported that kura clover allocates a majority of its resources to root and rhizome development during the establishment phase, resulting in a root/shoot ratio of 2.0 to 3.0 after a year of growth. Spencer et al. (1975) found that the kura clover cultivar Summit had a root/shoot ratio of 2.4, whereas the white clover (*Trifolium repens* L.) cultivar Grassland Huia had a root/shoot ratio of only 0.2. They also found that white clover had ten times the foliar production of kura clover in the first growing season. Spencer and Hely (1982) reported that kura clover had up to 300% greater root yield than white clover after 1 yr, but that white clover had 30% greater shoot production than kura clover over a 5 yr period. After studying seedling development of kura clover, Genrich et al. (1998) concluded that since kura clover seedlings partition a significant amount of dry matter to root and rhizome growth, increased seedling vigor might be achieved by selecting for increased shoot growth.

Selecting for reduced root/shoot ratio is an obvious method to use for increasing dry matter partitioning to the shoot. However, the results of selection on a ratio

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Abbreviations: ANOVA, analysis of variance; C_0, parent population; C_1, first selection cycle; C_2, second selection cycle; C_3, third selection cycle; HiRS, high root/shoot ratio; LoRS, low root/shoot ratio; LP, large plant biomass; DAP, days after planting.

are often not intuitive. Theory developed by Rowe (1995) can be used to predict the outcome of selection on a ratio. Assuming that shoot and root weight have similar genetic variances and are highly correlated, selection for reduced root/shoot ratio will rapidly reduce root weight while having little effect on shoot weight. Selecting for both low root/shoot ratio and large total plant weight would be a reasonable method to insure that selection would increase shoot weight while also reducing the root/shoot ratio.

Genrich (1995) initiated breeding for increased kura clover seedling vigor by completing one cycle of divergent selection for root/shoot ratio and selection for large plant biomass. When evaluated in the greenhouse, all three selected populations had significantly larger shoot mass than the parents, but no divergence was detected between the large and small root/shoot ratio populations. The favorable results obtained from a single cycle of selection indicated that shifting resource allocation of kura clover from the root to the shoot might be a reasonable mechanism of increasing seedling vigor in the species. Therefore, additional cycles of selection were warranted. The objective of this experiment was to study the potential of recurrent phenotypic selection in the greenhouse to increase total plant weight and reduce root/shoot ratio to increase kura clover seedling vigor.

MATERIALS AND METHODS

Greenhouse Selection

Two additional cycles of recurrent selection (C_2 and C_3) were performed on the three populations created by Genrich (1995): low root/shoot ratio (LoRS), high root/shoot ratio (HiRS), and large plant biomass (LP). Genrich (1995) had selected equal numbers of plants for LoRS, HiRS, and LP from 'Rhizo', ARS-2678, KZ1, and Erect Spreader populations. Rhizo was developed by selection for vigorous rhizomatous growth, persistence, disease, and insect resistance (Henry and Taylor, 1989). Population ARS-2678 is a germplasm selected for drought tolerance, winter hardiness, rhizome production, forage and seed yield, nodulation, and N_2 fixation activity (Rumbaugh et al., 1991). The New Zealand breeding population KZ1 was selected from 'Monaro' (Anonymous, 1983) for uniform flowering. The breeding population Erect Spreader was developed by the University of Minnesota from plant introductions by selections for erect growth habit and rapid rhizomatous spreading.

We established 1080 seedlings of the LoRS, HiRS, and LP populations in 15 cm deep sand benches. Seeds were scarified with sandpaper and planted 1.3 cm deep and 2.5 cm apart in rows 90 cm long spaced 7.6 cm apart. Two to four seeds were placed at each 2.5 cm spacing, and the plants were randomly thinned to 1080 seedlings at the unifoliolate leaf stage by removing all but one seedling from each 2.5 cm spacing. *Rhizobium leguminasarium* biovar *trifolii* (LiphaTech, Milwaukee, WI) was surface applied after planting and the sand benches were thoroughly watered. Greenhouse temperature was maintained between 22 and 26°C. Supplemental lighting was provided for 16 h d^{-1} at about 110 mmol s^{-1} m^{-2}. Before planting, the benches were steamed and treated with 1.1 g m^{-2} Terrachlor (pentachloronitrobenzene) fungicide. Before the C_2 selection, the sand was amended with 67 g m^{-2} $CaCO_3$, 4.5 g m^{-2} P, and 8.6 g m^{-2} K. Prior to the C_3 selection, the sand was amended with 67 g m^{-2} $CaCO_3$, 1.0 g m^{-2} P, 27.7 g m^{-2} K, and 900 g m^{-2} Micromax slow release micronutrient fertilizer (Grace-Sierra Horticultural Products Company, Milpitas, CA), and the sand was flushed thoroughly with water before planting to reduce surface salinity. Other than the fertility treatments that were adjusted to prevent nutrient availability from limiting seedling growth, the protocol was identical for both cycles of selection.

