In recent decades there has been considerable research to develop alternative methods for selection to accelerate the development of cultivars and improve breeding populations. Fundamental to those efforts is the quest for methods that can improve the response to selection or increase accuracy of estimates of breeding values (Falconer and Mackay, 1996). One of the earliest results of those efforts was the use of BLUP methodology for estimating breeding values with additional information from pedigree data or coancestry (Henderson, 1984). Earlier applications of BLUP in plant breeding have shown that it can result in higher percentages of superior crosses in soybean relative to traditional midparent value (Panter and Allen, 1995), and it can predict performance of untested single crosses of maize using relationship...
data from relatives (Bernardo, 1996). However, pedigree-based relationships do not account for random Mendelian segregation within families. This segregation is important because, when using inbred lines, as is common in plant breeding, a progeny will not necessarily receive an equal contribution from its two parents.

An alternative method is to use genome-wide association studies (GWAS) to identify markers associated with quantitative trait loci (QTL) and to incorporate those markers into prediction of breeding values as in the methods for MAS described by Lande and Thompson (1990). A GWAS-based MAS can reduce erroneous estimation of QTL effects caused by changes in genetic background, a problem that can arise in traditional MAS that is based on QTL estimated in biparental populations (Heffner et al., 2009). Those interactions can be avoided in GWAS-MAS through the use of a large panel of representative germplasm, such that discovered QTL are likely to have additive effects in diverse genetic backgrounds (Bernardo, 2008; Xu and Crouch, 2008; Heffner et al., 2011b). However, due to the fact that selection is based only on a subset of markers, GWAS-MAS may capture only a portion of the total genetic variation. Furthermore, QTL effects are often overestimated through bias introduced by choosing the largest observed effects (Beavis, 1994). Hence, GWAS-MAS can still result in an inaccurate estimate of the breeding values. An alternative to MAS methods is genomic selection (GS) or genome-wide selection, which predicts the breeding values of individuals using all available markers throughout a genome (Meuwissen et al., 2001). By using all available markers, selection is based on a larger proportion of the genetic variation that includes smaller QTL, resulting in more accurate estimates of breeding values (Goddard and Hayes, 2007). Furthermore, the use of all markers traces the Mendelian segregation for each QTL and prediction of breeding values within families is feasible (Daetwyler et al., 2007).

Comparative studies of GS, MAS, and phenotypic selection measured by response to selection \( R = i r \sigma_A \), where \( R \) is the response, \( i \) is the intensity, \( r \) is the accuracy of selection and \( \sigma_A \) is the additive genetic standard deviation; Falconer and Mackay, 1996) revealed that GS had a consistent advantage over the other methods. For example, in simulated breeding programs in maize, there was higher response to selection using GS than recurrent MAS (Bernardo and Yu, 2007; Mayor and Bernardo, 2009). In empirical cross-validation studies, GS showed greater accuracy than conventional MAS conducted among segregating progeny of biparental crosses, and was superior to GWAS-MAS in a multifamily wheat population (Lorenzana and Bernardo, 2009; Heffner et al., 2011a, 2011b). In comparisons of GS and phenotypic selection using pedigree information, the accuracy of GS was also higher (Nielsen et al., 2009; Crossa et al., 2010). In contrast, the accuracy of GS was no better than that of phenotypic selection in empirical studies of multifamily wheat populations (Heffner et al., 2011b). These studies support the idea that the primary advantage of GS would probably come from the cumulative response generated by several selection cycles per year (Jannink, 2010; Mayor and Bernardo, 2009).

However, there are few studies about the impact of these selection methods on maintaining genetic variation (Daetwyler et al., 2007). Change in genetic variance that is attributed to selection could either be caused by factors such as changes in allele frequencies, level of inbreeding or coancestry, or negative linkage disequilibrium (LD), i.e., the Bulmer effect (Robertson, 1960; Hill and Robertson, 1966; Bulmer, 1971; Sorensen and Kennedy, 1984; Falconer and Mackay, 1996). In a simulation study by Bastiaansen et al. (2012), the genetic variance for a polygenic trait decreased after short-term selection but had similar magnitude for GS and BLUP phenotypic selection. In addition, both Bastiaansen et al. (2012) and Daetwyler et al. (2007) have shown that there was higher inbreeding of animals undergoing phenotypic BLUP selection than those animals under GS. These studies suggest that higher coancestry of selected lines can affect the genetic variance of succeeding cycles of selections.

