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Cytoplasmic Effect on Groat Protein Content in Interspecific Matings of *Avena sativa* L. and *A. sterilis* L.[1]

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Thirty sets of reciprocal isopopulations, each with 20 F_2-derived oat lines from the BC_0, BC_1, and BC_2 of all possible matings among five *Avena sterilis* L. accessions and two *A. sativa* cultivars, were evaluated in a replicated field trial to determine whether groat protein content was influenced by cytoplasmic inheritance and to study associations between protein content and agronomic traits. *A. sterilis* cytoplasm had no direct effect on groat protein content, but significant interactions between the cytoplasms and nuclear genes from *A. sativa* and *A. sterilis* were detected. Thus, the potential may exist for improving groat protein content of cultivated oats by exploiting specific intra and interspecific nucleo-cytoplasmic combinations. Generally, associations of various traits with groat protein content showed no trend for change over successive backcrosses, but phenotypic and genotypic correlations between protein percentage and all traits except harvest index tended to be larger for lines with *A. sterilis* cytoplasm than those with *A. sativa* cytoplasm.
INDEX DESCRIPTORS: Cytoplasm, oats, genotypic correlation, phenotypic correlation.

Although extranuclear inheritance was first reported in 1909 by Correns (cited in Kirk and Tilney-Bassett 1967), the importance of cytoplasmic effects was not fully realized until cytoplasmic male sterility in maize (*Zea mays* L.) (Rhoades 1931) and the cytoplasmic-nuclear system of male sterility and fertility restoration in sorghum [*Sorghum bicolor* (L.) Moench] (Stephens and Holland 1954) were reported. Kihara (1973) introduced the term nucelo-cytoplasmic heterosis to describe the superiority of alloplasmic lines to the nucleus donor lines. Kihara (1980) found that alloplasmic lines of wheat gave nuclear cytoplasmic heterosis for grain yield. Further, a phenomenon akin to nuclear-cytoplasmic heterosis was reported by Robertson and Frey (1984) in BC_2 cytoplasmic "isopopulations" (Day et al. 1955) of oats (*Avena sativa* L.).

Several studies have shown that grain or seed composition may be affected by cytoplasmic inheritance. Singh and Hadley (1972) reported 3-4% differences in seed protein content for reciprocal crosses between high- and low-protein soybean lines [*Glycine max* (L.) Merr.], and Garwood and Lambert (1967) found reciprocal differences for protein content in maize. Sasaki et al. (1978) reported an increase in grain protein content of the Chinese spring wheat (*Triticum aestivum* L.) when the nuclear genome was put into several *Aegilops* cytoplasms, but the increase was a secondary effect due to yield-component compensation.

Brown and Argeetey (1973) showed significant maternal but no cytoplasmic effect for groat oil content of oats. Tantivit and Frey (1974), who studied reciprocal F_1 crosses of *A. sativa* x *A. sterilis* matings, found several instances of significant cytoplasmic effects on groat-protein content, but Ohm and Patterson (1973) found no reciprocal effect in similar matings.

Robertson and Frey (1984) reported that both direct cytoplasmic and nuclear-cytoplasmic interaction effects influenced seven traits measured on BC_xF_4 isopopulations from reciprocal crosses of matings among two *A. sativa* cultivars with five *A. sterilis* accessions. Also, Beavis and Frey (1987) evaluated 76 cytoplasmic isopopulations of oats for seven traits and concluded that all traits exhibited significant nuclear-cytoplasmic interactions, but they found no consistent cytoplasmic effect. Neither of these studies evaluated protein percentage.

The objectives of this study were to evaluate cytoplasmic effects on (a) groat protein percentages of F_2-derived lines of oats from BC_0, BC_1, and BC_2 of reciprocal crosses and (b) associations between protein percentage and agronomic traits.