Selection was performed using a form of recurrent restricted phenotypic selection (Burton 1974). Each population was divided into 12 plots consisting of 90 plants each (three rows) to reduce the effects of variable greenhouse lighting and temperature. Selections were made about 42 DAP. Seedlings were harvested and washed with water. The seedlings were clipped 0.5 cm above the crown, and the fresh shoot and root portions were weighed separately. Selection was performed by independent culling. Thirty plants with the largest total biomass were selected from each plot of 90 plants. Next, out of these 30, four plants were selected for high root/shoot ratio, low root/shoot ratio, or large total biomass (both root and shoot) in the HiRS, LoRS, and LP populations, respectively. Overall, the proportion of individuals selected was 4.4% for each of the three selection criteria.

Roots from the selected plants (four plants from each of 12 plots = 48 per population) were planted in 10-cm diameter pots and allowed to grow in the greenhouse until mid March when the plants were placed in a growth chamber for vernalization. The chamber was set at 4°C, and lighting was provided for 12 h d^{-1} at 130 mmol s^{-1} m^{-2}. In early May, the populations were transferred to isolated crossing blocks at St. Paul and Rosemount, MN. Seeds were harvested from the crossing blocks in August, threshed, and cleaned. A composite was constructed from each population by combining an equal quantity of seed from each plant by mass. Seed weight of each population was determined by weighing four replicates of 200 seeds from each population.

Greenhouse Evaluation

The first greenhouse evaluation was performed in 1996 using the same C_2 plants as were used in the third cycle of selection. Therefore, the protocol was the same as previously described in the third selection cycle. The four parent populations and the three C_2 populations were grown in a randomized complete block design with 12 replicates. A second greenhouse evaluation was performed in 1997 using the four populations making up the Cycle 0 (C_0) (Rhizo, ARS-2678, KZ1, and Erect Spreader) and the nine selected populations resulting from three selection cycles using each of the three selection criteria. The design was a randomized complete block with 15 replicates and 30 plants of each parent and selected population per replicate. Before planting, the sand was amended with 67 g m^{-2} $CaCO_3$, 1.0 g m^{-2} P, 13.9 g m^{-2} K, and 90 g m^{-2} Micromax micronutrient mix. All other procedures were as in the selection methods above. The four parent populations were used as an estimate for the C_0 because Genrich (1995) made the initial selections using equal numbers of plants from each of the four parent populations. Seeds from all populations were stored in a controlled environment at 4°C and less than 40% humidity to prevent loss of seedling vigor.

Field Evaluation

Four field evaluations were conducted during 1997 at St. Paul and Becker, MN. At each location, two separate experiments were conducted in which the four parent populations and the six C_1 and C_2 populations (two cycles by three selection criteria) were planted in randomized complete block designs with eight replications. One experiment at each location was space planted, and a second experiment was seeded in rows. In the space-planted experiments, seeds were planted to a 1 cm depth and seedlings were randomly thinned at the unifoliolate leaf stage to form a grid with 5 cm equal spacings and 56 plants plot^{-1}. The row-planted experiments were seeded at 0.15 g m^{-1} in rows 178 cm long and 15 cm apart with each plot consisting of two rows. At St. Paul, both experiments were planted on May 21. At Becker, the row-planted experiment was planted on May 22 and the space-planted experiment was planted on July 15. All plots were inoculated with *Rhizobium leguminosarum* biovar *trifolii* and irrigated as needed. The soil at St. Paul was a Waukegan silt loam (fine-silty over sandy, mixed Typic Hapludoll) and at Becker the soil was a Hubbard loamy sand (sandy, mixed, Udorthentic Haploboroll). At both locations, pH, P, and K levels were greater than 6.5, 35 kg ha^{-1}, and 200 kg ha^{-1}. The silt loam soil was derived from loess parent material and has an organic matter content of about 30 g kg^{-1} whereas the loamy sand was derived from glacial outwash and has an organic matter content of about 10 g kg^{-1}. Shoot and root fresh weight was determined 42 DAP by harvesting, washing, and weighing the seedlings. All seedlings in the space-planted plots were harvested, and in the row-planted plots seedlings were harvested from two randomly selected 30 cm lengths of row. Weeds were removed by hand from all plots except those in the St. Paul row-planted experiment. These plots had heavy weed pressure from *Portulaca oleracea* L. and *Digitaria sanguinalis* L. that reached a height of 15 cm by harvest.

