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Simultaneous selection for grain yield and protein percentage in backcross populations from *Avena sterilis* × *A. sativa* matings by using the independent culling levels procedure*

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Summary. The method of independent culling levels was applied for simultaneous improvement in grain yield, protein percentage, and protein yield in a population of oat lines from the BC_0–BC_5 of the interspecific mating *Avena sativa*×*A. sterilis.* Grain yield and protein percentage were subjected to selection with various combination of culling levels to give samples with 2% of the lines from the original population. – Intense selection for grain yield resulted in samples with high grain and protein yields but low protein percentage. Intense selection for protein percentage resulted in samples with high protein percentage but low grain and protein yields. The sample selected for protein yield only showed no significant difference from the recurrent parent for any trait. The recommended regime for improving protein percentage and grain and protein yields simultaneously was one that initially saved approximately 25–50% of the original population on the basis of protein percentage, and then provided intense selection for grain yield on the lines that remained. At least three backcrosses were necessary to obtain lines with high protein percentage and acceptable agronomic traits.

Key words: Oats – Selection – Interspecific cross – Protein – Grain yield – *Avena*

Introduction

A negative association between grain yield and protein percentage is common for cereal grains (Frey 1977). As a result, the primary route for improving protein yield of cereals has been via increasing grain yield (Takeda and Frey 1979). However, elevating the protein percentage is also an important breeding objective for cereals, such as oats (*Avena sativa* L.), that are used in the diets of humans and monogastric animals. In such cases, selection for the best combination of grain yield, protein percentage, and protein yield becomes the objective.

In this study, we utilized the breeding procedure of independent culling levels with various combinations of selection intensities to determine the best strategy for optimizing grain yield and protein percentage at levels that would maximize protein yield of oats. Also, we determined the effect of the various combinations of selection intensities upon the expression of agronomic traits.

Materials and methods

Materials

Material for this study was a group of 554 lines of oats, consisting of 79 F_2-derived lines from each of the generations BC_0 through BC_5 from an interspecific mating, C.I. 8044×B445, and 80 lines from C.I. 8044, which was used as the recurrent parent. C.I. 8044 is an *A. sativa* line adapted to midwestern USA and B445 is a high-protein *A. sterilis* collection. When all backcrosses were completed, 79 F_2 seeds from each BC generation and 80 seeds from C.I. 8044 were space planted into the field. The bulk progeny from each plant became an F_2-derived or parent line that was used for an evaluation experiment.

Field experiment

The 554 oat lines were evaluated in a randomized-block experiment with two replicates grown on a fertile Clarion-Webster-Nicollet loam soil at the Agronomy Field Research Center near Ames, Iowa, USA. A plot was a hill sown with 30 seeds, and hills were spaced 30.5 cm apart in perpendicular

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directions. The experiment was surrounded by two rows of hill plots to provide competition for peripheral plots. The experiment was hand weeded, and a fungicide was sprayed onto the plants at weekly intervals from anthesis to maturity to control foliar disease.

Thirteen traits were measured or calculated for each plot. Heading date was recorded as days after May 31 when 50% of the panicles in a plot were fully emerged. Plant height was the distance (cm) from the ground surface to the panicle tips. Biomass and grain yields were the air-dry weights (g) of the bundle of culms and grain from a plot. A sample of 20 seeds was dehulled and groat weight (g) was recorded as the weight of 100 groats. A 100-mg sample of groats was analyzed for nitrogen percentage by using the micro-Kjeldahl procedure[1], and protein percentage was computed as nitrogen percentage × 6.25. Straw yield was computed as biomass minus grain yield, and harvest index was calculated as grain yield divided by biomass yield and expressed as a percentage. Vegetative growth rate was computed as straw yield divided by days from sowing to heading, unit straw weight was obtained by dividing straw yield by plant height, and grain number was approximated by dividing grain yield by groat weight (this overestimates grain number because hull weights affect the numerator but not the denominator). Protein yield (g) was computed as protein percentage times grain yield (this overestimates protein yield because protein percentage of the groats was greater than the protein percentage of the hulls), and protein per groat (mg) was calculated as protein percentage times groat weight.