MATERIALS AND METHODS

Five *A. sterilis* accessions (PI 324725, PI 217512, PI 317982, PI 324819, and PI 317757) and two *A. sativa* cultivars (CI 9170 and 'Otee') were mated in a Design II (Comstock and Robinson 1952) with reciprocals to give 20 crosses. The 20 hybrids were backcrossed twice to their respective *A. sativa* parents to give 20 BC_1 and BC_2 crosses (BC_0, BC_1, and BC_2 refer to single cross, backcross one, and backcross two, respectively).

All crosses were made in the greenhouse, and BC_xF_2 seeds were space sown in the field. Twenty nonshattering plants were harvested from the BC_0, BC_1, and BC_2 of each reciprocal cross of each mating, and the bulk seed from a plant was used to establish an F_2-derived line in the F_3. There were 20 lines per population (a population represents one reciprocal cross of a mating in a generation), except in six combinations: (a) PI 319782 x CI 9170 BC_0 had 14 lines, (b) PI 324725 x Otee BC_0 had 16, (c) PI 217512 x Otee BC_1 had 17, (d) Otee x PI 317982 BC_0, CI 9170 x PI 324725 BC_0, and CI 9170 x PI 317982 BC_0 had 19. This technique for comparing two alternative cytoplasms is referred to as an "isopopulation method" (Day et al. 1955) because on average, the sets of lines with *A. sterilis* and *A. sativa* cytoplasms for a common mating are expected to have equivalent samples of nuclear genes: Thus, any difference between trait means for the populations of a mating should be caused by cytoplasms either directly or indirectly as they interact with nuclear genes. Each F_2-derived line was grown in a hill to advance it to the F_4 for use in the evaluation experiment. Thus, there were 60 populations (i.e., 20 BC_0, 20 BC_1, and 20 BC_2) each with 20 F_2-derived lines except for the six crosses noted.

In 1979, the 1184 F_2-derived lines were evaluated in a field experiment arranged in a randomized complete-block design with three replicates grown at Ames and three at Sutherland, Iowa. Soil types were Moody silty loam (fine-silty, mixed, mesic Udic Haplustolls) at Sutherland and clay silty loam (fine-loamy, mixed, mesic, Typic Haplaquoll) at Ames. The previous crop at both locations was soybeans. Nitrogen, P_2O_5, and K_2O were applied at Ames and Sutherland at rates of 28-56-55 and 17-68-34 kg ha^{-1}, respectively. A plot consisted of 30 seeds sown in a hill, and hills were spaced 30.5

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Table 1. Protein percentage means for 60 populations of F_2-derived lines representing the BC_0, BC_1, and BC_2 in different *A. sativa* and *A. sterilis* cytoplasms from 10 interspecific matings of oats.

		Generation											
		BC_0			BC_1			BC_2			Cytoplasm		
A. sativa parent	*A. sterilis* parent	*A. sterilis*	*A. sativa*	Mean	*A. sterilis*	*A. sativa*	Mean	*A. sterilis*	*A. sativa*	Mean	*A. sterilis*	*A. sativa*	Mean
Otee	PI 324725	20.8**[a]	19.7	20.2	18.2	19.8**	19.0	19.5	19.8	19.7	19.4	19.8	19.6
	PI 217512	19.0	19.1	19.0	19.0	18.6	18.8	18.8**	18.1	18.5	18.9	18.6	18.7
	PI 317982	20.3	20.4	20.3	20.1	20.6**	20.3	19.5	19.7	19.6	20.0	20.2	20.1
	PI 324819	18.6	19.1	18.9	19.5	19.1	19.3	19.3	19.2	19.3	19.1	19.1	19.1
	PI 327757	20.0	20.1	20.1	19.2**	18.5	18.8	19.6**	19.0	19.3	19.6	19.2	19.4
	Mean	19.7	19.7	19.7	19.2	19.3**	19.3	19.4**	19.2	19.3	19.4	19.4	19.4
CI 9170	PI 324725	19.5	19.9	19.7	19.2	19.2	19.2	17.7	17.8	17.7	18.9	18.9	18.9
	PI 217512	18.4	18.5	18.5	16.4	18.2**	17.3	17.3	17.8	17.6	17.4	18.2**	17.8
	PI 317982	19.1	19.2	19.2	18.3	18.1	18.2	17.5	17.9	17.7	18.2	18.4	18.3
	PI 324819	17.7	17.8	17.7	16.5	21.1**	18.8	17.0	17.9**	17.4	17.0	18.9**	18.0
	PI 317757	19.0**	18.1	18.5	17.7	18.2	18.0	16.9	18.2**	17.6	17.8	18.2	18.0
	Mean	18.7	18.7	18.7	17.6	19.0**	18.3	17.3	17.9**	17.6	17.8	18.5**	18.2
Overall mean		19.2	19.2	19.2	18.4	19.1	18.8	18.3	18.5	18.4	18.6	19.0**	18.8
LSD (5%)		0.6			0.5			0.5			0.5		
LSD (1%)		0.7			0.7			0.6			0.7		

