RESEARCH

Selection for Nutritional Function and Agronomic Performance in Oat

A. A. Chernyshova, P. J. White, M. P. Scott, and J.-L. Jannink*

ABSTRACT

The soluble fiber (1→3),(1→4)-β-D-glucan has been identified as an active component of oat (*Avena sativa* L.) that lowers serum cholesterol, reduces the risk of heart disease and colon cancer, and alleviates the symptoms of diabetes. Those beneficial effects may be caused by the ability of β-glucan to generate viscosity in the digestive system. The objective of this study was to estimate the genetic components of variance for β-glucan content and oat slurry viscosity in a population derived from the cross of high β-glucan with elite agronomic oat lines. Twelve high β-glucan inbred lines were crossed with 12 inbred lines with good agronomic performance. The $F_{3:4}$ generation was evaluated in 2005 at two Iowa locations. The range in β-glucan content was 37.1 g kg^{-1} to 73.5 g kg^{-1}. A positive correlation (r^2 = 0.38) was found between β-glucan content and log-transformed viscosity. High β-glucan lines tended to have low grain yield and biomass. Significant variation among families and among lines within families were observable for most traits, suggesting selection for β-glucan content, viscosity, and viscosity deviation should be feasible.

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Abbreviations: GCA, general combining ability; GOPOD, glucose oxidase/peroxidase reagent; IBD, identical by descent; RVA, rapid viscosity analyzer; RVU, rapid viscosity units; SCA, specific combining ability.

Dietary fiber is an essential component in the human diet (Kirby et al., 1981, Davidson et al., 1991). Increased consumption of this component results in a decreased risk of coronary heart disease. It also reduces the risk of colon cancer, and alleviates the symptoms of diabetes. Standard oat (*Avena sativa* L.) cultivars contain from 4.5 to 5.0% of the soluble fiber β-glucan. Large amounts of oat products are not consumed habitually in a Western diet. However, oat varieties with a more concentrated soluble fiber would reduce the bulk necessary to provide a sufficient amount of this effective component. Therefore, β-glucan content has been proposed as a target for plant breeding programs (Lim et al., 1992; Peterson et al., 1995; Humphreys and Mather, 1996; Doehlert et al., 1997).

An important mechanism whereby soluble dietary fiber lowers glucose, blood cholesterol, and insulin concentrations is their capacity to increase the viscosity of intestinal chime (Welch, 1995). Viscosity is the thickness or resistance to flow of a liquid. Because

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of its functional importance, we propose that the viscosity of oat flour solutions should be itself a target of selection. Relative to oat β-glucan content, two approaches can be considered to enhance viscosity of solutions. First, regression analyses have shown a positive effect of β-glucan content on viscosity (Colleoni-Sirghie et al., 2004; Doehlert et al., 1997), reinforcing the argument in favor of selecting for high β-glucan content. Second, viscosity does not correlate completely with β-glucan content. Indeed, regression analyses have also shown that the viscosity of some oat lines deviated significantly from the regression prediction, indicating that, for a given β-glucan content, some lines generate more viscosity and some less viscosity (e.g., Doehlert et al., 1997). Besides β-glucan content, viscosity depends on other oat flour constituents, and on β-glucan molecular weight and structure (Colleoni-Sirghie et al., 2003). Little research has been devoted to understanding the precise relationship between β-glucan molecular weight and structure and its ability to generate viscosity. Nevertheless, differences in the β-glucan structure and molecular weight among lines may contribute to whether the lines generate higher or lower viscosity than predicted based on β-glucan content. We call the deviation of viscosity from its prediction based on β-glucan content the *viscosity deviation*.

To our knowledge, the inheritance of viscosity per se and of the viscosity deviation has not been studied. Plant breeding programs designed to improve the nutritional function of oat germplasm would benefit from the information on the inheritance of viscosity per se and of viscosity deviation. Previous study has shown that oat β-glucan content is a polygenic trait under the control of genes with mainly additive effects, and no interallelic interactions have been found (Holthaus et al., 1996; Kibite and Edney, 1998).

The objectives of this study were (i) to estimate genetic components of variance for β-glucan content, viscosity, and viscosity deviation in elite agronomic lines, high β-glucan lines, and in their progeny; (ii) to evaluate the differences between elite agronomic lines and high β-glucan lines for these traits; and (iii) to use a mating design to detect epistatic interactions affecting these traits. These results will enable design of breeding methods to target not only β-glucan content, as has been done in the past, but, more generally, to improve oat nutritional function, thus enhancing the grain's value for producers, processors, and consumers.

MATERIALS AND METHODS

Plant Material

Crosses were made in 2003 between two germplasm sources (Fig. 1). Germplasm with high agronomic quality (hereafter referred to as the "agronomic population") came from the Early and Mid-Season Uniform Oat Performance Nurseries coordinated by the USDA (http://wheat.pw.usda.gov/ggpages/UE-MOPN.html). Germplasm with high β-glucan content (hereafter referred to as the β-glucan population) came from a β-glucan selection study (Cervantes-Martinez et al., 2001). That program was designed to select oat lines with increased β-glucan content without regard to agronomic performance; therefore those lines were not necessarily agronomically desirable. Twelve lines from each germplasm source were used (Table 1). A North Carolina Design II (Lynch and Walsh, 1998) with three sets each containing four agronomic parents and four β-glucan parents was used to cross inbred lines (Fig. 2). This resulted in 48 crosses. Seed from these crosses were advanced by single-seed descent and $F_{3:4}$ lines were obtained from each cross. In 2005 384 lines derived from the 48 crosses plus the 24 inbred parents were grown at the Ames Research Farm of Iowa State University and the Northern Research Center near Kanawha, IA, with four replications in each location. The preceding crop was soybean for both locations. The soil types were Webster loam (fine loamy, mixed, superactive, mesic Typic Endoaquoll) for Ames and Canisteo loam (fine- loamy, mixed, superactive, calcareous, mesic Typic Endoaquoll) for Kanawha. Sowing dates were 4 Apr. 2005 for Ames and 5 Apr. 2005 for Kanawha. The harvest dates were 13 July 2005 for Ames and 27 July 2005 for Kanawha. The lines were planted in a sets in replications design with each set containing four agronomic lines, four β-glucan lines, and one representative $F_{3:4}$ line of each of the 16 possible agronomic · β-glucan crosses. Entries were planted in hill plots containing 30 seeds per plot. Hills were spaced 0.3 m apart in perpendicular directions. Each experiment was surrounded by two rows of hills of a standard variety to provide the competition for border plots. Heading date was noted for a plot when half the heads had emerged above the flag leaf. Height was measured at maturity. At harvest, plants were bundled and cut at ground level then dried against a fence for a week. Bundles were weighed to obtain plot biomass then threshed to measure grain yield. The β-glucan content and viscosities of a representative of each sample were measured as follows.