Selection regime

Since variation of protein yield in this population of oat lines was nearly completely determined by the trait components grain yield and protein percentage, we used independent culling levels to select protein yield via these components.

To compute the expected gain (ΔG) for this study, we used the formulas of Young and Weiler (1960). Genetic gain (ΔG_D) from direct selection for a single trait was estimated as:

$$\Delta G_D = h^2 \cdot s \cdot k,$$

where h^2 and s are heritability and standard deviation, respectively, for the trait, and k is the selection differential in standard units. Genetic gain (ΔG_I) from indirect selection through a single trait was estimated as:

$$\Delta G_I = h_1 \cdot h_2 \cdot r_g \cdot s_2\, k,$$

where h_1 and h_2 are square roots of heritabilities for the selected and responding traits, respectively, r_g is the genotypic correlation between the traits, s_2 is the standard deviation of the responding trait, and k is the selection differential in standard units for the selected trait (Falconer 1981). Expected genetic gains (ΔG_{IC}) in a responding trait from independent culling for two other traits were computed as follows:

$$\Delta G_{IC} = k_1 (W_1 \alpha_1 s_1 + W_2 \beta_1 s_2) + k_2 (W_1 \alpha_2 s_1 + W_2 \beta_2 s_2),$$

where W_1 and W_2 are the genotypic regressions of the responding trait on the selected traits 1 and 2, respectively, s_1 and s_2 are the standard deviations of traits 1 and 2, respectively, α and β describe relationships between heritabilities and genotypic correlations for traits 1 and 2 (Young and Weiler 1960), and k_1 and k_2 are selection differentials in standard units for traits 1 and 2, respectively.

Table 1. Regimes and intensities of selection used to obtain actual protein yields from selection

Regime no.	Selection intensity for			
	Grain yield	Protein percentage	Protein yield	Total
1	0.02[a]	–	–	0.02
2	0.10[a]	0.20	–	0.02
3	0.14[a]	0.14	–	0.02
4	0.25[a]	0.08	–	0.02
5	0.50[a]	0.04	–	0.02
6	0.75[a]	0.03	–	0.02
7	–	0.02[b]	–	0.02
8	0.20	0.10[b]	–	0.02
9	0.14	0.14[b]	–	0.02
10	0.08	0.25[b]	–	0.02
11	0.04	0.50[b]	–	0.02
12	0.03	0.75[b]	–	0.02
13	–	–	0.02	0.02

[a] Grain yield selected initially
[b] Protein percentage selected initially

Table 2. Means, phenotypic variances, and heritability values of 474 F_2-derived oat lines for protein percentage, grain yield, and protein yield

Traits	Mean	Variance	Heritability
Protein percentage (%)	18.1	3.70	0.39
Grain yield (g/plot)	31.3	91.48	0.47
Protein yield (g/plot)	5.9	2.54	0.45

Table 3. Genotypic correlations among protein percentage, grain yield, and protein yield of oat lines

Traits correlated	Correlation
Protein percentage and grain yield	–0.39
Protein percentage and protein yield	–0.17
Grain yield and protein yield	0.97

Heritabilities and genotypic correlations for use in these prediction formulas were computed with variances and covariances obtained from analyses of covariance on data from the evaluation experiment. Heritability values were expressed on a plot basis.

To evaluate the actual gains from selection, oat lines were selected on the basis of line values in replicate 1 and the success from selection was evaluated on the basis of line values in replicate 2 and vice versa. We adopted a selection intensity of ca 2.0%, which gave a sample of 19 lines (474 F_2-derived lines × 2 replicates × 0.02 selection intensity) for each regime. Culling levels for selected and responding traits are shown in Table 1. In regimes 1, 7, and 13, only a single trait was selected. In regimes 3 and 9, grain yield and protein

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percentage were selected with equal intensities but in reverse order. Regime 13 was the direct selection check against which the various samples from independent culling were compared.