[a]Significant at the 1% level of probability is denoted for the larger mean of comparison between mating with *A. sterilis* and *A. sativa* cytoplasms.

cm apart in perpendicular directions. Two rows of border hills were sown around the experiment at each site to provide competition for peripheral plots. Plants were sprayed with a fungicide at weekly intervals from anthesis to maturity to control foliar diseases. The Ames and Sutherland sites were planted on 28 and 22 April, respectively.

Seven traits were measured or computed on a plot basis. Heading date was recorded as the number of days from planting until 50% of the panicles were completely emerged. Plant height was recorded in centimeters from ground level to the tips of the panicles. Biomass was the weight of the air-dried bundle of culms harvested at ground level. The threshed grain was weighed to give grain yield. Straw yield was obtained by subtracting grain from biomass, and harvest index was computed as the ratio of grain yield to biomass expressed as a percentage. Vegetative growth rate until maturity was calculated as straw yield divided by number of days to heading. Heading date, plant height, and vegetative growth rate until maturity were measured only at Ames. For each entry, 5-g samples from each of the three plots at a test site were bulked, and this sample was dehulled to provide ca. 10 g of groats for nitrogen (N) analysis. Thus, there were two samples (replicates), one from each test site, per entry for N analyses. The analyses were conducted with a Neo-Tech model 41 near infrared analyzer. N contents were multiplied by 6.25 to obtain groat protein contents.

Analyses of variance were conducted over generations. Oat lines within populations were considered random, but parents, populations, generations, and matings were considered fixed. Separate analyses also were computed for each backcross generation, each *A. sativa* parent, and each *A. sativa* parent within each backcross.

Mean squares for cytoplasms, *A. sterilis* and *A. sativa* parents, and backcross generations and the interactions of these factors were obtained from an unweighted means analysis (Snedecor and Cochran 1967). Replications, populations, lines/populations, and error mean squares were obtained from analyses of complete data sets. The mean squares for lines/populations were used in tests of significance for comparisons between *A. sativa* and *A. sterilis* cytoplasms for individual matings in each backcross generation and overall generations.

Phenotypic correlations of groat protein content with heading date, plant height, grain yield, harvest index, straw yield, unit straw weight, and vegetative growth rate were calculated by using line means. Genotypic variances and covariances, obtained by subtracting the error variance and covariance from line variance and covariance, respectively, were used to calculate genotypic correlations between the same traits. Correlations were computed for individual populations and then pooled across populations.

RESULTS

Populations of oat lines differed significantly for all traits (analyses not presented). All protein percentage mean squares were significant for cytoplasms, matings, generations of backcrossing, and all interactions among the main effects were significant, except for cytoplasms in BC_0. Mean squares for main effects were much larger than those for interactions.