Two additional field evaluations were performed at St. Paul in 1998 using the four parent populations and the nine populations from three cycles of selection for HiRS, LoRS, and LP in randomized complete block designs with 8 plants plot^{-1}. The first experiment was planted on June 23 and had six replications. The second experiment was planted on July 1 and had eight replications. All plots were inoculated with *Rhizobium leguminosarum* biovar *trifolii* and irrigated as needed. The soil characteristics were as in 1997 at St. Paul. Plots were seeded at a 1 cm depth and seedlings were randomly thinned at the unifoliolate leaf stage to form a grid with 5 cm equal spacings. Seedling shoot and root fresh weights from both experiments were determined after harvesting and washing all the seedlings on August 4, 1998.

Data Analysis

We determined changes in plant biomass due to selection by measuring seedling fresh weights. While fresh weight may be subject to variation due to changes in plant moisture status, it provided a rapid approach to measuring response to selection. We verified the association of fresh and dry weights in a preliminary experiment and found the correlation to be $r = 0.98$ ($P < 0.001$). Increased seedling size is needed to compete with other species during the establishment phase, and whether the increased size is due to higher water or dry matter content may be irrelevant to successful establishment of kura clover. Seedling fresh weights have been commonly used to evaluate seedling vigor in birdsfoot trefoil (McKersie and Tomes, 1982; Twamley, 1967, 1970, 1972, 1974).

Analyses of variance (ANOVA) were performed on plot means of fresh shoot weight, fresh root weight, root/shoot ratio, and total plant biomass from each experiment. For the preliminary greenhouse evaluation, nonorthogonal contrasts were used to compare the selected populations to the C_0. In the other experiments, orthogonal polynomial contrasts were used to evaluate the response to selection. Due to heterogeneity of error, results from the two locations in the 1997 field evaluation were analyzed separately, but the two planting methods within a location were analyzed together according to the technology adaptation experiment procedure as described by Gomez and Gomez (1984). For the 1998 field evaluation, the two planting dates were also analyzed together using the same procedure. A correlation analysis was conducted by calculating Spearman's rank-order correlation coefficients between the population means of greenhouse and field grown plants. Although the individual plant weights were often not normally distributed, all statistical calculations were performed using plot means, which were normally distributed. All statistical analyses were performed with SAS software (SAS Institute, 1990).

RESULTS AND DISCUSSION

Greenhouse Experiments

In the 1996 greenhouse evaluation, the C_2 LP population had shoot, root, and total biomass weights that exceeded those of the C_0 by 27, 20, and 24%, respectively (Table 1). Two cycles of selection for LoRS reduced the root/shoot ratio by 11% when compared to the C_0, and two cycles of selection for HiRS increased the root/shoot ratio by 11% when compared with the C_0. The C_2 LP population also had a 7% reduction in root/shoot ratio.

In the 1997 greenhouse experiment, three cycles of LoRS selection produced responses in all four measured traits (Table 2). Selection for LoRS resulted in a linear reduction in root/shoot ratio and linear increases in root weight and total plant weight. The selection method produced a large linear increase in shoot weight, but the response also contained a small quadratic component. The quadratic component was detected because the C_2 had a shoot weight only 7% larger than the C_0, while the C_3 shoot weight was 25% larger than the C_0 (Table 3). Three cycles of LP selection produced linear and quadratic increases in root, shoot, and total plant weight (Table 2). The quadratic responses were due to a lack of progress in the second cycle of selection, and the large gain in seedling size that was achieved in the third cycle. For example, shoot weight was 11, 11, and 39% larger than the C_0 in the C_1, C_2, and C_3, respectively. These results, along with the quadratic response in shoot weight due to LoRS selection mentioned above, point to a consistent lack of progress in the C_2 and superior response to selection in the C_3. The most obvious explanation for lack of progress in the second cycle and excellent progress in the third cycle was the difference in selection conditions. The new fertility regime used in the third selection cycle resulted in plant weights (shoot and root) being almost twice as large in the third cycle than in the second. The larger plants growing in a nutrient rich environment probably had better expression of their genotypes than the plants in the previous cycles. Furthermore, when the plants were smaller, true differences were more likely to be masked by sampling error. The results indicate that successful phenotypic selection can be highly dependent upon environmental conditions. Given this limitation, phenotypic selection was

Table 1. Population means of kura clover seedling traits in the 1996 greenhouse evaluation of the parents (C_0) and C_2 progeny populations resulting from two cycles of selection for high root/shoot ratio (HiRS), low root/shoot ratio (LoRS) and large total plant weight (LP).

Population	Ratio†	Root	Shoot	Total
		g		
Parents	1.085	0.108	0.104	0.212
HiRS	1.201	0.113	0.096	0.209
LoRS	0.967	0.106	0.113	0.219
LP	1.009	0.130	0.132	0.263
LSD (0.05)	0.053	0.006	0.007	0.011

† Fresh root weight/fresh shoot weight (Ratio), fresh root weight (Root), fresh shoot weight (Shoot), and fresh weight of whole plant (Total).

still effective. The average gain in fresh shoot weight of 13% per cycle obtained by the LP selection was similar to the gains in seedling vigor obtained by Twamley (1974) using geno-phenotypic selection in birdsfoot trefoil. Because phenotypic selection was so successful, the kura clover populations used in this experiment must have possessed high genetic variance for seedling vigor.

