Genetic Variances and Selection Potential for Selenium Accumulation in a Rapid-cycling *Brassica oleracea* Population

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ABSTRACT. Beneficial effects of selenium (Se) can be delivered to humans through enriched plant foods. Plants in the Brassicaceae are good sources of sulfur (S) and can be enriched with Se. Breeding plants to be more efficient at Se accumulation may complement enrichment efforts. Because Se and S are chemically similar and can compete in plant metabolic pathways, S levels must be considered when attempting to manipulate Se, and vice versa. The objectives of this study were to establish genetic variances for S and Se accumulation, and to determine if simple recurrent selection could be used to manipulate Se accumulation in a rapid-cycling (*Brassica oleracea* L.) population. Progeny from a North Carolina Design II mating scheme were grown in two seleniferous environments and expressed variability for Se and S accumulation. Narrow sense heritability estimates for Se and S accumulation were moderate (0.55 to 0.75), which suggested progress was possible. However, standard errors were large and may influence expected progress during improvement efforts. Plants of a rapid-cycling *B. oleracea* were also subjected to two cycles of divergent selection for Se accumulation in leaf tissues. Realized heritabilities were high during selection for both high and low Se accumulation. Simultaneous evaluation of all populations revealed actual gains from selection to be 4.8% and 4.0% per selection cycle for high and low Se accumulation, respectively. Predicted gains for Se accumulation in the plants were 6.8%. Selection for Se accumulation was successful and indicates population improvements for such traits are possible within the *B. oleracea* analyzed. Breeding plants that are more efficient at accumulating Se could be a useful tool towards Se enrichment.

More than two billion people consume diets that are less diverse than 30 years ago, leading to deficiencies in micronutrients, especially Fe, vitamin A, I, Zn, and selenium (Se) (Welch and Graham, 1999). An emerging strategy for reducing micronutrient malnutrition is use of plant breeding to improve the nutrient content of the food people consume. Micronutrient qualities of improved crops have rarely been objectives in breeding programs (Ruel and Bouis, 1998). Results from recent studies, however, provide compelling evidence for enhancing nutritional quality in breeding programs (Graham et al., 1999). Substantial genetic variation was reported for mineral content (Kopsell and Randle, 1997; Quintana et al., 1996), vitamin content, (Tigchelaar, 1986) and phytochemical content (Wang and Goldman, 1996) in plants. Screening procedures for micronutrient content in crops are now becoming more efficient, making selection for nutritional traits more feasible (Graham and Welch, 1996).

Selenium is an essential micronutrient for maintaining mammalian health (Combs, 1989). Epidemiological studies have pointed to inverse associations between nutritional Se status and cancer risks (Combs and Gray, 1998), cardiovascular diseases (Oldfield, 1991), and immune system functions (Baum et al., 1997). Currently, there are more than 40 diseases and conditions in humans related to Se deficiency (Clark et al., 1998). Average Se intakes of most countries are sufficient to meet US recommended daily allowances of 50 to 70 mg·d⁻¹ (Clark et al., 1998). However, several countries, such as New

Zealand, Finland, United Kingdom, and areas of China, have inadequate Se intake (Reilly, 1998). Because the soil is the primary source of Se, levels of Se in plant foods, and in the animals that consume them, show considerable variation because of fluctuations in soil Se that occur throughout the world (Combs, 1989; Reilly, 1998).

One recognized strategy for reducing micronutrient malnutrition is fortification of dietary foods (Maberly et al., 1994). Selenium fortification of food products has been approved, and products are available in Australia, New Zealand, Japan, and China (Reilly, 1998). Several problems may arise in the Se fortification of foods. First, Se is a micronutrient with a narrow safety margin for consumption. The U.S. National Academy of Sciences has recommended Se intakes up to 200 mg·d⁻¹ as safe and adequate (National Research Council, 1983). But, sustained consumption at levels exceeding 750 mg·d⁻¹ can cause Se poisoning, or selenosis (Department of Health, 1991). Second, the chemical form of Se in foods and supplements determines absorption, metabolic efficiency, and toxicity within the body (Thomson, 1998). Inorganic selenite or organic selenomethionine are the forms most commonly used in Se supplements, which provide Se only in a single form. Prolonged consumption of single forms of Se can produce side effects, such as changes in cellular glutathione homeostasis (selenite) and exaggerated accumulation in body tissues (selenomethionine) (Ip and Lisk, 1994a).

One of the goals of Se chemopreventative research is finding a way to deliver Se safely and efficiently to human diets in a food form (Ip and Lisk, 1994a). While most fruits and vegetables contain Se at <0.01 mg·g⁻¹ (Morris and Levander, 1970), some can be Se enriched. Selenium enriched vegetables (Ip et al., 1992) and nuts (Ip and Lisk, 1994b) can reduce certain carcinomas as effectively as traditional Se supplements while also providing multiple forms of Se when ingested (Ganter, 1986). In addition, some of these naturally synthesized selenocompounds have high anticarcinogenic activity (Ganter and Lawrence, 1997). Enrichment of vegetables

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Table 1. ANOVA of Design II (Comstock and Robinson, 1948) used for Se and S accumulation in rapid-cycling *Brassica oleracea* grown in two seleniferous environments.