Sample Preparation

Oats were dehulled with an air-pressure dehuller (Codema, Eden Prairie, MN). Samples were submitted to dehulling for 90 s, after which grains with hulls remaining were removed. Whole groats were ground in an ultra centrifugal mill (ZM-1, Retch GmbH&Co, Haan, Germany) with a 0.5-mm sieve and with the speed selection set at 1. Strict control of time of milling (30 s), and position of the sieve in the mill were applied.

Viscosity Evaluation

Apparent viscosity (hereafter simply referred to as viscosity) as a function of temperature, time, and stirring was measured using a Rapid Visco Analyser (RVA, Newport Scientific, Warriewood, Australia). Four grams of flour were weighed, and deionized water was added to reach a total weight of 28 g. Flour weights were not adjusted to account for moisture content, but we found very little variability in the latter (data not shown). The temperature and stirring profiles selected were a constant 55°C temperature, Stirring speed was 960 for the first 10 s then 160 until the end of the measurement. We selected the constant 55°C temperature to aid solubilization of β-glucan, but to

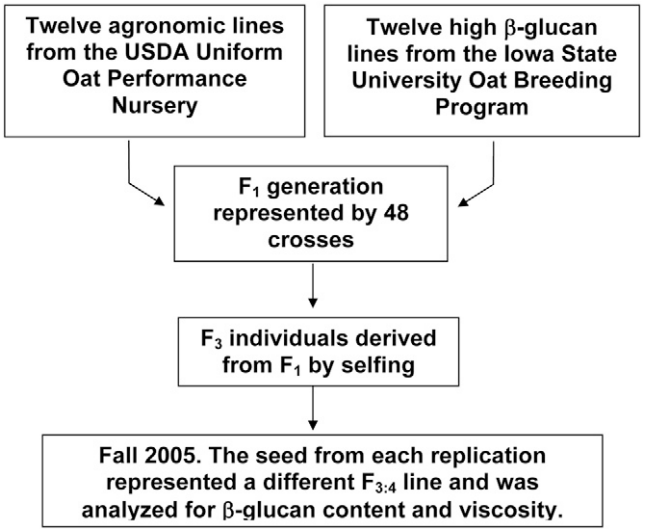

Figure 1. Population development and experiment timeline.

avoid starch gelatinization such that the contribution of starch to sample viscosity was minimal (Chernyshova, 2006). The measurement of viscosity was recorded every 4 s over a total time of 10 min. Curves were obtained, and the viscosity was expressed in rapid viscosity units (RVU). Some samples showed an identifiable peak viscosity, but for most samples the viscosity reached a plateau in the first few minutes such that it was difficult to define a peak. The viscosity measurement analyzed below was the average viscosity from 5 to 7 min on the curve. All measurements were taken in two replications, and the average between two replications was considered the actual value for each sample.

β-glucan Evaluation Using a Micro Enzymatic Method

The β-glucan content in flours was determined enzymatically using a mixed linkage β-glucan kit (Megazyme Int., Wicklow, Ireland). Modifications were made to the Approved Method 32–23 (AACC, 2000), which allowed us to increase the number of samples analyzed per unit time and to decrease cost. Solubilization and hydrolization steps were performed according to the standard method (Step 8 in the kit). Thus oat flour suspension, and β-glucan solubilization and hydrolysis with lichenase all follow the Approved Method 32-23 (AACC, 2000). From there, 5-μL aliquots were dispensed into the cells of a 96-well plate.

Table 1. Phenotypic means and standard errors (in parenthesis) for the 12 agronomic and 12 β-glucan parents across four replications in each of two locations.

	β-glucan concentration		Log (apparent viscosity)		Viscosity deviation		Biomass		Grain yield	
	g kg^{-1}		log(RVU)†		log(RVU)		g m^{-2}		g m^{-2}	
Agronomic parents										
P971A9-7-4	40.8	(1.6)	4.17	(0.08)	−0.17	(0.13)	1238	(69.9)	542	(34.2)
Winona	43.6	(1.6)	4.47	(0.08)	−0.02	(0.13)	1156	(71.1)	506	(35.9)
WIX7509-5	46.0	(1.6)	4.60	(0.08)	0.04	(0.14)	1377	(72.9)	580	(33.4)
SD000731	39.1	(1.6)	4.06	(0.08)	−0.23	(0.14)	1177	(71.9)	503	(34.6)
Woodburn	43.3	(1.5)	4.37	(0.08)	−0.10	(0.13)	1196	(68.9)	529	(33.5)
IL96-10351	45.3	(1.6)	4.49	(0.08)	−0.06	(0.13)	1297	(67.8)	565	(34.8)
Kame	40.6	(1.6)	4.49	(0.08)	0.05	(0.13)	1263	(66.9)	550	(33.0)
SD97575-5-29	42.0	(1.5)	4.35	(0.08)	−0.10	(0.13)	1364	(72.9)	608	(37.6)
Spurs	44.2	(1.5)	4.42	(0.08)	−0.06	(0.13)	1221	(67.2)	526	(34.2)
OA1021-1	38.7	(1.6)	4.45	(0.08)	0.06	(0.13)	1448	(80.5)	639	(37.6)
SD96024A	44.3	(1.6)	4.47	(0.08)	−0.03	(0.13)	1225	(69.9)	518	(34.1)
SD000843	39.2	(1.6)	3.95	(0.08)	−0.30	(0.14)	1238	(67.5)	537	(33.9)
Average	42.3	(0.5)	4.36	(0.02)	−0.08	(0.04)	1267	(20.4)	550	(10.0)
β-glucan parents										
IA95029-3-8	63.2	(1.7)	5.29	(0.08)	0.34	(0.18)	1068	(72.8)	461	(40.0)
IA94031-6-5	59.8	(2.2)	4.85	(0.10)	0.01	(0.19)	918	(70.1)	353	(36.6)
IA95148-3-9	70.9	(1.9)	5.47	(0.09)	0.29	(0.20)	919	(70.9)	364	(38.2)
IA95172-1-4	62.5	(2.0)	4.98	(0.09)	0.12	(0.18)	948	(71.3)	385	(37.6)
IA94190-10-9	64.5	(1.6)	4.70	(0.08)	−0.23	(0.18)	1078	(72.6)	461	(37.9)
IA94031-7-5	70.4	(3.7)	4.38	(0.17)	−0.34	(0.31)	745	(77.6)	206	(41.4)
IA95029-3-2	63.6	(1.9)	4.98	(0.09)	0.10	(0.20)	988	(69.7)	394	(38.1)
IA94031-6-6	64.3	(1.8)	5.43	(0.09)	0.44	(0.18)	938	(70.7)	363	(37.2)
IA95148-3-5	60.5	(2.5)	5.13	(0.10)	0.25	(0.20)	852	(72.8)	316	(40.1)
IA95172-1-3	66.5	(1.7)	4.96	(0.08)	−0.02	(0.18)	1041	(72.1)	445	(36.7)
IA94190-10-1	66.8	(1.9)	4.97	(0.09)	0.02	(0.19)	1053	(72.0)	440	(37.5)
IA94031-7-1	76.0	(4.5)	5.25	(0.15)	−0.07	(0.28)	825	(70.5)	273	(38.9)
Average	65.8	(0.7)	5.03	(0.03)	0.08	(0.06)	948	(20.8)	372	(11.1)