The HiRS selection method did not produce linear responses to selection in the 1997 greenhouse evaluation (Table 2). There were quadratic responses observed in shoot weight and total plant weight. These quadratic responses were due to smaller plant size in the C_2 followed by a return to plant sizes similar to the C_0 in the C_3 (Table 3). This reduction in plant size in the C_2 parallels the poor performance of C_2 populations produced by LoRS and LP. The poor C_2 performance across all selection methods may have been caused by the environmental conditions under which the C_2 seed was produced. However, we did not observe any conditions that could have been particularly detrimental to seedling vigor. The other possibility is that selection was highly ineffective in the second cycle of selection due to poor growing conditions.

Field Experiments

The two soil types used in the 1997 field experiment produced large differences in plant growth. Shoot weight on the silt loam soil was more than two times larger than shoot weight on the loamy sand. These large differences resulted in heterogeneity of error that precluded a combined analysis across locations. Plants growing on the loamy sand lacked vigor and were somewhat chlorotic. These symptoms may be related to limited N_2 fixation and low N fertility of the soil. Nitrogen fertilization has been shown to increase the growth of kura clover seedlings (Seguin et al., 2000) because of ineffective symbiosis during the first 50 DAP.

Table 2. Mean Squares from ANOVA of kura clover seedling traits in the 1997 greenhouse evaluation of the four parent (C_0) populations and nine progeny populations resulting from three cycles of selection for high root/shoot ratio (HiRS), low root/shoot ratio (LoRS), and large total plant weight (LP).

Source	df	Ratio†	Root	Shoot	Total
			×1000 g		
Block	14	0.52***	0.80***	0.04***	1.09***
Population	12	0.26***	1.01***	0.26***	2.17***
HiRS linear‡	1	0.15	0.02	0.01	0.00
HiRS quadratic	1	0.08	0.07	0.05**	0.23*
LoRS linear	1	0.84***	0.27**	0.56***	1.61***
LoRS quadratic	1	0.00	0.06	0.03*	0.17
LP linear	1	0.03	4.59***	1.23***	10.59***
LP quadratic	1	0.00	0.47***	0.13***	1.10***
Error	168	0.05	0.04	0.01	0.06

* Indicates significance at $P < 0.05$.
** Indicates significance at $P < 0.01$.
*** Indicates significance at $P < 0.001$.
† Fresh root weight/fresh shoot weight (Ratio), fresh root weight (Root), fresh shoot weight (Shoot), and fresh weight of whole plant (Total).
‡ Single df test for response to selection using orthogonal polynomials.

Seeding method affected root, shoot, and total plant weight at both locations (Table 4). On the silt loam, total plant weights in the row-planted plots were 40% smaller than total plant weights in the space-planted plots. This reduction in plant growth was most likely due to the weed pressure in the row-planted plots. In contrast to the silt loam, total plant weights were 37% smaller in the space-planted plots than they were in the row-planted plots. Seeding date was probably the largest factor contributing to these differences in plant growth because the row-planted plots were seeded earlier in the season when soil N is more abundant.

Although seeding method effects were large, there were no seeding method by population interactions for root, shoot, or total plant weight observed at either location (Table 4). The lack of interactions indicates the stability of population performance across diverse conditions caused by weed pressure, seeding date, and

Table 3. Population means of kura clover seedling traits in the 1997 greenhouse and 1998 field evaluations of the parents (C_0) and nine progeny populations resulting from three cycles of selection for high root/shoot ratio (HiRS), low root/shoot ratio (LoRS) and large total plant weight (LP).

		Greenhouse				Field			
Population	Cycle	Ratio†	Root	Shoot	Total	Ratio	Root	Shoot	Total
				g				g	
Parents	C_0	2.116	0.059	0.028	0.087	0.444	0.476	1.127	1.603
HiRS	C_1	2.268	0.061	0.027	0.088	0.440	0.471	1.092	1.563
	C_2	2.230	0.056	0.025	0.081	0.438	0.474	1.105	1.578
	C_3	2.251	0.062	0.028	0.090	0.452	0.525	1.226	1.751
LoRS	C_1	1.991	0.060	0.030	0.090	0.400	0.519	1.305	1.824
	C_2	1.982	0.060	0.030	0.090	0.391	0.510	1.360	1.869
	C_3	1.831	0.064	0.035	0.099	0.375	0.543	1.525	2.068
LP	C_1	2.148	0.066	0.031	0.097	0.413	0.497	1.225	1.723
	C_2	2.085	0.064	0.031	0.095	0.470	0.562	1.213	1.775
	C_3	2.085	0.081	0.039	0.120	0.411	0.591	1.505	2.096
LSD (0.05)		0.150	0.004	0.002	0.005	0.044	0.084	0.191	0.259

† Fresh root weight/fresh shoot weight (Ratio), fresh root weight (Root), fresh shoot weight (Shoot), and fresh weight of whole plant (Total).