Source of variation	df	MS	Expected MS ^z
Environments	1		
Set	3		
Set × environment	3		
Replication/set/environment	24		
Male/set	12	M ₇	$\sigma^2 + r \sigma_{fme}^2 + rf \sigma_{me}^2 + re \sigma_{mf}^2 + ref \sigma_m^2$
Female/set	12	M ₆	$\sigma^2 + r \sigma_{fme}^2 + rm \sigma_{fe}^2 + re \sigma_{mf}^2 + rem \sigma_f^2$
Male × female/set	36	M ₅	$\sigma^2 + r \sigma_{fme}^2 + re \sigma_{mf}^2$
Male/set × environment	12	M ₄	$\sigma^2 + r \sigma_{fme}^2 + rf \sigma_{me}^2$
Female/set × environment	12	M ₃	$\sigma^2 + r \sigma_{fme}^2 + rm \sigma_{fe}^2$
Male × female/set × environment	36	M ₂	$\sigma^2 + r \sigma_{fme}^2$
Pooled error	360	M ₁	σ^2
Total	504		

^zm, f, and e refer to males, females, and environments, respectively. $\sigma_m^2 = \sigma_f^2$ = covariance of half-sibs = $1/4 \sigma_A^2$. σ_{mf}^2 = (covariance of full-sibs) – (covariance of half-sibs of males and females) = $1/4 \sigma_D^2$. $\sigma_{me}^2 = \sigma_{fe}^2$ = interaction of covariance of half-sibs with environment = $1/4 \sigma_D^2$. σ_{fme}^2 = interaction of females × males with environments = $1/4 \sigma_{DL}^2$. σ^2 = experimental error.

with Se will allow consumers to reap the beneficial effects without altering their diets or having to take supplements (Ip and Lisk, 1994a).

Breeding plants that are enriched with beneficial mineral nutrients and vitamins could substantially reduce the recurrent costs associated with fortification (Bouis, 1996). Successful programs are now in place for improving the Zn (Ruel and Bouis, 1998) and Fe (Bouis, 1996) content of wheat (*Triticum aestivum* L.) and other grain crops. Breeding plants to be more efficient at Se accumulation may complement Se enrichment efforts. Members of the Brassicaceae can be enriched with Se for delivery of beneficial organic forms to human diets, but information on the genetic variances associated with Se accumulation in these plants is limited. Investigation into the genetic variation for Se content in tall fescue (*Festuca arundinacea* Shreb.) revealed that progress from selection for Se content is possible and that the trait was heritable (McQuinn et al., 1991). The genetic variances of Se accumulation in *B. oleracea* would be useful before efficient selection programs for selenized crops can be developed. Because of the competitive nature of Se and S, the genetic variances of S should also be determined. Sulfur accumulation can actually be enhanced by low level Se fertilization in *Allium* (Kopsell and Randle, 1997) and *Brassica* (Kopsell and Randle, 1999). S compounds in *Brassicaceae* are responsible for many of their flavor attributes. Therefore, our first objective was to identify the genetic variances associated with Se and S accumulation in a model system of *B. oleracea* grown in seleniferous environments. A second objective was to evaluate progress from divergent selection for both low and high Se accumulation within the same model system of *B. oleracea*, and to determine their realized heritabilities.

Materials and Methods

PLANT MATERIAL. Seeds were randomly selected from a genetically heterogeneous rapid-cycling base population (RCBP) of *B. oleracea* (Crucifer Genetics Cooperative, Department of Plant Pathology, The University of Wisconsin, Madison). Selection within the population had been performed only for rapid reproduction rate. Seeds were sown into sheets of growing cubes (Grodan A/S Dk-2640, Hedehusene, Denmark), covered with fine vermiculite and watered twice daily. Seeds were germinated in a greenhouse (28 °C) under metal halide lamps providing continuous irradiation of 1540.0 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at canopy height with a integrating quantum radiom-

eter/photometer (LI-188B; LI-COR, Inc., Lincoln, Neb.) (Williams and Hill, 1986). 1 L of a 200 mg·L⁻¹ solution of 20N–8.8P–16.6K water soluble fertilizer (Peter's; Grace-Sierra Co., Milpitas, Calif.) was applied 5 d after sowing to each sheet of cubes.