When averaged over backcrosses, mean protein percentages (Table 1) ranged from 17.0 for PI 324819 x CI 9170, to 20.2 for Otee x PI 317982, and the highest mating mean over cytoplasms at 20.1% involved Otee and PI 317982 as parents. When averaged across matings, the decrease in mean protein percentage was linear and significant over generations of backcrossing (19.2, 18.8, and 18.4% in BC_0, BC_1, and BC_2, respectively). Over all generations of backcrossing and matings, the mean protein percentage for lines with *A. sativa* cytoplasm (19.0%) was significantly greater than that for lines with *A. sterilis* cytoplasm (18.6%). When averaged over backcross generations the population with *A. sativa* cytoplasm had greater protein percentage than its counterpart with *A. sterilis* cytoplasm in six matings, and two of these were significantly ($P<0.05$) greater. In two matings, populations with *A. sterilis* cytoplasms had greater protein percentage than their counterparts with *A. sativa* cytoplasm, and in two the populations were equal.

The significance of interactions involving cytoplasms with other main effects indicated that (a) repeated backcrossing to an *A. sativa* parent caused protein percentage differences due to cytoplasm to vary (cytoplasms x generations of backcrossing) and (b) specific interac-

tions occurred between cytoplasms and nuclear-genomes (cytoplasms x matings). When matings and matings x cytoplasm mean squares were subdivided into among *A. sativa* parents, among *A. sterilis* parents, and *A. sativa* x *A. sterilis* interactions, all three of the subdivided mean squares were significant. These results suggest (a) that mating and cytoplasm differences were due to both *A. sativa* and *A. sterilis* parents and (b) that specific parental combinations influenced differences between cytoplasms. The significance of the mating x generations of backcrossing mean square also indicated that specific interactions occurred between level of backcrossing and mating.

When backcrosses are considered separately, the protein percentage means for lines with *A. sativa* cytoplasm were significantly greater than those for *A. sterilis* cytoplasm in BC_1 and BC_2. The means were equal in BC_0. The significant mean square for cytoplasms x generations of backcrossing suggests that interaction occurs between cytoplasm and certain levels of combination of *A. sativa* and *A. sterilis* genomes. This complex interaction was confirmed by the significance of mean square for cytoplasms x *A. sterilis* x *A. sativa* in all three backcross generations.

Because different proportions of *A. sativa* and *A. sterilis* genomes occur in each backcross generation, a variance analysis was conducted on data from each generation separately. In each analysis, the mean squares for mating and cytoplasm x mating were subdivided into sources due to *A. sativa* parents, *A. sterilis* parents, and *A. sativa* x *A. sterilis* interactions. All the mean squares, for cytoplasm, cytoplasm x *A. sativa* parents, cytoplasm x *A. sterilis* parents in BC_0, and cytoplasm x *A. sterilis* parents in BC_2, were significant ($P<0.05$). The mean square for *A. sativa* parents in BC_2 was much greater than its counterpart in BC_1 and BC_0, indicating the important effect of the *A. sativa* genome on groat protein percentage. However, the significance of mean squares for *A. sterilis* parents and *A. sativa* x *A. sterilis* interactions in all generations of backcrossing, shows that *A. sterilis* parents and specific interactions of parents also affect this trait, even though their effects are minor compared with *A. sativa* parents.

When considered over the whole study, there were 15 pairs of isopopulations that gave comparisons for *A. sterilis* vs. *A. sativa* cytoplasms for each *A. sativa* parent (Table 1). For the Otee parent, the isopopulation with *A. sterilis* cytoplasm had greater protein percentage than its counterpart in 7 of 15 pair comparisons (four were significant), but for CI 9170, in only 3 of 15 was the *A. sterilis* population superior (one was significant). The greatest effect of *A. sterilis* on groat protein percentage occurred in the BC_0 of the PI 324725 x Otee mating. Its protein percentage was 1.1% greater than the counterpart with *A. sativa* cytoplasm. The greatest superiority for *A. sativa* cytoplasm occurred in the BC_1 of the CI 9170 x PI 324819 mating. The population with *A. sativa* cytoplasm had a protein percentage 1.8% greater than its counterpart with *A. sterilis* cytoplasm.