Table 4. Mean Squares from ANOVA of kura clover seedling traits of the four parent (C_0) populations and six progeny populations resulting from two cycles of selection for high root/shoot ratio (HiRS), low root/shoot ratio (LoRS), and large total plant weight (LP) when evaluated at two field locations in 1997.

		Silt Loam				Loamy Sand			
Source	df	Ratio†	Root	Shoot	Total	Ratio	Root	Shoot	Total
			g				g		
Seeding method‡	1	0.086	30.77***	426.8***	686.8***	2.61*	15.34***	38.27**	91.83***
Blocks within method	14	0.261	0.29	4.2	6.1	0.61	0.60	2.29	5.09
Population	9	0.135***	0.47***	1.47***	20.4***	0.84***	0.45***	2.37***	4.28***
HiRS linear§	1	0.046	0.00	0.0	0.0	0.54	0.17	0.02	0.30
HiRS quadratic	1	0.000	0.01	1.2	1.4	0.44	0.01	0.04	0.01
LoRS linear	1	0.240*	0.09	5.2***	6.6**	0.59	0.00	0.18	0.23
LoRS quadratic	1	0.024	0.00	0.6	0.6	0.06	0.03	0.38	0.64
LP linear	1	0.091	0.21	7.0***	9.7***	0.95*	0.03	1.38*	1.83
LP quadratic	1	0.068	0.21	0.4	0.5	1.02*	1.09**	0.63	3.37*
Method × population	9	0.051	0.00	0.6	0.8	0.40*	0.09	0.33	0.57
Error	126	0.038	0.06	0.4	0.7	0.17	0.11	0.29	0.68

* Indicates significance at $P < 0.05$.
**Indicates significance at $P < 0.01$.
***Indicates significance at $P < 0.001$.
† Fresh root weight/fresh shoot weight (Ratio), fresh root weight (Root), fresh shoot weight (Shoot), and fresh weight of whole plant (Total).
‡ Seeding was performed in rows and at 5 cm equal spacings.
§ Single df test for response to selection using orthogonal polynomials.

planting method. On the loamy sand, there was an interaction between seeding method and population for root/shoot ratio. This interaction was due to changes in rank between the seeding methods (Table 5) and indicates that root/shoot ratio may be less stable across environments than are shoot, root, and total plant weight.

The LP selection method produced a linear increase in shoot weight across both seeding methods at both locations in the 1997 field evaluation (Table 4). The C_2 LP population had a shoot weight that exceeded the C_0 by 10% on the loamy sand and by 12% on the silt loam (Table 5). The LP selection also resulted in a linear increase in total plant biomass on the silt loam soil; the C_2 LP population total plant weight was 11% larger than the C_0. The quadratic responses in root/shoot ratio, root weight, and total plant weight on the loamy sand were due to poor performance of the C_2 on this soil. Whereas the C_1 had a total plant weight 15% larger than the C_0, the C_2 was only 7% larger than the C_0. This poor performance of the C_2 is consistent with results obtained in the 1997 greenhouse experiment.

On the silt loam, LoRS selection produced a linear increase in shoot and total plant weight and a linear reduction in root/shoot ratio (Table 4). The C_2 LoRS population had a shoot weight and total plant weight that were 11 and 10% greater than the C_0 and a root/shoot ratio that was reduced by 7% (Table 5). The LoRS selection did not result in any changes in seedling traits from the C_0 when evaluated on loamy sand. A probable explanation for this result is the inadequacy of N_2 fixation and low N availability of the loamy sand. Under conditions where fertility limits plant growth, a reduced

Table 5. Population means of kura clover seedling traits of the parents (C_0) and six progeny populations resulting from two cycles of selection for high root/shoot ratio (HiRS), low root/shoot ratio (LoRS), and large total plant weight (LP) when evaluated at two field locations in 1997.