Results

Variability among oat lines was highly significant for protein percentage, grain yield, and protein yield, and the heritability values for the three traits were quite similar, ranging from 0.39 for protein percentage of 0.47 for grain yield (Table 2). Grain yield was nearly perfectly correlated with protein yield, but protein percentage was negatively correlated with both grain and protein yields (Table 3). The correlations suggest that protein yield of oats was due primarily to grain yield and not at all to protein percentage.

Predicted and realized genetic gains for protein percentage, grain yield, and protein yield from selection with various combinations of culling levels are shown in Figs. 1, 2, and 3, respectively. Each value plotted in these figures is the mean of 19 selected lines: The standard error used to estimate the last significant difference (LSD) at the 5% level between regimes was computed by using the mean within-sample variation for all 13 selection regimes.

The sample of lines selected only for protein percentage (regime 7) showed a very high protein percentage mean, whereas samples selected intensively for grain yield (regimes 1, 2, 11, and 12) had protein percentage means below the original population mean (Fig. 1). The sample selected only for protein yield had a protein percentage ca 0.7 lower than the population mean.

Generally, observed values exceeded the predicted ones when protein percentage was selected intensively. For example, with regime 7, the actual protein percentage was 2.0 higher than the predicted value. The discrepancy between observed and predicted values was due to the frequency distribution of protein percentage being biased. Because the population was skewed with the long tail of the distribution toward high protein percentage, genetic progress was greater than expected when selection for this trait was intensive. The realized heritability values were 0.65, 0.59, and 0.58 in regimes 7, 8, and 9, respectively, values that were much higher than the heritability value of 0.39 estimated from variance component analysis (Table 2). Protein percentage means for samples of oat lines selected initially for protein percentage greatly exceeded the mean for samples selected initially for grain yield. Probably this difference was due to the fact that protein percentage was not distributed normally. If the frequency distributions for these two traits had been normal, predicted and observed values for samples selected initially for

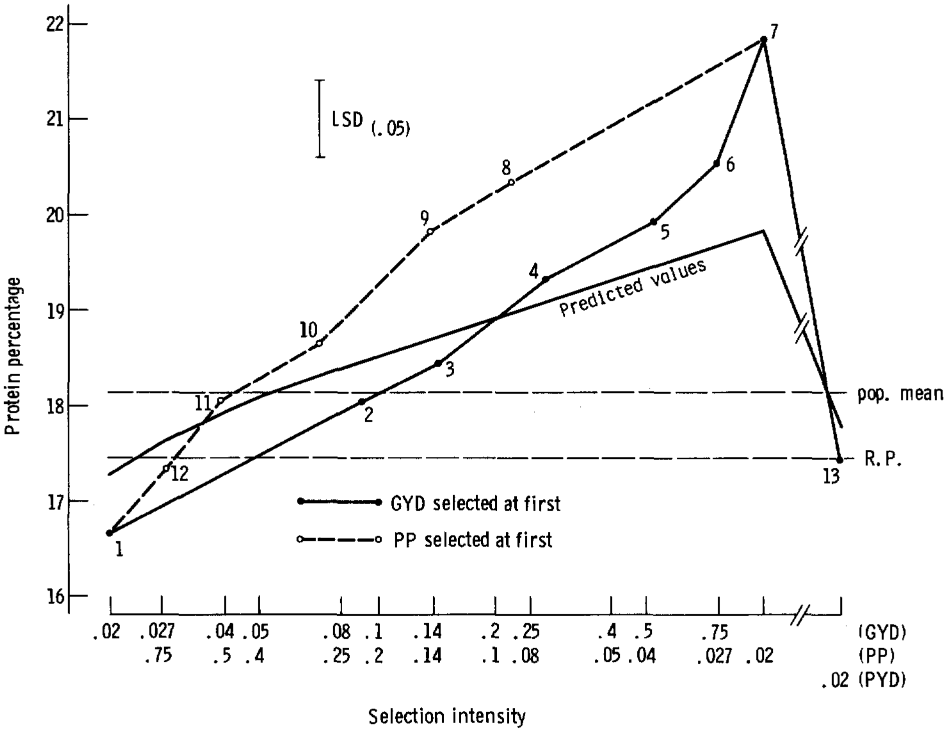

Fig. 1. Predicted and realized means for protein percentage from various regimes of independent culling for grain yield (*GYD*) and protein percentage (*PP*). Numerals that accompany the realized means denote selection regimes

