Genetic Variation for Selenium Content in Tall Fescue

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ABSTRACT

Selenium is an element necessary for good animal health. No information is available on inheritance of Se concentration in tall fescue (*Festuca arundinacea* Shreb.). Therefore, the purpose of this investigation was to determine the nature of genetic variation of Se concentration in tall fescue. To accomplish this objective, 15 parental clones were randomly chosen from a broad-based population, and their half-sib (HS) progenies were generated using a polycross mating design. Selenium analysis was determined flourometrically by digesting dried, ground herbage samples in 3:1 $HNO_3/HClO_4$. Significant genetic variation was observed among HS families in the fall for Se concentration. Heritability, calculated using parent-offspring (PO) regression, gave estimates of 18% for summer and 68% for the fall. Heritability and genetic gain estimates were maximized in the fall, as compared with the summer, largely because of a large environmental component. Genetic gain, expressed as a percentage of the mean of parents, predicted an advance of 19% for fall herbage. Progress in selecting for improved concentrations of Se in the fall in this population of tall fescue is likely.

INHERITANCE of mineral concentrations in cool-season forage grasses has largely concentrated on the elements Mg, Ca, and K (Sleper et al., 1989). These three elements have received attention by forage grass breeders because of their importance in grass tetany or hypomagnesemia. Grass tetany of ruminants is associated with low blood-serum Mg levels (Kemp, 1960). Kemp and t'Hart (1957) and Butler (1963) demonstrated that the incidence of grass tetany was reduced when the K/(Ca + Mg) ratio (meq basis) was <2.2. Selection has occurred for high Mg in tall fescue (Sleper et al., 1989) and annual ryegrass (*Lolium multiflorum* Lam.) (Hides and Thomas, 1981). In most cool-season forage grasses, relatively high heritability values have been obtained for Mg and Ca (Sleper et al., 1989). Potassium has been shown to be more variable in inheritance because it is highly influenced by the environment for most cool-season forage grasses. To date, no information is available on the inheritance of Se in cool-season forage grasses.

Selenium, an element similar to sulfur, is required by animals, but not by plants (Mayland et al., 1989). In some areas, available soil Se may not be sufficient for forage plants to accumulate the 50 to 100 ng Se g^{-1} needed by animals. These Se-deficient areas often coincide with the distribution of tall fescue (Mayland et al., 1989; Cook, 1975). Selenium may be relatively unavailable in neutral and acid soils, and any attempt to increase crop Se uptake by shifting to some other plant species has been viewed with some skepticism (Ehlig et al., 1968). Selenium concentrations may differ between plant species, as Mayland et al. (1989) reported for plants grown on alkaline soils containing moderate to high levels of Se. Such differences may result from differences in rooting depth, especially where the subsoil contains greater amounts of soluble Se. It appears from our work that breeding plants such as tall fescue for improved Se concentration is a worthy long-term objective. The objective of this study

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was to determine the genetic variation for Se concentration in a broad-based tall fescue population.

MATERIALS AND METHODS

Fifteen parental plants of tall fescue were randomly chosen from a broad-based population, and their HS progenies were generated in isolation using a polycross mating design (Nguyen and Sleper, 1983b). The broad-based population from which the 15 parents were chosen originated from progeny after randomly mating 70 parents for one generation. The 70 parents were largely plant introductions chosen at random. The theory and application of HS matings for forage grass breeding was detailed by Nguyen and Sleper (1982a).

Field plots were established in August 1979 at Mt. Vernon, MO, as described by Nguyen and Sleper (1983b). The randomized complete-block design had four replications. Each replicate had 30 entries (15 parents and 15 HS families), with each plot consisting of seven seedlings (HS progenies) or clones (parents) planted 30 cm apart within a row and 90 cm between rows.

Reproductive tillers were removed in early May of each year to establish a uniform robust vegetative growth for the subsequent June (1983) and October (1984) harvests. A flail-type forage harvester was used to cut the herbage to a height of 10 cm. The plant material was then dried in a forced-air oven (38 °C) and ground to pass a 1-mm sieve in an intermediate Wiley mill. Subsamples of plant tissue were digested in 3:1 $HNO_3/NClO_4$ and Se determined fluorometrically (Olson et al., 1972). A National Bureau of Standards (NBS; now NIST) wheat flour, No. 1567, certified at 1100 ± 20 ng Se g^{-1}, was analyzed as 1020 ± 80 ng. The NBS citrus leaf, No. 1572, reported to contain 25 ng Se g^{-1}, was analyzed as 37 ± 3 ng. All data were reported on a dry-matter basis.