		Silt Loam							
		Spaced				Rows			
Population	Cycle	Ratio†	Root	Shoot	Total	Ratio	Root	Shoot	Total
			g				g		
Parents	C_0	0.258	0.284	1.115	1.398	0.254	0.167	0.679	0.846
HiRS	C_1	0.272	0.300	1.092	1.392	0.231	0.147	0.631	0.777
	C_2	0.261	0.284	1.091	1.374	0.238	0.167	0.711	0.879
LoRS	C_1	0.241	0.297	1.229	1.526	0.239	0.172	0.735	0.907
	C_2	0.247	0.308	1.241	1.550	0.228	0.169	0.745	0.914
LP	C_1	0.242	0.294	1.209	1.502	0.243	0.177	0.731	0.908
	C_2	0.248	0.311	1.258	1.569	0.236	0.174	0.757	0.932
LSD (0.05)		0.029	NS	0.098	0.132	0.023	0.026	0.097	0.120
		Loamy Sand							
Parents	C_0	0.539	0.176	0.331	0.507	0.551	0.259	0.480	0.739
HiRS	C_1	0.488	0.173	0.356	0.529	0.573	0.265	0.467	0.732
	C_2	0.546	0.195	0.361	0.556	0.596	0.267	0.456	0.723
LoRS	C_1	0.495	0.176	0.359	0.535	0.531	0.277	0.535	0.812
	C_2	0.479	0.165	0.349	0.515	0.546	0.272	0.496	0.768
LP	C_1	0.518	0.195	0.381	0.575	0.617	0.328	0.534	0.862
	C_2	0.494	0.187	0.380	0.567	0.519	0.257	0.515	0.772
LSD (0.05)		0.043	0.019	0.036	0.051	0.065	0.044	NS	0.131

† Fresh root weight/fresh shoot weight (Ratio), fresh root weight (Root), fresh shoot weight (Shoot), and fresh weight of whole plant (Total).

Table 6. Mean Squares from ANOVA of kura clover seedling traits in the 1998 field evaluation of the four parent (C_0) populations and nine progeny populations resulting from three cycles of selection for high root/shoot ratio (HiRS), low root/shoot ratio (LoRS), and large total plant weight (LP).

Source	df	Ratio†	Root	Shoot	Total
			g		
Seeding date	1	0.814***	50.26***	24.60***	518.7***
Blocks within date	12	0.115	0.25	4.4	6.4
Population	12	0.073***	0.26***	3.6***	5.7***
HiRS linear‡	1	0.000	0.11	0.5	1.2
HiRS quadratic	1	0.003	0.13	1.2	2.1
LoRS linear	1	0.286***	0.28*	10.8***	14.5***
LoRS quadratic	1	0.015	0.00	0.0	0.0
LP linear	1	0.029	1.28***	10.5***	19.1***
LP quadratic	1	0.008	0.02	1.4	1.7
Date × population	12	0.018	0.05	0.6	0.9
Error	142	0.018	0.07	0.6	0.7

* Indicates significance at $P < 0.05$.
** Indicates significance at $P < 0.01$.
*** Indicates significance at $P < 0.001$.
† Fresh root weight/fresh shoot weight (Ratio), fresh root weight (Root), fresh shoot weight (Shoot), and fresh weight of whole plant (Total).
‡ Single df test for response to selection using orthogonal polynomials.

root/shoot ratio is not likely to be an effective method for improving seedling vigor because maximum root production will be necessary for plant nutrition. In a nutrient limited environment, selection for both increased shoot and root weight would be the best mechanism for increasing seedling vigor, and this theory is consistent with the results of the field evaluation. Two cycles of HiRS selection did not produce a significant response in any of the measured seedling traits in the 1997 field evaluations (Table 4). This result is consistent with the 1997 greenhouse evaluation, in which no linear responses were produced by HiRS selection. Because HiRS selection failed to produce a consistent increase in root/shoot ratio, we conclude that kura clover may already be near the upper limits of biomass allocation to the root. If this is true, root mass is not likely to be increased without a corresponding increase in shoot mass.

The 1998 field experiments included the C_0 and populations from all three cycles of selection. Results were similar to those obtained in the 1997 greenhouse experiment, which included the same populations (Tables 2 and 6). The primary difference was that quadratic responses to selection were found in the greenhouse, but only linear responses were seen in the field. After three cycles of selection, LoRS and LP selection had increased shoot weight by 35 and 34%, respectively, and LoRS selection had reduced the root/shoot ratio by 16% (Table 3). Highly significant seeding date effects were due to the plants from the different seeding dates being harvested at different ages. Seeding date by population interactions were not significant, indicating that the populations performed consistently across seeding dates and growth stages.

Correlations

Shoot, root, and total plant weight for the C_0, C_1, and C_2 populations measured in the greenhouse were highly correlated with shoot and total plant weights from these populations across two planting methods in two field locations (Table 7). These strong correlations suggest that greenhouse measurements of seedling weight appear to be good predictors of seedling weight in diverse field conditions. Greenhouse root/shoot ratio was not correlated with any of the seedling measurements performed in the field, indicating that greenhouse selection for root/shoot ratio alone is not likely to increase seedling vigor in the field. Therefore, increases in field shoot weight in response to LoRS selection may be a result of selection for consistently large shoot weight rather than selection for low root/shoot ratio. This is not surprising because the LoRS selection protocol included not only selection for low root/shoot ratio, but also selection for large plant weight. However, the importance of population correlations should not be overestimated. McKersie and Tomes (1982) found that in birdsfoot trefoil correlations using population means cannot be extrapolated to correlations of individual plants within populations.