When averaged over generations of backcrossing and matings, the mean protein percentage for lines with Otee cytoplasm (19.4%) was significantly greater ($P<0.05$) than that for lines with CI 9170 cytoplasm (18.5%). The mean protein percentage for lines with CI 9170 cytoplasm was significantly higher than that for counterpart lines with *A. sterilis* cytoplasm, whereas there were no cytoplasm effects for Otee matings.

In general, associations between protein percentage and heading date, straw yield, and vegetative growth rate were low, even if significant (Table 2). Protein content was negatively associated with grain yield and harvest index in all generations and both cytoplasms. Protein percentage had low negative association with biological yield and low positive correlation with plant height. The associations of protein percentage with other traits tended to be of greater magnitude both negatively and positively in *A. sterilis* than in *A. sativa* cytoplasm. There was no trend toward either increasing or decreasing correlations with advance in generations of backcrossing.

DISCUSSION

Because *A. sativa* and *A. sterilis* have been separated geographically for two millenia or more (Coffman 1961), it is possible that they have evolved divergent cytoplasms or plasmagenes. Robertson and Frey (1984) and Beavis and Frey (1987), who investigated cytoplasmic isopopulations representing reciprocal crosses from *A. sativa* x *A. sterilis* matings, found an expression of nuclear-cytoplasmic interaction effects for all seven traits studied. In general, Beavis and Frey (1987) found no consistent cytoplasmic effect on any trait.

If direct cytoplasmic effects occur, there should be no interaction between nuclear genomes and cytoplasms. In our study, no cytoplasm had a consistent superiority or inferiority for groat protein percentage. This was true with respect to *A. sterilis* cytoplasms across *A. sativa* parents and *A. sativa* cytoplasms across *A. sterilis* parents. Therefore, we concluded that the significant differences for protein

Table 2. Phenotypic (above) and genotypic (below) correlations between protein percentage and eight agronomic traits for F_2-derived lines within *A. sterilis* and *A. sativa* cytoplasms in each of three backcrosses.

	BC_0			BC_1			BC_2			Cytoplasm		
Traits	*A. sterilis*	*A. sativa*	Combined	*A. sterilis*	*A. sativa*	Combined	*A. sterilis*	*A. sativa*	Combined	*A. sterilis*	*A. sativa*	Combined
Heading date	0.04	0.04	0.04	0.03	0.00	−0.03	0.20**	0.20**	0.18**	0.09	0.05	0.05
	0.02	0.05	0.04	0.02	0.00	−0.04	0.22	0.22	0.20	0.09	0.07	0.05
Plant height	0.24**	0.22**	0.22**	0.23**	0.00	0.09**	0.08**	−0.05	0.02	0.25**	0.10**	0.16**
	0.30	0.26	0.28	0.27	−0.04	0.09	0.08	−0.07	0.02	0.29	0.11	0.19
Biomass	0.01	−0.02	0.00	−0.03	0.18**	−0.14**	0.05	0.09	0.06	−0.03	−0.11**	−0.08**
	0.02	−0.04	0.00	−0.06	−0.29	0.20	0.08	0.15	0.09	−0.04	−0.16	−0.11
Grain yield	−0.18**	−0.04	−0.11**	−0.24**	−0.28**	−0.30	−0.14**	−0.01	−0.10	−0.24**	−0.19**	−0.24**
	−0.31	−0.05	−0.18	−0.31	−0.41	−0.39	−0.18	−0.01	−0.13	−0.33	−0.27	−0.32
Harvest index	−0.37**	−0.07	−0.21**	−0.42**	−0.34**	−0.39**	−0.40**	−0.28**	−0.26**	−0.44**	−0.25**	−0.35**
	−0.80	−0.05	−0.39	−0.65	−0.57	−0.62	−0.61	−0.70	−0.64	−0.72	−0.45	−0.61
Straw yield	0.13**	0.00	0.07*	0.11**	−0.08**	−0.01	0.18**	0.16**	0.17**	0.12**	−0.03	0.04
	0.22	−0.02	0.11	0.12	−0.17	−0.04	0.24	0.28	0.26	0.19	−0.05	0.06
Unit straw	0.03	−0.09**	0.03	−0.01	−0.09**	−0.07*	0.17**	0.19**	0.17**	0.02	−0.07**	−0.03
weight	0.10	−0.22	0.03	−0.04	−0.14	−0.13	0.29	0.33	0.29	0.04	−0.12	0.06