The combined ANOVA was computed separately for parents and HS families using plot means. The split-plot in time with years as subplots was used according to Steel and Torrie (1980). Expected mean squares were based on a random effects model for replicates, years, parents, and HS families (Table 1). Components of variance were estimated by computing appropriate linear functions of the mean squares as determined from expectations of mean squares in the ANOVA (Steel and Torrie, 1980). The standard error of an estimated variance component was calculated as described by Anderson and Bancroft (1952), as follows:

standard error of variance

$$(S^2) = [2/\Sigma a_i^2 (M_i^2/f_i + 2)]^{1/2}$$

where the a_i's are the linear coefficients used in computing the variance component (S^2), M_i's are the mean squares, and f_i's are the degrees of freedom associated with the M_i's.

Broad- (H_b) and narrow-sense (H_n) heritabilities were estimated on a phenotypic mean basis averaged over years and replications (Nguyen and Sleper, 1983a), as follows:

$$H_b = S_p^2/S_{pm}^2 = S_p^2/[S_p^2 + S_{py}^2/y + S_e^2/ry]$$

and

$$H_n = S_f^2/S_{pfm}^2 = S_f^2/[S_f^2 + S_{fy}^2/y + S_e^2/ry]$$

Table 1. Expectation of mean squares from the analysis of variance for parents and half-sib families.

Source	df	Expected mean squares†
Reps (R)	3	$\sigma_e^2 + 2\sigma_{gr}^2 + 15\sigma_{rr}^2 + 30\sigma_r^2$
Genotypes (G)	14	$\sigma_e^2 + 2\sigma_{gr}^2 + 4\sigma_{gy}^2 + 8\sigma_g^2$
G × R (Error a)	42	$\sigma_e^2 + 2\sigma_{gr}^2$
Year (Y)	1	$\sigma_e^2 + 4\sigma_{gy}^2 + 60\sigma_y^2$
G × Y	14	$\sigma_e^2 + 4\sigma_{gy}^2$
G × R × Y (Error b)	42	σ_e^2

† $r = 4$ replications, $g = 15$ genotypes (parents or half-sibs), $y = 2$ years.

where S_p^2 and S_f^2 = variance components due to parents and HS families, respectively; S_{py}^2 and S_{fy}^2 = variance components due to parents × year and HS families × year, respectively; S_{pm}^2 and S_{pfm}^2 = total phenotypic variance of parents and HS families estimated from variance components of plot means averaged over year and reps, respectively; S_e^2 = variance components due to genotype × replication × year within the same location; and y and r = number of years and replications, respectively.

Approximate standard error (SE) estimates of H_b and H_n were computed as a ratio of the SE of the genetic variance in the numerator to the corresponding phenotypic variance according to Dickerson (1969), as follows:

$$\text{SE } H_b = \text{SE}(S_p^2)/S_{pm}^2 \text{ and SE } H_n = \text{SE}(S_f^2)/S_{pfm}^2.$$

Narrow-sense heritability (h^2) on a phenotypic mean basis was estimated as twice the genotypic regression coefficient $(h_{po}^2 = 2b_G)$, according to Falconer (1981).

Expected gains per cycle (G) were calculated for a selection intensity of 20%, as follows (Nguyen and Sleper, 1983a):

$$G_p = Kh^2(S_{pm}^2)^{1/2}$$

where K = standardized selection differential for selection intensity of 20%, which was 1.4; S_{pm}^2 = phenotypic variance among parent mans; and h^2 = narrow-sense heritability estimated using the PO regression. This genetic gain was expressed as a percentage of the mean of the parents.

RESULTS AND DISCUSSION

Table 2 shows the means for HS families and parents for each season when pooled over years. Selenium concentrations ranged from 82 to 147 ng g^{-1} for Parent 146 and HS 144 sampled in the fall, respectively. When pooled across years, the HS and parental means were nearly identical for both seasons; however, the year source of variation was highly significant (Table 3). Mean values of Se content averaged across parents and HS families were 109 and 129 ng g^{-1} for summer and fall, respectively, in 1983 and 125 and 102 ng g^{-1} for summer and fall, respectively, in 1984. The environmental data indicated that rainfall was probably the main source of variation. In 1983, precipitation from April through June was considerably higher than for this same period in 1984. Although July and August of 1983 received more rainfall than 1984, Sep-

Table 2. Means and standard errors of Se content across years for summer and fall for parents and half-sib families from a broad-based population of tall fescue.