Seed weight was correlated with shoot, root, and total plant weight on the silt loam soil, but only with shoot weight on the loamy sand (Table 7). This indicates that there is potential to increase seedling vigor through selection for seed weight and that selection for increased seedling weight should also produce increased seed weight. Our results support this hypothesis because three cycles of selection for LP also increased seed weight by 15%, and the C_3 LP population had a seed weight which exceeded that of the high seed weight population created by Smith (1995) after two cycles of recurrent selection for seed weight. However, the correlations between shoot weight in the field and seed weight were lower and less consistent than those with

Table 7. Spearman's rank-order correlation coefficients between kura clover seedling traits in the greenhouse, weight of 200 seeds, and seedling traits measured in two field environments. Correlations include means of the four parent, three C_1, and three C_2 populations averaged across two seeding methods on each soil.

	Silt Loam				Loamy Sand			
	Ratio†	Root	Shoot	Total	Ratio	Root	Shoot	Total
Greenhouse Ratio	0.10	0.04	−0.08	−0.08	0.07	0.52	0.05	0.10
Root	−0.58	0.79**	0.72*	0.72*	−0.59	0.52	0.88***	0.81*
Shoot	−0.81**	0.85**	0.88***	0.88***	−0.70*	0.49	0.95***	0.89***
Total	−0.71*	0.88***	0.83**	0.83**	−0.62	0.54	0.94***	0.89***
Weight of 200 Seeds	−0.66*	0.81**	0.68*	0.68*	−0.53	0.37	0.68*	0.62

* Indicates significance at $P < 0.05$.
** Indicates significance at $P < 0.01$.
*** Indicates significance at $P < 0.001$.
† Fresh root weight/fresh shoot weight (Ratio), fresh root weight (Root), fresh shoot weight (Shoot), and fresh weight of whole plant (Total).

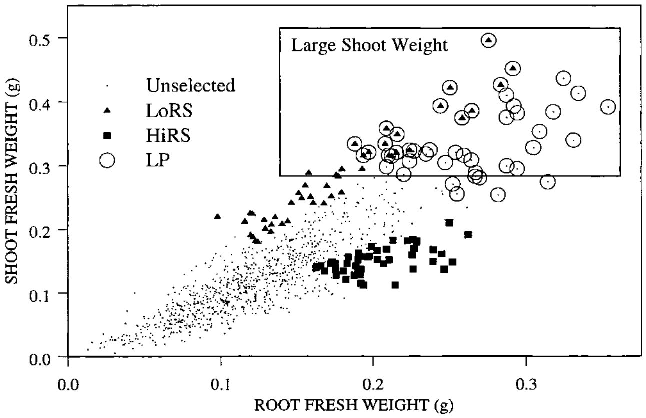

Fig. 1. Theoretical selections for low root/shoot ratio (LoRS), high root/shoot ratio (HiRS), large total plant weight (LP), and a proposed selection for large shoot weight in Kura clover indicated by the rectangle. The data presented are from greenhouse-grown C_2 LP plants.

greenhouse shoot or total plant weight. Furthermore, as Twamley (1967) stated regarding selection for seedling vigor in birdsfoot trefoil, large seed selection always suffers from the possibility of being confounded with large maternal effects.

Additional Cycles of Selection

Additional cycles of selection will be necessary to make agronomically valuable improvements in the seedling vigor of kura clover. Although we have demonstrated the effectiveness of LP and LoRS selection for increasing shoot weight of kura clover, three cycles of selection yielded populations with shoot weights similar to the most vigorous parent population. The LP selection method currently appears to be most promising because of its consistent performance under diverse environments. If shoot mass can be increased without sacrificing root mass, the plants may have a competitive advantage when nutrients are limiting, but LoRS selection may be beneficial in other environments. Therefore, the most conservative strategy would be to continue both selection methods until their strengths in different environments can be more fully determined.

Selecting simply for high shoot weight may be an efficient compromise between the LoRS and LP selection methods. Because the correlation between shoot weight and total plant weight is high ($r = 0.97$), selection for high shoot weight would resemble LP selection. But compared to LP selection, selection for large shoot weight would eliminate several plants with modest shoot weights and large root weights and include several plants with large shoots and low root/shoot ratios (Fig. 1). Selection for high shoot weight also would demand fewer resources by eliminating the need to carefully dig, wash, and weigh roots.