GENETIC VARIANCE. Seeds for the genetic variance experiment were sown 29 Sept. 1998. Once the first true leaves emerged 7 Oct. 1998, the seedlings were planted into 38-L containers (Rubbermaid, Inc., Wooster, Ohio). Eight plants were put into holes on each lid, and the containers were filled with 30 L of a half-strength modified Hoagland's nutrient solution (Hoagland and Arnon, 1950). Solutions were aerated via an air stone connected to a conventional air compressor. The containers were placed in the greenhouse and grown under conditions described previously for seed germination. On 12 Oct. 1998, sodium selenate at 2.0 mg·L⁻¹ (Na₂SeO₄; ICN Biochemicals, Cleveland, Ohio) was added to each of the containers. Selenium concentrations were based on results from a previous study (Kopsell and Randle, 1999). Deionized water was added as needed to maintain the initial volume.

Crosses were made according to a North Carolina Design II factorial mating design (Comstock and Robinson, 1948). The eight plants in each container were divided equally as male and female parents. Each container was considered a set. The design consisted of four sets with four male and four female parents each, giving a total of 16 male and 16 female plants. Each male parent was crossed to four different females, resulting in a total of 16 full-sib families per set. Mature seeds were collected from each different F₁ full-sib family.

The F₁ seeds were sown on 1 Feb. 1999 and the seedlings grown in nutrient solutions as described previously. Progeny were evaluated under two different environmental conditions. The first environment (E1) was similar to that described for the mating design, while the second environment (E2) used natural photoperiods for February (35 °N latitude). In addition, temperature in E2 was set at 8 °C. The experimental design was a randomized complete block, with four single plant blocks containing each of the 64 F₁ full-sib families. Plants in E1 matured earlier and were harvested when flower buds formed 3 Mar. 1999, while plants in E2 were harvested 21 Mar. 1999.

Leaves were rinsed with deionized water, placed in paper bags, dried in a forced-air oven at 70 °C for 24 h to obtain dry weights (DWs), and then ground through a 0.5-mm screen (Cyclotec sample mill model 1093; Tecator, Höganäs, Sweden). To prepare the

samples for Se analysis, ground tissues were placed into a 125-mL flask with 10 mL of concentrated nitric acid (70% HNO₃) and placed on a hot plate (model 2200; Thermolyne, Dubuque, Iowa) for 4 h at 165 °C. The flasks were cooled to room temperature, the volume adjusted to 50 mL with deionized water, and filtered (Whatman no. 1 filter paper). Selenium was measured using graphite furnace atomic absorption spectrophotometry (GFAA) (model 4100ZL; Perkin-Elmer Corp., Norwalk, Conn.) with a detection limit for Se of 4.0 µg·L⁻¹. Total S was measured for each plant using a S determinator (model SC-232; LECO, St. Joseph, Mich.). Ground tissue was combined with vanadium pentoxide accelerator (LECO) and combusted with oxygen at 1371 °C. Total S was measured as SO₂ by an infrared cell detector. Due to limited amounts of tissue from some F₁ progeny, S was not analyzed for these progeny and numbers are reflected in the total degrees of freedom in the analysis of variance (ANOVA).

A mixed model was used for the ANOVA of Design II for sets repeated over environments as described by Searle (1971). Mean square (MS) expectations and degrees of freedom (df) for the sources of variation in the ANOVA are outlined according to Hallauer and Miranda (1981) (Table 1). The variability due to progeny was partitioned into variation due to males within sets, females within sets, and male × female interactions within sets. Direct F tests were straight forward for all sources of variation in the ANOVA except for the male and female components. Approximate F tests, with the appropriate df, were constructed based on the MS and used to test the effects of the male and female components of variance (Satterthwaite, 1946).

Variance component estimates were calculated by equating the expected MS to the corresponding observed MS and then solving through simple algebraic equations (Hallauer and Miranda, 1981). The components of genetic variance were estimated according to the expectations set by Cockerham (1956): $\sigma_m^2 = \sigma_f^2 = \text{cov HS} = (1/4)\sigma_A^2$, and $\sigma_{fm}^2 = \text{cov FS} - \text{cov HS}_f - \text{cov HS}_m = (1/4)\sigma_D^2$ where σ_m^2 , σ_f^2 , and σ_{fm}^2 are the variance due to males, females, and female × male interactions, respectively. The genetic variance components of σ_A^2 and σ_D^2 are the additive and dominance variance. The cov HS and cov FS are the covariance of half-sib and full-sib progeny, respectively.

Variance components are most often positive, but estimates obtained from the ANOVA can be negative (Searle, 1971). Negative variance components were calculated for the ANOVA in the current study and their contributions to heritability estimates were taken to be zero. Estimates of narrow sense heritability (h^2) were calculated for male and female components of variance according to Hallauer and Miranda (1981). Estimates of h^2 unbiased by genotype-environment interactions can be calculated for plants grown across all environments as $h^2 = 4 \sigma_m^2 / (\sigma^2 / \text{rfe}) + 4 \sigma_{mf}^2 / \text{fe} + 4 \sigma_{me}^2 + 4 \sigma_{mf}^2 / f + 4 \sigma_m^2$ with a standard error (SE) of $\text{SE}(h^2) = 4 \text{SE}(\sigma_m^2) / (\sigma^2 / \text{rfe}) + 4 \sigma_{mf}^2 / \text{fe} + 4 \sigma_{me}^2 + 4 \sigma_{mf}^2 / f + 4 \sigma_m^2$ where m, and e refer to the number of males and environments, respectively. A similar estimate of h^2 was calculated for the female source of variation, and for an average of both parents.