Parent genotype†	Summer		Fall	
	Half-sibs	Parents	Half-sibs	Parents
		— $n\,g^{-1}$ —		
144	130 ± 35	137 ± 10	147 ± 45	135 ± 37
137	130 ± 26	120 ± 21	134 ± 46	146 ± 31
125	121 ± 46	117 ± 22	131 ± 49	127 ± 34
166	136 ± 41	113 ± 20	127 ± 28	112 ± 27
118	126 ± 28	108 ± 22	125 ± 41	105 ± 33
170	126 ± 21	136 ± 41	120 ± 30	140 ± 57
149	112 ± 24	123 ± 26	114 ± 38	130 ± 27
159	111 ± 25	117 ± 24	110 ± 43	105 ± 34
173	118 ± 26	112 ± 22	110 ± 49	89 ± 19
133	108 ± 24	111 ± 32	108 ± 31	98 ± 14
141	109 ± 17	123 ± 24	106 ± 29	132 ± 47
163	96 ± 17	119 ± 19	106 ± 23	129 ± 45
135	104 ± 20	123 ± 26	102 ± 24	126 ± 45
164	108 ± 16	98 ± 14	101 ± 23	83 ± 14
146	106 ± 30	101 ± 31	98 ± 41	82 ± 21
Mean	116	117	116	116

† Fifteen parental clones randomly chosen.

tember (which corresponds with the period of active fall growth for tall fescue) accumulated >7.5 cm more in 1984 than in 1983. Whenever rainfall was higher during spring and fall growing seasons, Se content was lower. This may be explained by the oxidative state of Se and by dilution of the soluble Se in soil water and then dilution by the increased photosynthate of the rapidly growing plants (Albasel et al., 1989). In alkaline, dry, well-aerated soils, Se tends to form selenate (+6), while acid, wet, and poorly drained soils have a higher selenite (+4) concentration (Gissel-Nielson et al., 1984; Albasel et al., 1989). Uptake and translocation of selenate is generally more efficient than selenite because it is able to utilize the uptake sites and transport mechanism of sulfate. Also, selenite retention by clay particles is greater than selenate retention (Albasel et al., 1989).

Mean squares for parents were not significant; for HS families, significance occurred only during the fall (Table 3). Provided that genetic variation was adequate, this suggests that fall would be the time in which selection for high Se concentration would likely be successful. It is likely that selection for high Se concentration in the summer would not be useful in most of the tall fescue growing regions of the USA because tall fescue usually goes dormant during this period because of high temperatures and lack of moisture.

Advancement of any trait within a mixed population depends upon whether genetic variability exists among individual plants and if this variability is a result of additive genetic effects. The genetic variance component for HS families was significantly greater than zero in the fall, suggesting the existence of genetic variation for Se concentration during this season (Table 4). Other genetic variance components were not significant. Genotype × year interaction variance components were significantly different from zero only for parents in the fall. Negative genotype × year variance components were observed for parents in the summer and HS families in the fall. Dudley and Moll (1969) stated that if a particular component of variance is in reality very small, negative experimental estimates are not unexpected. Because variance components are obtained via subtraction of mean squares, slight inaccuracies can be magnified, even to the extent of causing negative variance components (which in our analysis were interpreted to be zero).

According to Levings and Dudley (1963), the best estimate of heritability in outcrossing species of autotetraploids and diploids results from doubling the

regression of offspring on parent. Heritability based on the regression of HS progeny means on one of their parents contains only additive genetic variance and additive type of epistasis, allowing for a less restrictive genetic model and reducing the bias associated with the estimated components of genetic variance (Falconer, 1981). A practical problem with any heritability estimate is that we must interpret genetic transmission in terms of individual genes, and we know that gene interactions are extremely important (Gilbert, 1973 and Simmonds, 1984). Furthermore, genotype × environmental interactions also influence heritability estimates. An underlying assumption of PO regression is that all genotype × environment interactions between parents and progenies are zero (Hanson, 1963; Dudley and Moll, 1969; Vogel et al., 1980; Casler, 1982). A way to remove this potential bias is to regress progeny means on parental means evaluated under different environments (Buckner et al., 1981). In this investigation, only one location was used, but data were collected over a 2-yr period; however, it must be realized that data collected from one location may bias heritability estimates upward (Nguyen and Sleper, 1983a).