CONCLUSIONS

The LoRS and LP selection methods effectively created kura clover populations that had increased shoot growth in the greenhouse and in several field environments. Successful phenotypic selection for seedling size may be dependent upon growing the seedlings in an environment that will allow vigorous growth. Selection for seed size in kura clover may be beneficial because population mean seed size was correlated with shoot and root growth in the field. However, the correlations were greater for greenhouse shoot weight than for seed size, so gains are likely to be obtained more rapidly by direct phenotypic selection for shoot growth. Because the greatest increases in fresh shoot and total plant weight were obtained in the third cycle of selection, we expect that additional selection cycles will result in increased seedling growth. Selection for reduced root/shoot ratio does not appear to result in more rapid gains in seedling vigor than simply selecting the largest plants by weight. Selection based only on large shoot weight would be an effective compromise between the LoRS and LP selection methods used thus far, and reduce the resources necessary for conducting the selection.

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Genetic Relationships of Crown Rust Resistance, Grain Yield, Test Weight, and Seed Weight in Oat

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ABSTRACT

Integrating selection for agronomic performance and quantitative resistance to crown rust, caused by *Puccinia coronata* Corda var. *avenae* W.P. Fraser & Ledingham, in oat (*Avena sativa* L.) requires an understanding of their genetic relationships. This study was conducted to investigate the genetic relationships of crown rust resistance, grain yield, test weight, and seed weight under both inoculated and fungicide-treated conditions. A Design II mating was performed between 10 oat lines with putative partial resistance to crown rust and nine lines with superior grain yield and grain quality potential. Progenies from this mating were evaluated in both crown rust-inoculated and fungicide-treated plots in four Iowa environments to estimate genetic effects and phenotypic correlations between crown rust resistance and grain yield, seed weight, and test weight under either infection or fungicide-treated conditions. Lines from a random-mated population derived from the same parents were evaluated in three Iowa environments to estimate heritabilities of, and genetic correlations between, these traits. Resistance to crown rust, as measured by area under the disease progress curve (AUDPC), was highly heritable ($H = 0.89$ on an entry-mean basis), and was favorably correlated with grain yield, seed weight, and test weight measured in crown rust-inoculated plots. AUDPC was unfavorably correlated or uncorrelated with grain yield, test weight, and seed weight measured in fungicide-treated plots. To improve simultaneously crown rust resistance, grain yield, and seed weight under both lower and higher levels of crown rust infection, an optimum selection index can be developed with the genetic parameters estimated in this study.

Crown rust, one of the most widespread and damaging diseases of oat (Harder and Haber, 1992), can reduce grain yields (Endo and Boewe, 1958; Frey et al., 1973) and grain quality traits such as seed weight and groat percentage (Simons and Browning, 1961; Simons et al., 1979). Host plant resistance is the most economical control measure of oat crown rust (Harder and Haber, 1992).

The most common form of resistance exploited by oat breeders to date completely prevents reproduction of the fungus on the host and segregates as a single gene. Monogenic resistance historically has not remained effective in North America longer than 5 yr after the resistance genes were released in pure-line cultivars (Holland, 1997). Monogenic resistances can be overcome rapidly by new races of the pathogen that emerge because of the selection pressure exerted by large areas of uniformly resistant hosts (Harder and Haber, 1992; Kolmer, 1997). Evolution of new races in crown rust populations can occur via accumulation of mutations in asexual populations or by sexual recombination on the alternate host, buckthorn (*Rhamnus cathartica* L.), which occurs naturally in North America (Chong and Kolmer, 1993; Dinoor et al., 1988).

Methods proposed to improve the durability of crown rust resistance in oat include gene pyramiding, gene deployment, multiline breeding, and selection for partial resistance. Gene deployment, wherein breeders in different regions agree to release cultivars with different sets of resistance genes, should exert disruptive, rather than directional, selection pressure on the pathogen population (Frey et al., 1973). Multilines are expected to exert stabilizing, rather than directional, selection on the pathogen (Frey, 1982). Both gene deployment and multiline breeding strategies depend upon the availability of large numbers of effective resistance genes, which are not currently available for crown rust resistance in oat. Compared with resistance from a single major gene, gene pyramiding may enhance the durability of resistance because it should be more difficult for virulence to two or more major resistance genes to develop in a single fungal genotype. The combination of resistance genes *Pc38* and *Pc39* was released in the Canadian cultivars Dumont, Riel, and Robert, and in the North Dakota cultivars Steele and Valley (McMullen and Patterson, 1992). This combination of genes was no more durable than typical single gene resistances. Simultaneous virulence to both *Pc38* and *Pc39* became frequent in Canadian rust populations after the release of cultivars with this gene combination (Chong and Kolmer, 1993).

Partial resistance should be more durable than race-specific, complete resistance because selection pressure on the rust population is reduced (Simons, 1972). Evolution of virulence to partial resistance is expected to be slower than to complete resistance, although *Puccinia recondita* Roberge ex Desmaz populations responded to

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Abbreviations: AUDPC, area under the disease progress curve; SCA, specific combining ability.