DIVERGENT SELECTION. Seeds for the divergent selection experiment were sown 10 Oct. 1998 and 14 Oct. 1998 to initiate populations for selecting low (LP0) and high (HP0) Se accumulation in leaves, respectively. Once the first true leaves emerged, 100 plants for each of the high and low Se populations were planted into two containers, holding 50 plants each. The containers were filled with 70 L of half-strength modified Hoagland's nutrient solution (Hoagland and Arnon, 1950) that was aerated as described earlier. The containers were placed in the greenhouse under conditions described previously for germination. Deionized water was added as

needed to maintain the initial solution volumes throughout the experiment.

After 3 d in nutrient solutions, Na₂SeO₄ was added to the containers for each population at a concentration of 2.0 mg·L⁻¹. Plants within the low and high Se populations were evaluated for Se accumulation on 6 and 9 Nov. 1998, respectively (17 d after treatment initiation). The third most recently expanded leaf was removed from each plant for nutrient analysis (Jones, 1972). Leaves were collected, digested, and analyzed for Se as described previously.

Phenotypic mass selection was performed in each population before pollination based on Se accumulation in the leaves. The 10 lowest Se accumulating plants were selected from the low population, and the 10 highest Se accumulating plants were selected from the high population. The remaining plants were discarded. The selected plants within each population were then randomly mated. Pollen was collected and bulked from the plants within each group and used to pollinate receptive flowers. Mature seeds were collected, cleaned, and bulked within each population to form the next generation.

Seeds for the next selection cycle were germinated as described previously 20 Jan. 1999 for the high Se accumulating population (HP₁) and 23 Jan. 1999 for the low Se accumulating population (LP₁). Plants were then evaluated for leaf Se, selected, and mated as described previously. Mature seeds were collected to form the second high Se accumulating population (HP₂) and the low Se accumulating population (LP₂).

Realized heritabilities (h^2) for the divergent selection for Se accumulation were calculated using the following formula (Falconer and Mackay, 1996): $h^2 = G/D$ where G = selection gain which is the difference between the mean of the newly derived population from the selected parents and the mean of the original population; and D = selection differential which is the difference between the mean of the selected parents and the population from which they originated. The SES for the estimates of realized heritabilities were calculated according to the formula by Prout (1962) for the variance of the selection response and then taking the square root: $\text{SE}(h^2) = (1/D^2 \cdot [h^2(1 - h^2) S_p^2/N_p + S_o^2/N_o])^{1/2}$ where D^2 = (difference between the mean of the selected parents and the population from which they originated)²; S_p^2 = phenotypic variance of population from which parents were drawn; N_p = number of selected parents; S_o^2 = Phenotypic variance of offspring population; and N_o = number of offspring measured.

PROGRESS FROM SELECTION. The initial and selected populations were evaluated under one environment to assess progress from selection. On 20 May 1999, seeds from the base populations (LP₀ and HP₀) and each of the high and low Se accumulation selection cycles (LP₁, HP₁, LP₂, and HP₂) were germinated as described previously. On 27 May 1999, seedlings were transferred to the nutrient solutions and grown in a growth room at 28 °C with continuous irradiation of 1540.0 µmol·m⁻²·s⁻¹ from metal halide lamps (Williams and Hill, 1986). The experimental design was a randomized complete block with eight replications and 10 plants per plot for each population. The populations were evaluated for Se accumulation in leaf tissues on 16 June 1999. All leaves were removed and analyzed for Se accumulation on a single plant basis. Leaves were rinsed with deionized water, placed in paper bags, dried and ground as described previously. One gram of tissue was digested and analyzed for Se content as described previously.

Data were subjected to ANOVA. Linear regression to estimate rate of progress from selection was used on the high and low Se accumulating populations according to (Eberhart, 1964): $Y_i = m_0 +$

Table 2. Population parameters for Se and S accumulation in leaves of a rapid-cycling *Brassica oleracea* grown in two greenhouse environments.