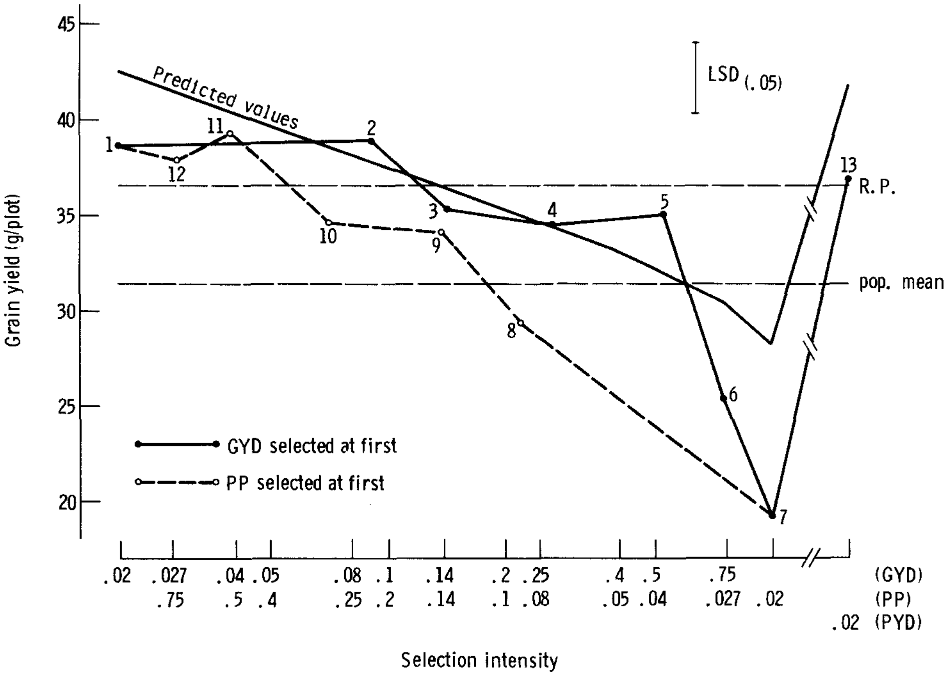

Fig. 2. Predicted and realized means for grain yield from various regimes of independent culling for protein percentage (*PP*) and grain yield (*GYP*). Numerals that accompany the realized means denote selection regimes