†RVU, rapid viscosity units.

Five μL β-glucosidase were added, and the plate was incubated for 10 min before 150 μL of glucose oxidase–peroxidase reagent (GOPOD) were added to each cell. With each plate glucose standards of five different concentrations in two replications were included. Therefore, 10 randomly-selected cells on the plate were filled with glucose standards. The concentrations were 125, 250, 375, 500, 625 μg mL^{-1} of glucose in 50 mM sodium acetate buffer. Ten μL glucose standard and 150 μL GOPOD reagent were added to each glucose standard cell. Glucose concentration of the standards was regressed on their optical density. This calibration curve was used to predict the amount of glucose in each sample aliquot, which was in turn converted to a β-glucan content using a modification of the formula given in the mixed linkage kit. Glucose standard concentrations were chosen so that optical densities from samples were located in the middle of the calibration curve. The absorbance at 510 nm was measured within an hour with a spectrophotometer. Because all samples were tested in duplicate, repeatability was assessed by the standard deviation of differences between duplicate tests of the same freshly ground subsamples. All results were expressed as grams of β-glucan per kg of moisture-free flour.

Experimental Design and Statistical Analysis

Lines were planted in an incomplete block design with four replications at each of two environments (Ames and Kanawha). A set, which consisted of four inbred agronomic parents, four inbred β-glucan parents, and 16 $F_{3:4}$ progeny lines, was assigned to each incomplete block. In each replication the inbred parents and the 16 families were constant for a given set, but different $F_{3:4}$ progeny lines within families were used to represent each family.

Variance components and predictors of line effects were estimated in a Bayesian analysis using WinBUGS (Spiegelhalter et al., 2004). WinBUGS code to fit the models is available from the corresponding author on request. Separate linear models were used to fit the inbred parents and the progeny lines, as follows.

$$P_{ijkm} = Blk_i + Pop_j + Parent_{k(j)} + Error_m \quad [1]$$

$$L_{iabcd} = Blk_i + \lambda + Agr_a + Bet_b + Agr^{*}Bet_{ab} + Line_{c(ab)} + Error_d \quad [2]$$

where P_{ijkm} is the effect of inbred parent k from population j (Pop$j$; j = agronomic or β-glucan) in block i (Blk_i), and L_{iabcd} is the effect of $F_{3:4}$ progeny line c derived from the cross between agronomic parent a (Agr_a) and β-glucan parent b (Bet_b) in block i. The linear model for the progeny lines necessitated an additional intercept, λ, that accounted for the fact that the overall progeny line mean might be different from the overall inbred parent mean. The shared term between these two models, Blk_i, allowed for improved estimation of block effects, since inbred parents were constant across blocks. The prior distribution for block effects was normal with a high variance: $Blk_i \sim N(0, 10^6)$. The large variance ensured that the prior distribution had essentially no influence on the block effect, and that block effects absorbed both location and replication effects (Edwards and Jannink, 2006). The prior for Pop_j with j = β-glucan was also distributed as $N(0, 10^6)$. To make this effect estimable, Pop_j for j = agronomic was set to zero. Prior parent effects were distributed as $Parent_{k(j = \text{agronomic})} \sim N(0, \sigma^2_{P\_A})$ and $Parent_{k(j = \beta\text{-glucan})} \sim N(0, \sigma^2_{P\_B})$, allowing the agronomic and β-glucan parents to have distinct variances. Moving to the effects modeling for progeny lines, the priors used were $\lambda \sim N(0, 10^6)$, $Agr_a \sim N(0, \sigma^2_{GCA\_A})$, $Bet_b \sim N(0, \sigma^2_{GCA\_B})$, $Agr^{*}Bet_{ab} \sim N(0, \sigma^2_{SCA})$, and $Line_{c(ab)} \sim N(0, \sigma^2_{L})$. Note that only a single replication of each progeny line was evaluated such that genetic variation among progeny lines within a cross was confounded with error variation. Such confounding, however, was not the case for the inbred parental lines. The estimation of σ^2_L depends on the assumption that error variances for the inbred parents and for the progeny lines are equal. In the model, this assumption was realized by assuming the same variance for $Error_m$ and $Error_d$ (σ^2). All variance components in turn were given uninformative inverse gamma priors $\sigma^2_{\cdot} \sim IG(0.001, 0.001)$ (Edwards and Jannink, 2006).

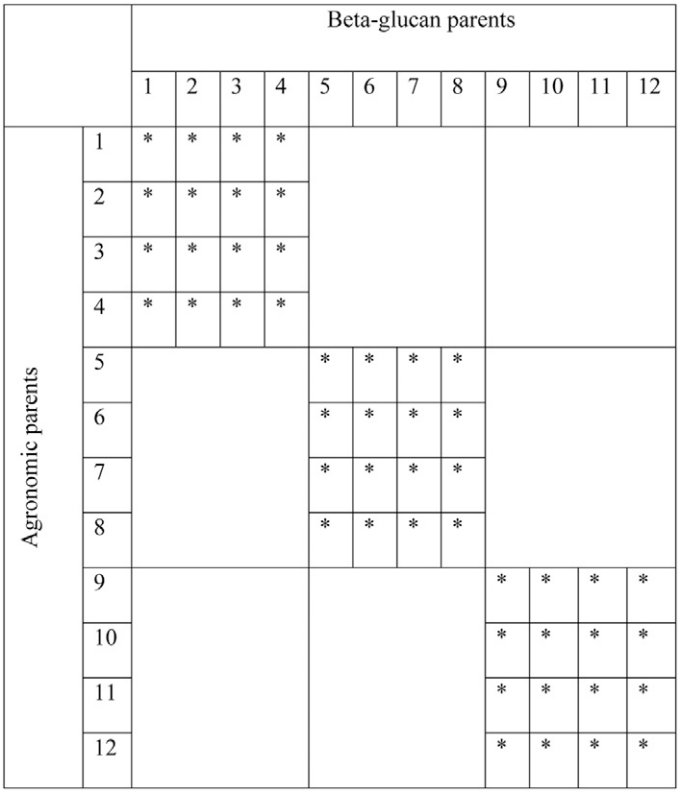

Figure 2. Crossing scheme between 12 parents with high β-glucan content and 12 parents with high agronomic performance. North Carolina Design II.