Parameter	Se ($\mu\text{g}\cdot\text{g}^{-1}$ dry wt)		S ($\text{mg}\cdot\text{g}^{-1}$ dry wt)	
	E1 ^z	E2 ^y	E1 ^z	E2 ^y
N	219	190	192	152
Mean	604.4	340.5	33.9	29.4
SE	± 7.7	± 5.7	± 0.39	± 0.34
Range	120.0–988.1	152.7–531.4	20.8–48.2	19.1–39.6
CV	18.9%	23.1%	16.1%	14.1%

^zPlants in E1 were grown at 28 °C with continuous irradiance ($1540.0 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).^yPlants in E2 were grown at 8 °C with natural photoperiods (35 °N latitude) and without supplemental light.

$b_i X_i$ where Y_i = are the observed means over cycles of selection ($i = 0, 1, \dots, c$); m_0 = estimates the original population mean; b_1 = linear regression coefficient, which expresses the rate of gain per cycle of selection; and X_i = cycles of selection. Paired t tests were used to determine significant differences between means of the selection cycles for each population (Ott, 1993).

Results and Discussion

GENETIC VARIANCES. Results demonstrated that significant variation in leaf Se accumulation exists in the rapid-cycling *B. oleracea* population, and that this trait is heritable. Differences in mean Se accumulation were demonstrated across two environmental conditions using nutrient solution culture. While the same Na_2SeO_4 concentrations ($2.0 \text{ mg}\cdot\text{L}^{-1}$) were used in both environments, mean Se and S accumulations in leaf tissues differed between E1 and E2 (Table 2). Selenium accumulations ranged from 120.0 to 988.1 $\mu\text{g}\cdot\text{g}^{-1}$ DW for E1 (longer photoperiod and warmer temperatures) and 152.7 to 531.4 $\mu\text{g}\cdot\text{g}^{-1}$ DW for E2. Sulfur accumulations ranged from 20.8 to 48.2 $\text{mg}\cdot\text{g}^{-1}$ DW for E1 and 19.1 to 39.6 $\text{mg}\cdot\text{g}^{-1}$ DW for E2. Mean Se content decreased by 44% from E1 to E2, while S content decreased by only 13%. This response was somewhat unexpected because S and Se are thought to use the same enzymes involved in their early uptake and metabolism (Leggett and Epstein, 1956). Outside the recommended environment for optimum RCBP reproductive performance, the competitive nature of S and Se changed. Sulfur accumulation was less affected compared to Se in the suboptimal environment (E2) and may indicate that S and Se metabolism are not so similar.

Differences in environments were highly significant in the ANOVA

($P=0.01$) for both Se and S accumulation (Table 3). Se accumulation for females within sets was significant in the ANOVA ($P=0.05$), while males within sets were not. This indicated greater contribution from the female parents for Se accumulation. Sulfur accumulations were significant for males within sets ($P=0.1$) and females within sets ($P=0.01$). This would indicate importance of both parents for S accumulation. There was also a significant interaction between environments and males within sets ($P=0.05$) for S accumulation. MSs due to females were much larger than MSs due to males for both Se and S accumulation (Table 3). The difference between the two estimates reflects the variability of the trait among the parents and indicates significant maternal and nonmaternal reciprocal influences on trait expression (Cockerham, 1963).

Estimates of additive and dominance variance components were made for plants grown across environments (Table 4). For leaf Se accumulation, the additive variance component due to females was 2.5 times greater than the additive variance due to males. A similar response was found for S accumulation. When estimates of maternal components of variance are greater than paternal components of variance, it indicates the possible presence of maternal effects on the trait of interest (Mather and Jinks, 1971). A negative estimate of dominance genetic variance was found for Se accumulation. Because seeds of the rapid cycling *B. oleracea* base population were generated from repeated random mating by the Crucifer Genetic Cooperative at the University of Wisconsin (personal communication), linkage disequilibrium that could affect the dominance variance was assumed to be minimal. In addition, substantial morphological variation was expressed in the base population (i.e., leaf shape, leaf color, and plant height) indicating the selection pressure placed on this population for rapid flowering cycles may not have significantly affected the allelic frequency of other plant traits.

Table 3. MSs from ANOVA of Design II (Comstock and Robinson, 1948) for Se [$\mu\text{g}\cdot\text{g}^{-1}$ dry weight (DW)] and S ($\text{mg}\cdot\text{g}^{-1}$ DW) in leaves of rapid-cycling *Brassica oleracea* grown in two greenhouse environments.

Source of variation	Se		S	
	df	MS	df	MS
Environments	1	7085085.1***	1	1697.3***
Sets	3	27546.8***	3	44.9
Sets $\times$ environments	3	12473.9	3	20.5
Replications/sets/environments	24	18069.3	24	27.5
Males/sets	12	14046.8	12	32.6*
Females/sets	12	23337.8**	12	72.9***
Males $\times$ females/sets	36	5646.6	36	24.4
Males/sets $\times$ environments	12	11104.9	12	35.3**
Females/sets $\times$ environments	12	11347.7	12	26.7
Males $\times$ females/sets $\times$ environments	36	8508.1	30	18.9
Pooled error	360	8679.4	198	20.2
Total	504		343	

***, **, * Significant at $P = 0.1$, 0.05, or 0.01, respectively.

Table 4. Estimates of additive (s^2_A) and dominance variance (s^2_D), narrow sense heritability (h^2) and their SEs for Se [$\mu\text{g}\cdot\text{g}^{-1}$ dry weight (DW)] and S ($\text{mg}\cdot\text{g}^{-1}$ DW) in leaves of a rapid-cycling *Brassica oleracea* across two greenhouse environments.