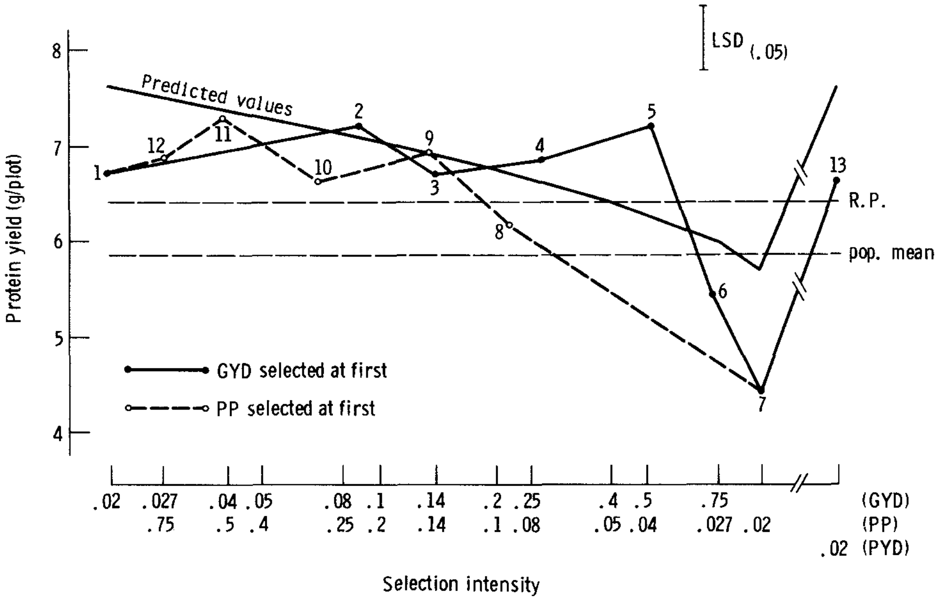

Fig. 3. Predicted and realized means for protein yield from various regimes of independent culling for protein percentage (*PP*) and grain yield (*GYD*). Numerals that accompany the realized means denote selection regimes

protein percentage and initially for grain yield would have coincided with each other.

Samples of oat lines selected for grain yield intensively (regimes 1, 2, 11, and 12) showed high grain yield means, whereas samples selected intensively for protein percentage (regimes 6 and 7) showed very low grain yield means (Fig. 2). The sample selected only for protein yield (regime 13) had a grain yield nearly identical to the sample selected for grain yield only (regime 1), which corroborates the genetic correlations in suggesting that grain yield is of paramount importance in determining protein yield. Observed means

Table 4. Means for the recurrent parent, the original population of oat lines, and samples selected according to 13 regimes for 13 traits, and means of backcross generations for lines in samples

Selection regime	Trait													
	Heading date (June)	Plant height (cm)	Biomass (g/plot)	Grain yield (g/plot)	Groat weight (g/1000)	Protein per-centage (%)	Harvest index (%)	Straw yield (g/plot)	Growth rate (g/plot/da)	Unit straw weight (g/cm)	Grain number (no./plot)	Protein yield (g/plot)	Protein per groat (mg/groat)	Backcross generation[a]
1	14	111	90	39	27	16.7	43	51	0.77	0.46	1,470	6.5	4.4	3.7
2	15	115	95	39	26	18.0	41	57	0.83	0.49	1,510	7.0	4.7	3.0
3	16	114	92	35	26	18.4	39	57	0.82	0.50	1,350	6.4	4.8	2.5
4	16	117	95	34	24	19.3	37	61	0.87	0.52	1,430	6.6	4.7	1.4
5	16	120	98	35	23	19.9	36	63	0.91	0.52	1,550	6.9	4.5	1.0
6	16	120	80	25	23	20.6	32	55	0.80	0.46	1,130	5.2	4.7	0.2
7	17	115	67	19	22	21.9	28	48	0.69	0.42	870	4.1	4.7	0.1
8	16	112	92	34	25	18.7	38	57	0.83	0.51	1,360	6.4	4.7	1.9
9	16	119	98	34	24	19.8	35	64	0.92	0.53	1,450	6.7	4.7	1.2
10	16	112	92	35	25	18.7	38	57	0.83	0.51	1,360	6.4	4.7	1.9
11	15	117	98	39	26	18.1	40	59	0.86	0.50	1,540	7.0	4.6	2.8
12	15	113	91	38	27	17.4	42	53	0.79	0.47	1,450	6.6	4.6	3.5
13	15	112	89	37	27	17.4	42	52	0.70	0.46	1,400	6.4	4.6	3.3
Population mean	14	111	78	32	26	18.2	40	46	0.69	0.42	1,220	5.9	4.7	2.5
Recurrent parent	14	107	85	37	28	17.4	43	48	0.72	0.45	1330	6.4	4.8	–
LSD[b]	2.3	4.9	8.2	3.6	1.3	0.8	3.0	6.3	0.08	0.05	150	0.64	0.29	

[a] Mean value for number of backcrosses

[b] Least significant difference at 5% level

Table 5. Means for the ten most promising lines for 13 traits and the independent culling level regimes in which they were selected