Viscosity deviations and their variance components were estimated by adjusting viscosity for β-glucan content using the following models.

$$P_{ijkm} = Blk_i + \delta^{*}\beta_{ijkm} + Pop_j + Parent_{k(j)} + Error_m \quad [3]$$

$$L_{iabcd} = Blk_i + \delta^{*}\beta_{iabcd} + \lambda + Agr_a + Bet_b + Agr^{*}Bet_{ab} + Line_{c(ab)} + Error_d \quad [4]$$

where β_{ijkm} and β_{iabcd} are the β-glucan contents for the specific parental and line experimental units, and δ is the regression coefficient of log(viscosity) on β-glucan content that was assumed the same for inbred parents and progeny lines. The prior for δ was $\delta \sim N(0, 10^6)$.

Bayesian analyses provide posterior distributions of parameters which can be used to evaluate probabilities of interest, such as $P(\lambda < 0)$. Because the posterior probabilities of variances are constrained to being greater than zero, however, they are not

well suited to evaluating the "significance" of these variances [indeed $P(\sigma^2 > 0) = 1$]. To decide whether a variance should be accounted for and is biologically relevant, a 95% highest posterior density interval can be constructed, and evaluated for its distance from zero (Gelman, 2004). Here, we used a simple approach that could easily be implemented with standard spreadsheet software to decide whether a variance's highest posterior variance interval contained zero. The variance's posterior median, *m*, was calculated and the posterior mass between zero and 1/5*m* determined. If that mass was less than half of the posterior mass in any 1/5*m* slice of the posterior distribution, the variance was declared positive (its highest posterior variance interval did not contain zero).

Relationship between Observable Variance Components and Causal Variance Components

Because all lines evaluated were inbred at least to the F_4 generation, we disregard here the possibility of dominance variance and assume traits depended only on additive and additive × additive gene action. There are consequently six relevant causal variances, two additive variances and four additive × additive variances. The additive variances are the variance among effects of alleles derived from the agronomic population (denoted V_A^A) and the variance among effects of alleles derived from the β-glucan population (denoted V_A^B). The additive × additive variances are the variances among effects of two-locus gametes wherein (i) alleles at both loci derive from the agronomic population (denoted V_I^{AA}), (ii) the first locus allele derives from the agronomic population and the second locus allele derives from the β-glucan population (denoted V_I^{AB}), (iii) the first locus allele derives from the β-glucan population and the second locus allele derives from the agronomic population (denoted V_I^{BA}), and finally, (iv) alleles at both loci derive from the β-glucan population (denoted V_I^{BB}). In addition to variances, the expected effects of alleles from the two populations are relevant. The additive effects of alleles from the agronomic and β-glucan populations are, respectively, α^A and α^B. By convention, their expectations are $E(\alpha^A) = -E(\alpha^B) = \alpha$. The additive × additive interaction effects of alleles are similarly denoted ε^{AA}, ε^{AB}, ε^{BA}, and ε^{BB}, and by convention their expectations are $E(\varepsilon^{AA}) = E(\varepsilon^{BB}) = -E(\varepsilon^{AB}) = -E(\varepsilon^{BA}) = \varepsilon$.

The variance among families can be deduced by considering the covariance between progeny lines within a family. The coefficient of coancestry between two lines within a family is 1/2 . If the sampled alleles of the two lines are identical by descent (IBD), there is a probability of 1/2 that the alleles derived from the agronomic parent and a probability of ½ that the alleles derived from the β-glucan parent. The covariance among lines within a family due to additive effects is consequently $1/2(V_A^A + V_A^B)$. For additive by additive epistasis, the probability of IBD at two independent loci is ¼. If sampled gametes are IBD at both loci, there are equal probabilities that the gametes carry two agronomic alleles, an agronomic and a β-glucan allele, a β-glucan and an agronomic allele, or two β-glucan alleles. The covariance among lines within a family due to additive × additive effects is consequently $1/4(V_I^{AA} + V_I^{AB} + V_I^{BA} + V_I^{BB})$. To deduce the general combining ability (GCA) of inbred parents, consider the covariance between progeny that share that parent. Their coefficient of coancestry is 1/4, so that their covariance due to additive effects is $1/2V_A^A$ if the shared parent is agronomic and $1/2V_A^B$ if the shared parent is β-glucan. Similarly, their covariance due to additive × additive effects is $1/4V_I^{AA}$ if the shared parent is agronomic and $1/4V_I^{BB}$ if the shared parent is β-glucan. Subtracting the GCA components from the overall among-family variance gives the specific combining ability (SCA) of $1/4(V_I^{AB} + V_I^{BA})$.

The among line within family variance can be obtained by deducing the overall among-line variance and subtracting the among family variance. Denoting the effect of a line by *L* and its genotype by G_L, the overall line variance can be deduced by

$$\mathrm{var}(L) = \mathrm{var}[E(L|G_L)] + E[\mathrm{var}(L|G_L)] \quad [5]$$

where $E(X|Y)$ is the expectation of *X* conditional on *Y*. Considering single-locus additive effects, *L* can be inbred with probability F_L, in which case it carries either two agronomic (G_L = AA) or two β-glucan alleles (G_L = BB), or L can be heterozygous (G_L = AB) with probability $(1- F_L)$. The first term on the right hand side of Eq. [5] requires calculating