Narrow-sense heritability obtained via regression gave estimates of 68% for the fall and 18% for summer (Table 5). The narrow-sense estimate obtained via regression for the fall exceeded its standard error by more than three times. Narrow-sense estimates from variance components were 0 and 91% for summer and fall, respectively. Broad-sense heritability estimates were 3 and 0% for summer and fall, respectively. Narrow-sense estimates obtained via regression and variance components for the fall were large enough to suggest progress in selecting for improved Se content. If parental or F_1 clones are to be selected and reproduced vegetatively, improvement may be estimated directly from the broad-sense heritability estimate and the clonal means. If clones are to be selected for inter-

Table 4. Estimates of various components and their standard errors for selenium content in parents and half-sib families from a broad-based population of tall fescue.

Variance component‡	Summer		Fall	
	Parents	Half-sibs	Parents	Half-sibs
		× 10⁻⁷†		
S_g^2	−4 ± 552	−148 ± 470	−942 ± 421	2717* ± 1680
S_{gy}^2	−485 ± 168	269 ± 135	1826* ± 246	−433 ± 209
S_e^2	3050 ± 635	2499 ± 524	3487 ± 791	3854 ± 893

* Significant at the 0.05 level of probability.
† Actual data multiplied by this to get the reported numbers.
‡ S_g^2 = variance components due to genotypes; S_{gy}^2 = variance components due to genotypes × year; and S_e^2 = variance components due to genotype × replication × year.

Table 3. Mean squares of parents and half-sib (HS) families for Se concentration in the summer and fall for a broad-based population of tall fescue.

Source	Summer		Fall	
	Parents	HS	Parents	HS
		× 10⁻⁷†		
Genotypes (G)	10292	9125	17409	36591*
G × Rep (R)	11714	8995	17421	16931
Year (Y)	69870**	71262**	295609**	157669**
G × Y	1852	3713	7890*	1856
G × R × Y	2976	2456	3708	4188

*,** Significant at the 0.05 and 0.01 level of probability, respectively.
† Actual data multiplied by this to get the reported numbers.

Table 5. Broad- (H_b) and narrow-sense (H_n) heritability estimated from variance components and narrow-sense heritability estimated by parent–offspring regression (h_{po}^2) and gain from selection (G) expressed as a percentage of the mean in a broad-based population of tall fescue.

Statistic	Summer	Fall
H_b	0.03 ± 0.41	0.0
H_n	0.0	0.91 ± 0.60
h_{po}^2	0.18 ± 0.16	0.68 ± 0.18
G (%)	3	19

crossing to establish a new breeding population, then narrow-sense heritability should be used to predict genetic gain from selection.

The response to selection that a plant breeder is primarily concerned with is the change of the population mean: the difference of the mean phenotypic value between the offspring of the selected parents and the whole of the parental generation before selection. Falconer (1981) cautions that estimated gain from selection is not a prediction but rather a description, because the regression of offspring on parent cannot be measured until the offspring generation has been grown. Also, the response depends on the heritability of the character, which includes the particular trait's interaction with the environment. As this study has revealed, yearly and seasonal differences in Se content can be obtained. Therefore, gain from selection is valid, in principle, for only one generation. However, by averaging a character's response across several seasons and years, one is able to approach a reasonable and practical trend toward predicting future responses to selection. Based on the 1983 and 1984 performance of a broad-based population of tall fescue, the estimated gain for fall and summer was 19 and 3%, respectively (Table 5).

In summary, a broad-based population of tall fescue genotypes did express variability in Se content. Results indicated that heritability and genetic gain were maximized in the fall, as opposed to summer selection, due in part to a large environmental component. Heritability obtained using PO regression gave us a fall estimate of 68%, with a predicted genetic advance of 19%. Genetic progress can be expected in a program to increase Se concentration in this population of tall fescue in the fall. Breeding procedures such as phenotypic restricted recurrent selection or simple mass selection, which utilize the additive genetic variance, are expected to be the methods of choice in selection for improved Se content. The expected gains are of significant importance to grazing animal health.

REFERENCES

Albasel, N., P.F. Pratt, and D.W. Wescot. 1989. Guidelines for selenium in irrigation waters. J. Environ. Qual. 18:253–258.