Backcross generation	Trait														Regimes in which each line was selected
	Line number	Heading date (June)	Plant height (cm)	Biomass (g/plot)	Grain yield (g/plot)	Groat weight (g/1000)	Protein percentage (%)	Harvest index (%)	Straw yield (g/plot)	Vegetative growth rate (g/plot da)	Unit straw weight (g/cm)	Grain number (no./plot)	Protein yield (g/plot)	Protein per groat (mg/groat)	
5	27	13	110	84	40	26	18.6	48	44	0.67	0.41	1,580	7.5	4.7	2, 10
	37	13	110	99	42	27	18.5	43	57	0.86	0.52	1,550	7.8	5.0	2, 3, 11, 12
	57	15	109	91	38	29	18.4	42	53	0.77	0.48	1,320	7.0	5.3	–
4	1	12	106	101	42	27	18.7	42	59	0.90	0.55	1,590	7.8	5.0	9, 10
	2	13	114	89	40	29	18.3	45	49	0.75	0.44	1,390	7.3	5.3	3, 10
	18	14	101	87	41	29	18.7	47	46	0.69	0.46	1,430	7.7	5.4	2, 3, 10, 11, 12, 13
	27	15	110	100	43	28	18.8	43	57	0.84	0.52	1,520	7.9	5.2	12
	29	11	107	87	38	25	18.5	44	49	0.76	0.45	1,490	7.0	4.7	2, 11
	42	15	111	88	40	28	18.6	45	48	0.71	0.43	1,390	7.3	5.3	–
3	43	12	112	92	40	28	19.5	43	52	0.80	0.47	1,420	7.7	5.4	4, 5, 9, 10
Recurrent parent		14	107	85	37	28	17.4	43	48	0.72	0.45	1,330	6.4	4.8	
LSD[a]		2.0	6.7	18.4	8.9	1.4	1.4	5.1	11.5	0.18	0.10	340	1.5	0.5	

[a] Least significant difference at 5% level

were smaller than predicted ones for many selection regimes, probably because the heritability value calculated from variance component (0.47) was overestimated. And, as expected, the realized heritability values were 0.33, 0.44, 0.26, and 0.27 for selection regimes 1, 2, 3, and 4, respectively. The frequency distributions of grain and protein yields were nearly normal.

For samples except those from regimes 6 and 7, in which protein percentage was selected intensively, protein yield means were greater than the population mean (Fig. 3). Selection regimes 2, 3, 4, 5, 9, 10, and 11 were very good for giving desirable combinations of protein yield, grain yield, and protein percentage. Means of 13 traits for the samples of oat lines selected via the 13 selection regimes, for the recurrent parent, and the original population are given in Table 4. Also shown in Table 4 are the means of generations of backcrossing for the selected samples. Note that regimes that placed high selection intensity on grain and protein yields (regimes 1, 2, 11, 12, and 13) selected lines from advanced backcross generations, whereas those that placed greatest intensity on protein percentage (regimes 5, 6, and 7), selected lines from the BC_0 and BC_1. This would be expected because the recurrent and donor parents were high for grain yield and protein percentage, respectively. The most desirable selection regimes seemed to be numbers 2 and 11. When compared with the recurrent parent, both had higher grain yields and higher protein yields and percentages, and significantly higher straw and biomass yields and vegetative growth rates. For both regimes, mean heading date was one day later, harvest index was reduced by 2.0 or 3.0%, and plant height was 8 to 10 cm taller. Mean backcross generation was 2.8 for regime 11 and 3.0 for regime 2. Groups of oat lines from regimes 5 and 9 are especially interesting materials for further breeding. They are two days later, 12–13 cm taller, and 6–8% lower in grain yield than the recurrent parent; however, they have 2.5% higher protein content and 25% greater vegetative growth rate and straw yield than the recurrent parent. Some of the lines from the samples from regimes 5 and 9 should be valuable for further crossing with adapted parents to produce segregates that combine increased vegetative growth rate with high harvest index and good protein percentage and agronomic traits. Means for backcross generations for the lines selected via regimes 5 and 9 were 1.0 and 1.2, respectively.

For more intensive study, we singled out ten promising lines that had high protein percentage and were comparable to the recurrent parent in heading date, plant height, grain yield, groat weight, and harvest index (Table 5). All of the lines were from BC_3 to BC_5 and half of them were selected by regime 10. Four were selected by regime 2, but it was disturbing that two lines, BC_5-57 and BC_4-42, were not selected by any regime with a selection intensity of 2%.