Parameter	Se ($\mu\text{g}\cdot\text{g}^{-1}$ DW)	S ($\text{mg}\cdot\text{g}^{-1}$ DW)
σ^2_A (male)	725.6 $\pm$ 910.0	1.04 $\pm$ 2.48
σ^2_A (female)	1856.4 $\pm$ 1278.4	5.08 $\pm$ 3.84
σ^2_A (both)	1291.0 $\pm$ 1094.6	2.02 $\pm$ 3.16
σ^2_D	-1430.8 $\pm$ 1213.2	2.76 $\pm$ 3.64
h^2 (male)	0.55 $\pm$ 0.66	0.0
h^2 (female)	0.75 $\pm$ 0.51	0.69 $\pm$ 0.52
h^2 (both)	0.65 $\pm$ 0.59	0.0

Table 5. Mean Se accumulation in the leaf tissue ($\mu\text{g}\cdot\text{g}^{-1}$ dry weight) of rapid-cycling *Brassica oleracea* during divergent selection for high and low Se accumulation.

Selection cycle ^z	N ^y	Mean ^x
High Se		
HP ₀	100	584.1 $\pm$ 14.4
Selected individuals	10	699.3 $\pm$ 5.8
HP ₁	100	731.2 $\pm$ 19.1
Selected individuals	10	939.6 $\pm$ 10.1
HP ₂	100	962.8 $\pm$ 16.3
Low Se		
LP ₀	100	560.0 $\pm$ 11.0
Selected individuals	10	401.4 $\pm$ 17.8
LP ₁	100	444.6 $\pm$ 8.9
Selected individuals	10	338.5 $\pm$ 6.6
LP ₂	100	376.8 $\pm$ 11.8

^zHP₀ = base population for selection for high Se accumulation, HP₁ = high population after one cycle of selection, HP₂ = high population after two cycles of selection, LP₀ = base population for selection for low Se accumulation, LP₁ = low population after one cycle of selection, and LP₂ = low population after two cycles of selection.

^yNumber of individuals.

^xMean value of Se in the third most recently expanded leaf $\pm$ the SE.

Negative variance components are not uncommon and are often found for dominance variance components (Hallauer and Miranda, 1981). Selection for total Se accumulation can therefore be made on individual plant performance rather than on hybrid combinations. Considerable dominance variance was established for S accumulation, and would indicate selection from hybrid crosses may be beneficial. Additive genetic variance was also important for S accumulation and shows that selection on individual plant performance may be plausible.

Narrow sense heritability expresses the extent to which phenotypes are determined by the genes transmitted from the parents (Falconer and Mackay, 1996). Selenium accumulation across environments showed moderate h^2 estimates (Table 4). Values of h^2 for Se accumulation were 55% for males, 75% for females, and 65% for the estimate of both parents. Because of negative variance estimates, only the female h^2 (69%) could be estimated for S accumulation across environments. Moderate h^2 estimates would suggest reasonable progress of both Se and S accumulation could be made. However, the h^2 estimates in the current study produced very large SEs and caution should be taken when interpreting these estimates (Table 4). If the large SEs represented the variability measured in the progeny generated from parents, selection progress for Se and S accumulation could be less than h^2 would indicate. In fact, actual

progress was less than the predicted value using divergent selection in our second objective. In a broad-based population of tall fescue, parent-offspring regression for shoot Se content produced heritabilities of 18% in the summer and 68% in the fall (McQuinn et al., 1991). Their results showed that progress from selection for Se content would be affected by yearly and seasonal differences. Similarly, S uptake and metabolism were greatly affected by many environmental factors in onion (*Allium cepa* L.) (Randle, 1997).

DIVERGENT SELECTION. Selection for both high and low Se accumulation in leaf tissue successfully increased and decreased mean Se accumulation in their respective populations. Mean Se accumulation increased during two cycles of selection for high Se accumulation (Table 5). During the first selection cycle, the mean Se of the highest accumulating parents was 115.2 $\mu\text{g}\cdot\text{g}^{-1}$ DW higher than HP₀, which was a 16.5% increase. In the second cycle of selection, the mean of the highest Se accumulating parents was 205.4 $\mu\text{g}\cdot\text{g}^{-1}$ DW higher than HP₁, which was a 22% increase. Mean Se accumulation from plants in HP₁ represented a 20% increase over the mean of HP₀ while HP₂ represented a 24% increase over the mean of HP₁. Conversely, mean Se accumulation decreased during two cycles of selection for low Se accumulation (Table 5). During the first selection cycle, mean Se of the lowest accumulating parents was 158.6 $\mu\text{g}\cdot\text{g}^{-1}$ DW lower than LP₀, which was a 28% decrease. In the second cycle of selection, the mean of the lowest Se accumulating parents was 106.1 $\mu\text{g}\cdot\text{g}^{-1}$ DW lower than LP₁, which was a 24% decrease. Mean Se accumulation from plants in LP₁ represented a 21% decrease over the mean of LP₀ while in LP₂ represented a 15% decrease over the mean of LP₁.