$$\mathrm{var}[E(L|G_L)] = E\{[E(L|G_L)]^2\} - \{E[E(L|G_L)]\}^2 \quad [6]$$

The expectations for *L* conditional on the three possible genotypes are $E(L|G_L = AA) = 2E(\alpha^A) = 2\alpha$; $E(L|G_L = BB) = 2E(\alpha^B) = -2\alpha$; and $E(L|G_L = AB) = E(\alpha^A) + E(\alpha^B) = 0$. Consequently, the first term on the right hand side of Eq. [6] is $E\{[E(L|G_L)]^2\} = 1/2F_L(2\alpha)^2 + 1/2F_L(-2\alpha)^2 = 4F_L\alpha^2$, and the second term is zero. The variances for *L* conditional on the three possible genotypes are $\mathrm{var}(L|G_L = AA) = \mathrm{var}(2\alpha^A)$; $\mathrm{var}(L|G_L = BB) = \mathrm{var}(2\alpha^B)$; and $\mathrm{var}(L|G_L = AB) = \mathrm{var}(\alpha^A + \alpha^B)$. Therefore $E[\mathrm{var}(L|G_L)] = 1/2F_L\mathrm{var}(2\alpha^A) + 1/2F_L\mathrm{var}(2\alpha^A) + (1- F_L)\mathrm{var}(\alpha^A + \alpha^B) = F_L(V_A^A + V_A^B) + (1- F_L)1/2(V_A^A + V_A^B)$. Contributions of additive effects to the variance of L are, therefore,

$$\mathrm{var}(L)_A = 4F_L\alpha^2 + F_L(V_A^A + V_A^B) + 1/2(1- F_L)(V_A^A + V_A^B) = 4F_L\alpha^2 + 1/2(1 + F_L)(V_A^A + V_A^B) \quad [7]$$

From that we must subtract the variance among families shown above to be $1/2(V_A^A + V_A^B)$, and the variance among lines within families due to additive effects is $4F_L\alpha^2 + 1/2F_L(V_A^A + V_A^B)$. A similar development for variances due to additive × additive effects shows that

$$\mathrm{var}(L)_I = 16(F_L)^2\varepsilon^2 + [1/2(1 + F_L)]^2(V_I^{AA} + V_I^{AB} + V_I^{BA} + V_I^{BB}) \quad [8]$$

From that we must subtract $1/4(V_I^{AA} + V_I^{AB} + V_I^{BA} + V_I^{BB})$, and the variance among lines within families due to additive × additive effects is $16(F_L)^2\varepsilon^2 + 1/4[F_L(2 + F_L)](V_I^{AA} + V_I^{AB} + V_I^{BA} + V_I^{BB})$.

The α^2 and ε^2 coefficients that enter into σ^2_L also appear in two other estimable parameters. In particular, given the definitions above, the expected value for an agronomic parent is $E(\mathrm{Pop}_{j = \mathrm{agronomic}}) = P^A = 2\alpha + 4\varepsilon$ and $E(\mathrm{Pop}_{j = \beta\text{-glucan}}) = P^B = -2\alpha + 4\varepsilon$, such that $[E(P^A - P^B)]^2 = 16\alpha^2$. Similarly, the expected value of the parental inbreds is $E(P_{ijkm}) = P = 0\alpha + 4\varepsilon$, while the expected value of the progeny $E(L_{iabcd}) = \lambda = 0\alpha + 0\varepsilon$, and $\lambda = -4\varepsilon$ or $\lambda^2 = 16\varepsilon^2$. Finally, because the inbred parent effects were random, and there were only 12 parents of each population, a small fraction of the variances among parents end up in these squared differences. A complete summary of the relationship

between causal and observable variance components is given in Table 2. Note that both the α and ε components are composite effects (Lynch and Walsh, 1998, Chap. 9). The underlying parts of a composite effect may be nonzero but cancel each other out leaving the composite effect itself close to zero.

RESULTS AND DISCUSSION

As expected, for all traits agronomic parents were significantly different from β-glucan parents (Table 1). Parental lines selected for high β-glucan content were on average 56% higher in β-glucan than the agronomic lines. Conversely, the agronomic lines yielded on average 48% more than the β-glucan lines (Table 1). Given that all progeny lines tested in this experiment derived from high β-glucan by agronomic crosses we expected evidence of linkage disequilibrium between alleles that increase β-glucan content and alleles that decrease yield. Such disequilibrium would express itself as a negative correlation between the two traits. While we did not formally evaluate the genetic correlation between β-glucan and yield, we observed that lines with the highest β-glucan content tended to show low rank for biomass and grain yield (Table 3). Similarly, lines with the lowest β-glucan content tended to show high rank for the biomass and grain yield (Table 3). Previous studies have not given a consistent picture of the correlation between β-glucan content and yield. A positive correlation between grain yield and β-glucan was observed in the generation means analysis of Holthaus et al. (1996). Those results were supported by research conducted in Finland (Saastamoinen et al., 1992), where a positive correlation between β-glucan content and grain yield among variety trials at eight locations for 2 yr was found. On the other hand, Brunner and Freed (1994) reported that correlations between groat β-glucan content and grain yield were mostly small or not significant. Cervantes-Martinez et al. (2002) reported that one cycle of selection for greater β-glucan content did not change the mean of agronomic traits under study. However, the second cycle of selection resulted in significant decreases in mean grain yield. Peterson et al. (1995) reported nonexistent or inconsistent correlations between β-glucan content and agronomic traits across years or nurseries. His conclusion was that β-glucan was not consistently correlated with any agronomic traits, and therefore there was little chance of undesirable shifts in other traits that would hinder the breeding process. Our study does not contradict these previous studies because the nature of our study material, consisting of lines derived from divergent parents, was different from the nature of the material in these previous studies.

The progeny lines studied displayed a large range of β-glucan content from 37.1 to 73.5 g kg^{-1} (Table 3). This range is comparable to that of previous studies values between 30 and 80 g kg^{-1} (Aman and Graham, 1987) and between 22 and 62 g kg^{-1} (Beer et al., 1997) have been reported.