percentage between isopopulations were not caused by unilateral effects of plasmagenes. Our results corroborate those of Elliott et al. (1985) who found that general cytoplasmic effects on protein percentage of oats studied were nonexistent. On the other hand, we did find evidence for nuclear-cytoplasmic interactions for groat protein content. Generally, plasmagenes could interact with nuclear genes in several ways: (a) the *A. sterilis* nuclear genome could interact with its own or *A. sativa* cytoplasm, (b) the *A. sativa* nuclear genome with its own or *A. sterilis* cytoplasm, and (c) the two nuclear genomes could interact with either *A. sativa* or *A. sterilis* cytoplasm.

On average, the BC_0, BC_1, and BC_2 would be expected to have 50, 75, and 87.5%, respectively, of the *A. sativa* parent nuclear genome, regardless of the direction in which the original cross was made. However, the cytoplasms should remain intact, i.e., either *A. sativa* or *A. sterilis*. Therefore, with successive backcrossing to the *A. sativa* parent, contributions to protein percentage due to interaction between *A. sterilis* nuclear genomes and cytoplasms should have decreased proportionally to the expected degree of *A. sterilis* nuclear genome.

We found no general superiority for *A. sterilis* cytoplasms. Also, no difference between the two cytoplasms was detected in the BC_0, and the superiority of the *A. sativa* cytoplasm decreased from BC_1 to BC_2. Therefore, we concluded that no interaction of *A. sativa* or *A. sterilis* nuclear genomes with their own cytoplasms was evident. On the other hand, continued backcrossing of the reciprocals of a mating, which would decrease the proportion of the *A. sterilis* nuclear genome, would increase or decrease effects due to *A. sativa, A. sterilis* nuclear-cytoplasmic interactions, depending upon whether the interacting nuclear genome was from the donor or recurrent parent. Because we did not find any consistent pattern of change in groat protein content over backcrosses, our results suggest the presence of complex or very specific interactions between the plasmagenes and nuclear genes from both parents. This complex interaction was further illustrated by the significant mean squares for generations of backcrossing x cytoplasm and cytoplasm x *A. sativa* x *A. sterilis* parents. Particular matings gave high groat protein percentages, which indicates the presence of variation in degree of interaction between *A. sterilis* cytoplasm and *A. sativa* nuclear genomes (e.g., PI 324725 x Otee) or *A. sterilis* nuclear genome and *A. sativa* cytoplasm (e.g., CI 9170 x PI 324819). Beal and Knowles (1978) reported the "mixing ability" for some nuclei and cytoplasms and gave an explanation of how specific combinations of the two could give superior performance.

The direct effect of a cytoplasm or its interaction with a nuclear genome could be useful for plant breeding, even when the trait superiority results from specific mixing ability in a particlular mating. Frey and Robertson (1984) found up to 17% yield advantage when the *A. sativa* genome was placed in *A. sterilis* cytoplasms in two matings, PI 317757 x CI9170 and PI 317982 x Otee. They referred to this superiority as an example of nuclear-cytoplasmic heterosis (Kihara, 1975). In our study, superior groat protein percentages were obtained when the Otee nuclear genome was placed in the PI 217512 or PI 327757 *sterilis* cytoplasms. These might be instances of nuclear-cytoplasmic heterosis for groat protein percentage.

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