Realized heritabilities (h^2) were high for each cycle of selection in both the high and low Se populations. After one cycle of divergent selection, h^2 was 1.27 $\pm$ 0.47 and 0.72 $\pm$ 0.20 for the high and low Se accumulating populations, respectively. In the second cycle of selection, h^2 was 1.11 $\pm$ 0.21 and 0.63 $\pm$ 0.31 in the high and low Se accumulating populations, respectively. Realized heritability is a description of the response to selection in a specific environment, and may not provide a valid estimate of the heritability of a character in the base population (Falconer and Mackay, 1996). Realized heritabilities for Se accumulation in tall fescue were high (0.68 $\pm$ 0.18) when selection was carried out in the fall, but low for summer selection (0.18 $\pm$ 0.16), suggesting a large environmental effect for Se accumulation for the crop (McQuinn et al., 1991).

PROGRESS FROM SELECTION. To determine the effectiveness of mass selection for divergent Se accumulation, all populations were grown and evaluated simultaneously in a single environment. By the second cycle of selection, population means for both the high and the low selected plants differed from each other and the mean of the unselected populations (Table 6). Poor seed germination in the base population selected for high Se accumulation caused us to evaluate only 40 individual plants. There was a 48.7 $\mu\text{g}\cdot\text{g}^{-1}$ DW increase in the mean of HP₁ over the Se mean of HP₀ with one cycle of selection which was a 7.8% gain. The Se mean of HP₂ was 9.4 $\mu\text{g}\cdot\text{g}^{-1}$ DW higher than the mean of HP₁. Overall, there was an increase of 9.2% in the mean Se accumulation over HP₀ after two cycles of selection. After the first cycle of selection for low Se accumulation, there was no decrease in the mean of LP₁ over the mean of LP₀. However, the Se mean of LP₂ was reduced by 46.1 $\mu\text{g}\cdot\text{g}^{-1}$ DW, which was a 8.0% lower than LP₀. The coefficient of variation changed little through each cycle of selection for divergent selection, indicating minimal loss in variation for Se accumulation in response to 10% selection pressure.

Low rates of gain can be expected due to the complexity of S (and

Table 6. Progress in mean leaf Se accumulation ($\mu\text{g}\cdot\text{g}^{-1}$ dry weight) from selection for high or low Se accumulation in rapid-cycling *Brassica oleracea* grown in a single environment.

Selection cycle ^z	N ^y	Mean ^x	Min	Max	CV	Gain (%) ^w
High Se						
HP ₀	40	572.2 $\pm$ 12.8	340.1	652.6	15.9%	---
HP ₁	78	620.9 $\pm$ 13.9	258.1	961.0	19.8%	7.8
HP ₂	79	630.3 $\pm$ 14.0	396.8	984.7	19.8%	9.2
Low Se						
LP ₀	78	572.7 $\pm$ 14.7	297.4	961.1	22.7%	---
LP ₁	78	572.8 $\pm$ 14.8	330.8	863.3	22.8%	0.0
LP ₂	80	526.7 $\pm$ 11.9	231.1	786.0	20.2%	8.0

^zHP₀ = base population for selection for high Se accumulation, HP₁ = high population after one cycle of selection, HP₂ = high population after two cycles of selection, LP₀ = base population for selection for low Se accumulation, LP₁ = low population after one cycle of selection, and LP₂ = low population after two cycles of selection.

^yNumber of individuals.

^xMean value for Se accumulation of all the leaves on a single plant basis $\pm$ SE.

^wGain over the original population for each divergent selection population.

Se) metabolism in these plants. Many enzymes are involved in the metabolism of S in plants, especially those with complex secondary S-metabolites. Moreover, Se can be an alternative substrate in these pathways. Sulfur uptake is mediated by a high affinity permease (Leggett and Epstein, 1956). Sulfate is then reduced by ATP sulfurylase (Shaw and Anderson, 1972). ATP sulfurylase may be the rate-limiting enzyme controlling the pathway for S assimilation (Leustek, 1996), and may also be the rate-limiting enzyme responsible for selenate-Se assimilation (Pilon-Smits et al., 1999; Zayed et al., 1998). Two enzymes, glutathione reductase and cysteine synthase reduce inorganic sulfite to cysteine, and also reduce inorganic selenite to selenocysteine (Ng and Anderson, 1978). Selenocysteine is further converted to selenomethionine by β -cystathionase and cystathionine γ -synthase (Dawson and Anderson, 1989). The complex genetic control of S and Se metabolism is further illustrated in multi-enzymatic pathways leading to cysteine sulfoxide accumulation and in the synthesis of the glucosinolates (Stoewsand, 1995). Such complex metabolic systems could be expected to limit progress from selection.