In our study, the relationship between flour β-glucan content and flour slurry viscosity was best linearized by taking the logarithm of slurry viscosity (Fig. 3). Doehlert et al. (1997) found a linear relationship between β-glucan content and raw slurry viscosity. The β-glucan contents of the lines that they tested, however, only ranged from about 27 to 45 g kg^{-1}. The smaller range of β-glucan contents may not have allowed them to detect nonlinearity in the relationship. In support of a nonlinear relationship between β-glucan content and flour slurry viscosity, however, they found an exponential relationship between flour concentration in the slurry and slurry viscosity (Doehlert et al., 1997). In that experiment, a two and a half-fold range of flour concentration was used. From our data, the regression equation relating flour β-glucan content and flour slurry viscosity was log(RVA) = 2.91 + 0.035(β-glucan content), and gave a coefficient of determination r^2 = 0.38 (Fig. 3). Higher coefficients of determination have been obtained in previous studies (Doehlert et al., 1997; Colleoni-Sirghie et al., 2004). Our study, however, was based on many more lines measured using more rapid techniques, as would be needed in a breeding program. The lower coefficient of determination in our study was therefore likely due to greater experimental error in the measurements rather than due to a difference in the importance of β-glucan in determining slurry viscosity. Nevertheless, our results suggest that viscosity may be used as a crude estimate of β-glucan content for the purpose of selection. It is equally interesting to note that viscosity displayed more transgressive segregrants than did β-glucan (Fig. 3). In particular, only two progeny lines exhibited a β-glucan content outside of the range of the inbred parents whereas more than 12 lines exhibited viscosity outside the range of the inbred parents (Fig. 3). Two factors may have contributed to the transgressive segregation shown for viscosity. First, in neither the agronomic nor the β-glucan population was viscosity

Table 2. Causal components of the variances observed in the statistical model.

Observable	Causal							
	V_A^A	V_A^B	V_I^{AA}	V_I^{BB}	V_I^{AB}	V_I^{BA}	α^2	ε^2
$\sigma^2_{P\_A}$	2	0	4	0	0	0	0	0
$\sigma^2_{P\_B}$	0	2	0	4	0	0	0	0
$\sigma^2_{GCA\_A}$	1/2	0	1/4	0	0	0	0	0
$\sigma^2_{GCA\_B}$	0	1/2	0	1/4	0	0	0	0
σ^2_{SCA}	0	0	0	0	1/4	1/4	0	0
σ^2_L	$1/2F_L$	$1/2F_L$	$1/4F_L$ $(2 + F_L)$	$1/4F_L$ $(2 + F_L)$	$1/4F_L$ $(2 + F_L)$	$1/4F_L$ $(2 + F_L)$	$4F_L$	$16(F_L)^2$
$(P^A - P^B)^2$	1/6	1/6	1/3	1/3	0	0	16	0
$(P - \lambda)^2$	1/24	1/24	1/12	1/12	0	0	0	16

Table 3. β-glucan and agronomic traits and standard errors (in parentheses) for lines with the highest and lowest β-glucan content among 289 experimental oat lines.

Line	Location	Beta-glucan content	Rank	LogRVA†	Rank	Biomass	Rank	Grain yield	Rank
		g kg^{-1}		log(RVU)‡		g m^{-2}		g m^{-2}	
IA03146-6	Kanawha	73.5 (0.43)	1	6.18 (0.19)	1	1000 (162)	204	434 (79.9)	173
IA03146-4	Ames	73.1 (0.43)	2	6.10 (0.19)	2	1055 (155)	165	455 (81.7)	143
IA03146-7	Kanawha	69.2 (0.38)	3	5.30 (0.19)	27	922 (160)	247	385 (82.1)	231
IA03187-5	Kanawha	68.5 (0.45)	4	5.34 (0.19)	24	1169 (172)	75	482 (88.7)	95
IA03164-5	Kanawha	68.4 (0.44)	5	NA§	NA	736 (160)	285	273 (86.2)	284
IA03188-7	Kanawha	68.2 (0.44)	6	5.00 (0.23)	67	753 (173)	284	250 (85.1)	286
IA03187-6	Kanawha	68.1 (0.46)	7	4.89 (0.24)	89	1022 (169)	189	406 (91.3)	211
IA03202-2	Ames	40.4 (0.44)	283	4.31 (0.19)	251	938 (169)	240	348 (89.4)	254
IA03176-3	Ames	39.7 (0.40)	284	4.59 (0.16)	179	1113 (134)	120	451 (67.2)	150
IA03199-5	Kanawha	39.2 (0.45)	285	4.47 (0.20)	215	1462 (174)	7	696 (92.4)	6
IA03199-4	Ames	39.0 (0.46)	286	4.74 (0.20)	132	1560 (181)	1	737 (96.1)	1
IA03199-6	Kanawha	39.0 (0.46)	287	4.34 (0.22)	247	1373 (180)	11	644 (96.0)	7
IA03176-2	Ames	37.7 (0.38)	288	4.74 (0.16)	130	1154 (135)	85	484 (66.2)	90
IA03150-5	Kanawha	37.1 (0.38)	289	4.15 (0.16)	266	1283 (157)	22	553 (74.5)	34

†RVA, Rapid viscosity analyzer.

‡RVU, rapid viscosity units.

§Rapid viscosity analyzer (RVA) for this line was not measured due to lack of sufficient seed.

an object of selection. Thus, both favorable and unfavorable alleles for viscosity might have been segregating in both populations. If favorable or unfavorable alleles recombined into single progeny lines, those lines could then be transgressive for viscosity. Second, while β-glucan content showed no evidence for epistatic interaction among alleles derived from the different parental populations (Table 4, specific combining ability), significant variance for interaction effects was observed for viscosity. Epistatic interactions lead to reduced resemblance of progeny to their mid-parent values and thus increase the probabilities of transgressive segregation (Lynch and Walsh, 1998).

We also examined viscosity profiles of oat lines with high and low viscosity deviation (Fig. 4). Note that, in these figures, the behavior of line IA03146-6 is of some concern because, after a sharp increase, its viscosity begins to decline (Fig. 4a). In previous studies of the viscosity/β-glucan relationship, this type of decline has been attributed to the action of endo-glucanase enzymes (Colleoni-Sirghie et al., 2004). In these previous studies, however, viscosity was measured for much longer periods than in our study (e.g., 4 h for Colleoni-Sirghie et al. [2004], and three to 8 h in Doehlert et al. [1997] as compared to 10 min in our study). Furthermore, in those studies the effects of endo-glucanases was not observed before 20 to 30 min into the viscosity profile. Finally, in a preliminary experiment to determine conditions for viscosity measurement, we had observed that the addition of silver nitrate (which inhibits endo-glucanases, Colleoni-Sirghie et al,. 2004) had virtually no effect on viscosity measurements (Chernyshova, 2006). If endo-glucanases had had an important impact on viscosity deviation, one would predict that lines with negative deviations would initially show increases in viscosity (as a result of high β-glucan content) and then declines (due to endo-