Discussion

In general, observed means for protein percentage, grain yield, and protein yield for samples of oat lines selected via various regimes of independent culling levels did not correspond very closely to the expected values. The discrepancies probably had two causes: (a) the frequency distribution for protein percentage deviated from normal and (b) the estimate of heritability for grain yield from the whole population of oat lines, computed via variance components, was quite different from realized heritability with a number of selection regimes.

Intensive selection for protein percentage resulted in samples with high protein percentage, but low grain and protein yields. Low grain yields in these samples were caused by poor plant vigor and low harvest index. For example, in regime 7, where selection was only for protein percentage, biomass was 67 g/plot and harvest index was only 27%. Strong negative genotypic correlations between protein percentage and harvest index have been reported previously by Takeda and Frey (1979) and Takeda et al. (1979).

Contrariwise, intensive selection for high grain yield resulted in samples with high protein yield and low protein percentage. The strong positive genotypic association between grain and protein yields, and the negative association between grain yield and protein percentage predicted the results from selection. Intensive selection for protein yield only gave sample means that were similar to the recurrent parent for all traits. Therefore, to improve protein percentage, grain yield, and protein yield simultaneously requires a procedure that selects for grain yield and protein percentage jointly. The procedure of independent culling levels meets this requirement.

Our results show that regimes 11 and 2 were best and efficient for our objectives and materials. With regime 11, the initial selection for protein percentage was rather relaxed at 50% retention of the population of oat lines and subsequent intense selection at 4% for grain yield. In a real oat breeding program, however, selection for protein percentage and grain yield probably would have been carried out in successive years, rather than in one year. Our results with regime 11 suggest that a low intensity of selection should be used for protein percentage during the first year. The first year testing of the total population of oat lines could be done in microplots (Frey 1965) and with few replicates because the heritability of protein percentage of oats is quite high (Frey 1975). The selected sample of lines would be tested with larger plots and more extensive replication in the second year and selection would be practiced for grain yield with a fairly high intensity. This suggested procedure does not fit the definition of "independent culling levels" exactly. It does, however, accomplish several objectives: (a) it utilizes the genotypic variability of both protein percentage and grain yield to increase their product, protein yield; (b) testing in the first year is rather inexpensive, and selection for protein percentage is not intense, with the result that genotypic variability for grain yield is not restricted very much; and (c) all three traits, protein percentage, grain yield, and protein yield, can be increased, or at least held at acceptable levels, simultaneously.

Another breeding procedure that would be applicable for the simultaneous selection of protein percentage and grain yield would be "index selection". Hazel and Lush (1942) have reported that the efficiencies of index selection and independent culling levels are very similar when the number of traits selected is small and selection intensity is high. Usually, however, the method of independent culling levels is less time consuming and more practical than index selection.

Two promising lines, BC_5-57 and BC_4-42, were not selected with any independent culling regime. These two lines had a good balance of all characters measured, but they were missed because the selection intensity was too high to include them in a selected sample. They would have been selected if the final selection intensity was 4% instead of 2%.

Our results show that from three to five backcrosses are necessary if one hopes to select from interspecific oat matings directly for lines that have high protein percentage and acceptable levels of agronomic traits. Lines with very high protein percentages occurred in early backcross generations of these *A. sativa* × *A. sterilis* matings and in the one reported by Takeda and Frey (1979), but they did not have acceptable levels of agronomic traits. However, regimes 5 and 9 selected samples of lines with very high protein and rather good levels of agronomic traits. The vegetative vigor of these lines averaged from 25% to 28% greater than the recurrent parent, and their mediocre grain yields were due to low harvest index. On average, oat lines selected by these regimes were in BC_1. They would be good candidate lines for further backcrossing. Such a strategy would permit the exploitation of genes from *A. sterilis* for high vegetative growth rate (Lawrence and Frey 1975; Takeda and Frey 1977) and high protein percentage in segregates with the high harvest index and good agronomic traits of the recurrent parent.

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