Anderson, R.L., and T.A. Bancroft. 1952. Statistical theory in research. McGraw-Hill Book Co., New York.

Buckner, R.C., P.B. Burrus, P.L. Cornelius, and L.P. Bush. 1981. Genetic variability and heritability of certain forage quality and mineral constituents in Lolium-Festuca hybrid derivatives. Crop Sci. 21:419–423.

Butler, E.J. 1963. The mineral element content of spring pasture in relation to the occurrence of grass tetany and hypomagnesaemia in dairy cow. J. Agric. Sci. 60:329–340.

Casler, M.D. 1982. Genotype X environment interaction bias to parent-offspring regression heritability estimates. Crop Sci. 22:540–542.

Cook, K.A. 1975. Determination of selenium in Missouri fescue by neutron activation analysis. MS thesis. Univ. of Missouri–Columbia.

Dickerson, G.E. 1969. Techniques for research in quantitative animal genetics. p. 36–79. In W.D. Hanson and H.F. Robinson (ed.) Techniques and procedures in animal science research. Am. Soc. Anim. Sci., Albany, NY.

Dudley, J.W., and R.H. Moll. 1969. Interpretation and use of estimates of heritability and genetic variances in plant breeding. Crop Sci. 9:257–261.

Ehlig, C.F., W.H. Allaway, E.E. Cary, and J. Kubota. 1968. Differences among plant species in selenium accumulation from soils low in available selenium. Agron. J. 60:43–47.

Falconer, D.S. 1981. Introduction to quantitative genetics. Ronald Press, New York.

Gilbert, N. 1973. Biometrical interpretation. Oxford Univ. Press, London.

Gissel-Nielsen, G., U.C. Gupta, M. Lamand, and T. Westermarck. 1984. Selenium in soils and plants and its importance in livestock and human nutrition. Adv. Agron. 37:397–460.

Hanson, W.D. 1963. Heritability. p. 125–139. In W.D. Hanson and H.F. Robinson (ed.) Statistical genetics and plant breeding. Natl. Acad. Sci.–Natl. Res. Council, Washington, DC.

Hides, D.H., and T.A. Thomas. 1981. Variation in the magnesium content of grasses and its improvement by selection. J. Sci. Food Agric. 32:990–991.

Kemp, A. 1960. Hypomagnesaemia in milking cows: The response of serum magnesium to alterations in herbage composition resulting from potash and nitrogen dressings on pasture. Neth. J. Agric. Sci. 8:281–304.

Kemp, A., and M.L. t'Hart. 1957. Grass tetany in grazing milking cows. Neth. J. Agric. Sci. 5:4–17.

Levings, G.S., and J.W. Dudley. 1963. Evaluation of certain mating designs for estimation of genetic variance in autotetraploid alfalfa. Crop Sci. 3:532–535.

Mayland, H.F., L.F. James, K.E. Panter, and J.L. Sonderegger. 1989. Selenium in seleniferous environments. p. 15–50. In L.W.G. Jacobs (ed.) Selenium in agriculture and the environment. SSSA Spec. Publ. 23. ASA, CSSA, and SSSA, Madison, WI.

Nguyen, H.T., and D.A. Sleper. 1983a. Theory and application of half-sib matings in forage grass breeding. Theor. Appl. Genet. 64:187–196.

Nguyen, H.T., and D.A. Sleper. 1983b. Genetic variability of seed yield and reproductive characters in tall fescue. Crop Sci. 23:621–626.

Olson, D.E., I.S. Palmer, and E.E. Cory. 1972. Modification of the official fluorometric method for selenium in plants. J. Assoc. Off. Anal. Chem. 58:117–121.

Simmonds, N.W. 1984. Principles of crop improvement. Longman, New York.

Sleper, D.A., K.P. Vogel, K.H. Asay, and H.F. Mayland. 1989. Using plant breeding and genetics to overcome the incidence of grass tetany. J. Anim. Sci. 67:3456–3462.

Steel, R.G.D., and J.H. Torrie. 1980. Principles and procedures of statistics. 2nd ed. McGraw-Hill Book Co., New York.

Vogel, K.P., F.A. Haskins, and H.J. Gorz. 1980. Parent-progeny regression in indiangrass: Inflation of heritability estimates by environmental covariances. Crop Sci. 20:580–582.