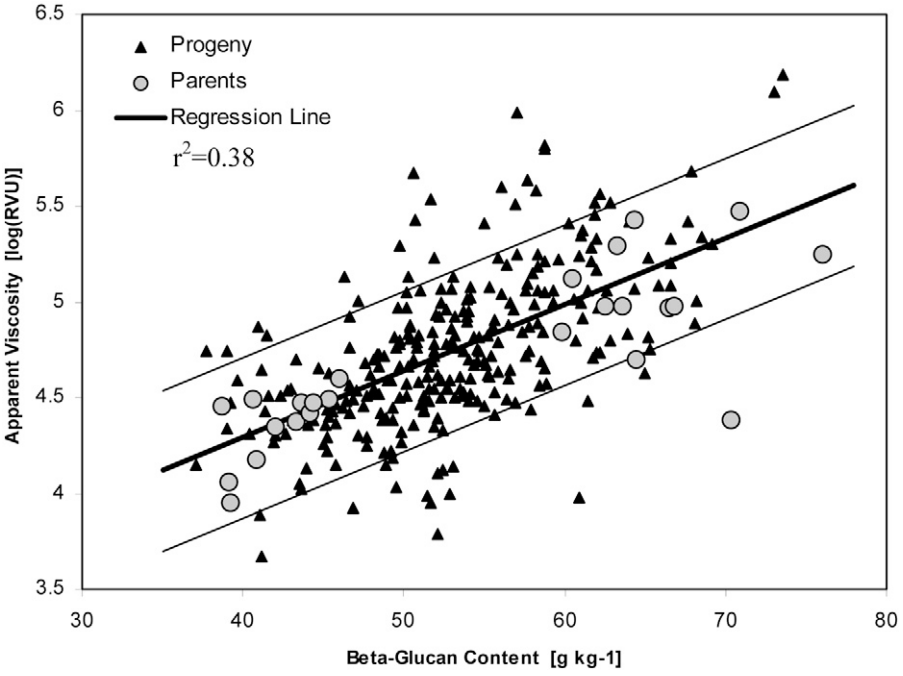

Figure 3. Correlation between the log-transformed apparent viscosity and β-glucan content. The bold line shows the regression of log viscosity on β-glucan content. Lighter lines are parallel and two standard errors from the regression line.

glucanases) before the 5 to 7 min interval during which viscosity was evaluated. This pattern was not observed in lines with negative viscosity deviation, which simply registered very little increase in viscosity over the profile (Fig. 4b). This observation suggests that endo-glucanases did not play an important role in determining viscosity deviation. Nevertheless, endo-glucanases could be a confounding factor in the measurement of the viscosity deviation, and we would recommend the inactivation of these enzymes before measuring viscosity.

To our knowledge, the viscosity deviation and its application to improve oat functional properties have not been studied. Viscosity deviation may be useful to identify oat lines with valuable β-glucan structure/function properties that could be increased through breeding. Clearly β-glucan content strongly affects the viscosity differences between agronomic vs. β-glucan parental lines (Table 1). To determine whether the agronomic lines have something to contribute to the improvement of viscosity, it is necessary to control for differences in β-glucan content. The difference in viscosity on a log scale between agronomic and β-glucan populations was 0.68, with a posterior probability of being zero or lower $P(\log RVA^B < \log RVA^A \mid \text{data}) < 0.001$. Once viscosity is adjusted for β-glucan content, however, the difference in viscosity between agronomic and β-glucan populations dropped to 0.15 with $P(\log RVA^B < \log RVA^A \mid \text{data}) > 0.1$. Similarly, loci segregating for alleles from either the β-glucan or agronomic populations generate an important component of the variance among progeny within families, and this variance is significant for log(RVA) (Table 4). This variance, however, loses significance for the viscosity deviation because, while alleles from the β-glucan population increase β-glucan content, and thus viscosity, they apparently do not specifically affect viscosity through other paths than β-glucan content. Nevertheless, there was significant genetic variation for viscosity deviation, as shown by the fact that the variance among families was of a similar order of magnitude as the variance for viscosity itself [Table 4, var(*Fam*)].

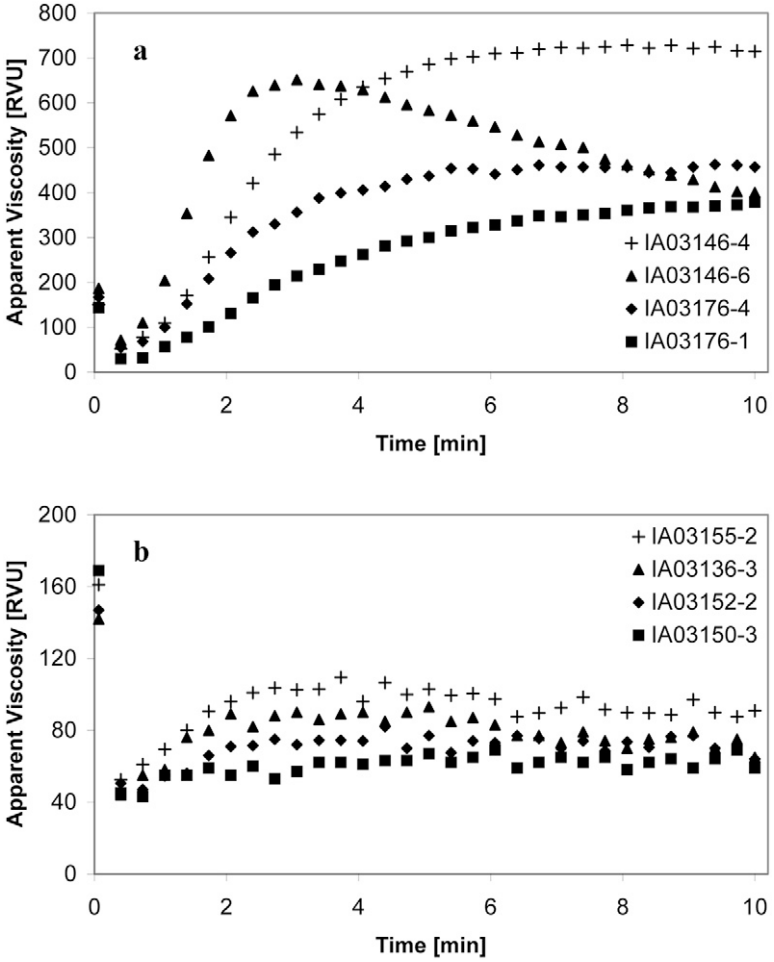

Figure 4. Viscosity profiles of (a) four oat lines with the highest viscosity deviations, and (b) four oat lines with the lowest viscosity deviations. Experimental line designations are given in the legends.