A linear regression model was successfully fit to both the high Se accumulating population ($Y = 579.7 + 28.5\text{Cycle}$; $P = 0.02$) and the low Se accumulating population ($Y = 560.5 - 23.1\text{Cycle}$; $P = 0.02$). Linear regression estimated the progress per selection cycle for high Se accumulation to be $28.5 \mu\text{g}\cdot\text{g}^{-1}$ DW, whereas progress from selection for low Se accumulation was $-23.1 \mu\text{g}\cdot\text{g}^{-1}$ DW. The standard t tests indicated that both slopes differed from zero ($P = 0.001$). Paired t tests revealed that the mean of HP₀ differed from the mean of HP₁ ($P = 0.005$) and the mean of HP₁ ($P = 0.001$). Mean Se accumulation for HP₁ also differed from the mean for HP₂ ($P = 0.1$). The mean LP₀ did not differ from the mean of LP₁, but differed from the mean of LP₂ ($P = 0.05$). The mean LP₁ differed from the mean for LP₂ ($P = 0.05$). Selection response for Se accumulation was effective for both directions, but the slopes in the regression lines revealed differences in the rate of response. Asymmetry in response has been found for many two-way selection experiments (Falconer and Mackay, 1996). Asymmetry in our study may be due to a similar behavior of Se and S in the metabolic pathways of a plant (Leggett and Epstein, 1956). Being a macroelement, S is used in large quantities by plants. More over, *B. oleracea* has significant secondary S metabolic pathways. Increasing S in the metabolic pathways, and Se by default, should therefore be beneficial to the plant and thus be favored. On the other hand, decreases in S metabolic enzymes, and therefore Se metabolic enzymes, would not only restrict growth, but also decrease the cysteine sulfoxides and glucosinolates associ-

ated with plant defense and flavor. This should be a disadvantage for *Brassica*. In addition, the *B. oleracea* plants used in the current study are known Se accumulators, and can tolerate high accumulations of Se. By excluding Se from S-protein incorporation, and accumulating Se in less toxic nonprotein Se-amino acids (Brown and Shrift, 1982), these plants are further able to tolerate greater Se levels resulting from the up-regulation of S metabolizing enzymes.

Expected progress from phenotypic mass selection can be calculated as: $\Delta G = k(1/2) \sigma_A^2 / (\sigma_A^2 + \sigma_D^2 + \sigma^2 + \sigma_{we}^2)^{1/2}$ where DG = predicted gain; k = selection differential for the selection intensity of 0.10 which is 1.755; σ_A^2 = additive genetic variance; σ_D^2 = dominance genetic variance; σ^2 = error variance; and σ_{we}^2 = environmental variance (Hallauer and Miranda, 1981). Using the derived genetic components from the genetic parameters study, predicted gain from selection was estimated to be 6.8%. Actual gain per cycle of selection for increased Se accumulation was 4.6%, whereas the actual gain per cycle of selection for decreased Se accumulation was 4.0%. Predicted gains from selection were higher, but within the range of actual gains.

Population gains made during selection indicated much greater progress than what was actually discovered when populations were evaluated simultaneously. This may have two possible explanations. First, selection for Se accumulation was performed on the third most recently expanded leaf, whereas populations were evaluated for Se accumulation using all leaves. Analysis of the third most recently expanded leaf allowed for selection and inter-mating of plants, but may not have been representative of Se accumulation in all the leaves of a plant. Second, an increasing natural photoperiod (winter through spring) during each cycle of selection could have favored and accentuated Se accumulation in the selected populations. Irradiance and photoperiod has influenced Se accumulation in *Brassica* (unpublished data).

In conclusion, the heterogeneous population of rapid-cycling *B. oleracea* expressed variability in Se and S accumulation in seleniferous environments. Based on narrow sense heritability estimates, progress for Se and S accumulation within the plants was suggested, although large ses may influence expected progress. Divergent selection for high and low Se accumulation in a model system of rapid-cycling *B. oleracea* revealed gains from selection. Realized heritabilities were high for both low and high Se accumulation over cycles of selection. However, estimated gain in Se accumulation was predicted to be 6.8% while actual gains from selection for increased and decreased Se accumulation were 4.8% per cycle of selection and 4.0% per cycle of selection, respectively. Selection for

Se accumulation was successful and indicates population improvements for such traits are possible within *B. oleracea*.

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