For the traits β-glucan content, biomass and grain yield, there was a difference between agronomic and β-glucan populations in terms of the correlation between the performance of the parents and their breeding values, as measured by the mean values of the parent's progeny (Table 5). Considering β-glucan content, the correlation was strong for the agronomic population (r = 0.75), but there was almost no correlation for the β-glucan population (r = 0.03). For biomass and grain yield, there was a slight negative correlation for the agronomic population (r = −0.08 for both traits), whereas for the β-glucan parent there was strong positive correlation (r = 0.90 and r = 0.87 for biomass and yield, respectively). For log(RVA), heading date and height the correlation was positive in all cases and not significantly different between agronomic and β-glucan populations (Table 5). The pattern that revealed itself in these correlations is that the correlation was essentially zero for a trait/population combination where the trait had been specifically selected in that population. Since the correlations are a function of the additive genetic variances for traits, the low correlations for selected traits suggest that selection has depleted much of that variance.

Table 4 shows genetic variances for the parental inbreds themselves, for the means of their crosses, and for the values of progeny within crosses. The general combining ability variances for the agronomic and β-glucan parents, $\sigma^2_{GCA\_A}$ and $\sigma^2_{GCA\_B}$ are the variances among half-sib families of agronomic and β-glucan parents, while var(*Fam*) is the variance among crosses between agronomic and β-glucan parents, $\text{var}(Fam) = \sigma^2_{GCA\_A} + \sigma^2_{GCA\_B} + \sigma^2_{SCA}$. For β-glucan content all three combining ability variances $\sigma^2_{GCA\_A}$, $\sigma^2_{GCA\_B}$, and σ^2_{SCA} were not clearly positive individually, although their sum var(*Fam*) was obvious (Table 4). Plot basis heritabilities can be calculated from the data in Table 4 as

Table 4. Genetic variances among inbred parents of agronomic and β-glucan populations and among their recombinant progeny. Abbreviations σ^2PAR_A and σ^2PAR_B refer to variances among inbred parents, σ^2GCA_A, σ^2GCA_B, and σ^2SCA refer to general and specific combining ability variances, var(*Fam*) is the sum of combining ability variances, var(*L*) is the variance among lines within families, and var(*Err*) is the error variance.

	$\sigma^2_{PAR\_A}$	$\sigma^2_{PAR\_B}$	$\sigma^2_{GCA\_A}$	$\sigma^2_{GCA\_B}$	σ^2_{SCA}	var(*Fam*)	var(*L*)	var(*Err*)
β-glucan conc. (g kg^{-1})	0.110†	0.339†	0.058NS‡	0.064NS	0.103NS	0.225†	0.485†	0.353†
Apparent visc. [log(RVU)]§	0.055†	0.132†	0.030NS	0.016NS	0.030†	0.075†	0.131†	0.044†
Viscosity Dev. [log(RVU)]	0.027†	0.094†	0.018†	0.015†	0.012NS	0.045†	0.016NS	0.084†
Biomass (g m^{-2})	15,600†	19,500†	11,900NS	9,000NS	35,500†	56,500†	19,500†	45,720†
Grain yield (g m^{-2})	3,800†	9,600†	2,400†	3900†	9,900†	17,000†	4,500†	10,676†
Heading date (dap)¶	3.38†	4.98†	0.90NS	0.13NS	0.12NS	1.16†	3.63†	1.66†
Height (cm)	42.8†	3.07NS	13.2†	10.1†	2.52NS	25.9†	1.45NS	32.1†

†Variance's highest posterior variance interval did not contain zero (see Materials and Methods).

‡NS, the interval did contain zero.

§RVU, rapid viscosity units.

¶dap, days after planting.

Table 5. Correlation between the values of the parents and the mean values of each parent based on the performance of its progeny.

Population	β-glucan concentration	Log (apparent viscosity)	Biomass	Grain yield	Heading date	Height	Viscosity deviation
	g kg^{-1}	log(RVU)†	—— g m^{-2} ——		dap‡	cm	log(RVU)
Agronomic	0.75*	0.82*	-0.08NS§	-0.08NS	0.44NS	0.75*	0.36NS
β-glucan	0.03NS	0.66*	0.90*	0.87*	0.54NS	0.75*	0.18NS

*Correlation significantly different from 0 ($P < 0.05$).

†RVU, rapid viscosity units.

‡dap, days after planting.

§NS, not significant.

$$H^2 = [\text{var}(Fam) + \text{var}(L)]/[\text{var}(Fam) + \text{var}(L) + \text{var}(Err)] \quad [9]$$

These heritabilities were high, ranging from 0.42 for the viscosity deviation to 0.82 for viscosity itself. The variance among progeny within families is a function of segregation of divergent alleles inherited from the agronomic and β-glucan parents. We may also exclude this variance because it is somewhat an artifact of our crossing design in that divergent populations will not usually be crossed in a breeding program. In that case, plot basis heritabilities are

$$H^2 = \text{var}(Fam)/[\text{var}(Fam) + \text{var}(Err)] \quad [10]$$

These heritabilities are still reasonable and range from 0.35 for the viscosity deviation to 0.63 for viscosity. Given adequate replication, it should therefore be feasible to select for any of the traits evaluated here.

Despite the power of the North Carolina II mating design to uncover interaction variances through the specific combining ability component, this component was too small to be observable for β-glucan content (Table 4). This result is in agreement with other studies that have found additive gene action affecting β-glucan content (Kibite and Edney, 1998; Holthaus et al., 1996). In contrast, observable interaction variance was identified for viscosity (Table 4). In general, higher specific combining ability variance suggests that for the improvement of a trait, more crosses should be attempted to identify those crosses that are more likely to generate transgressive segregants.

CONCLUSIONS

Progeny derived from crosses between agronomically elite parents and parents selected for high β-glucan content showed evidence for a negative correlation between β-glucan content and yield. This result indicates at least linkage between loci that affect β-glucan content and that affect yield, and it does not exclude the possibility of pleiotropic loci for which alleles that positively affect β-glucan content also negatively affect yield. Nevertheless, the negative correlation was low and should not impede progress in programs selecting for both β-glucan content and agronomic performance. The study found relatively high heritabilities for the traits β-glucan content, flour slurry viscosity, and viscosity deviation that are relevant for the selection of oat lines with high nutritional function. The study confirmed that the mode of gene action of loci affecting β-glucan was primarily additive, but found observable interallelic interaction variance for flour slurry viscosity. This interaction variance may further explain the transgressive segregation for flour slurry viscosity that was observed. Finally, the study showed for the first time that selection for viscosity deviation is feasible. While at this time we do not know the mechanisms generating viscosity deviations, the fact that the trait is selectable should allow us to divergently select for the trait and to study the differences among lines with high and low viscosity deviation.

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