Despite empirical cross-validation studies showing the advantages of molecular markers, there is still a need to test these results in actual breeding programs. Inferences from those comparisons are important because response to selection is dependent on the level of genetic variance for any trait (Falconer and Mackay, 1996). We conducted selection for \( \beta \)-glucan concentration in oat using BLUP (Henderson, 1984) phenotypic selection (BLUP-PS), GS, and MAS. \( \beta \)-glucan has been identified as the active component of soluble fiber in oat that lowers blood serum cholesterol levels—a major risk for heart disease (reviewed by Butt et al., 2008). Previous research on \( \beta \)-glucan concentration indicated that this trait is controlled by many genes acting in an additive manner (Holzhaus et al., 1996; Chernyshova et al., 2007; Cervantes-Martinez et al., 2001). Our objective was to compare marker-based and phenotypic selection strategies, and to develop empirical estimates of their relative efficacy before incorporation in larger breeding programs. We chose to compare BLUP-based phenotypic selection, a GWAS-MAS with re-estimation of marker effects between cycles, and GS. Our specific objectives were to (i) compare the short-term response and highest ranked progenies over two cycles of selection; (ii) examine changes in correlated response of heading date and plant height; and (iii) assess short-term changes in genetic variance for \( \beta \)-glucan concentration for the three selection methods.

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**MATERIALS AND METHODS**

**Marker Data for Cycles 0, 1, and 2**

Oat lines and their progenies were planted at the Iowa State University Agronomy greenhouse in January 2008 (Cycle 0), January 2010 (Cycle 1), and January 2011 (Cycle 2). Leaf samples were collected from the single plant of each line and DNA was extracted from Cycle 0 and 1 according to recommended diversity arrays technology (DArT) protocol (Diversity Arrays Technology, Yarralumla, Australia). For Cycle 2, DNA samples were extracted using a kit (QIAGEN, Valencia, CA). In every cycle, DNA was assayed for DArT polymorphisms by a service provider (Diversity Arrays Technology, Yarralumla, Australia) based on the 2500-clone array available at the time and protocols described by Tinker et al. (2009). Redundant markers were removed as described in Supplementary Method 1, leading to 866 polymorphic markers in Cycle 0 and 675 polymorphic markers in Cycle 1.

**Phenotypic Data of Base Population (Cycle 0)**

The Cycle 0 population was composed of 446 lines from various oat breeding programs in North America. These lines were tested in the Uniform Oat Performance (UOPN) and the Quaker Uniform Oat Nurseries (QUON) for agronomic traits and biochemical characters including β-glucan concentration from 1994 to 2007. Most β-glucan data (97%) in this study were from those nurseries and have been deposited in the Graingenes 2.0 database (Carollo et al., 2005). Minor sources of β-glucan data were included from Chernyshova et al. (2007), Colleoni-Sirghie et al. (2004), the Germplasm Resources Information Network (http://www.ars-grin.gov/npgs/acc/acc_queries.html verified 16 Nov. 2011), and the North Dakota State University Oat experiments (M. McMullen, personal communication, 2007).

**Genomic Selection of 12 Parents for Cycle 1**

The genotype matrix (M) in Cycle 0 was used to derive a marker-based relationship matrix equal to \( K = MM^t \sum \hat{p}_i (1 - \hat{p}_i) \) where \( \hat{p}_i \) is the frequency of allele 1 in marker \( k \) computed using the Spageti program (Hardy and Vekemans, 2002). Because there were negative relationship values, the resulting matrix was then scaled between 0 and 1. The same relationship matrix was also used to calculate principal components using SAS PROC Princomp (SAS Institute, 2008). Only the eigenvectors of the first three PC axes (denoted as P) were used in the association analysis because subsequent axes accounted for only a small proportion of the variation based on a scree plot (results not shown).

To compute the genomic estimated breeding values (GEBV), a mixed model methodology was implemented in PROC MIXED using the following model:

\[
 y = Xb + E + Zu + e
\]

where \( y \) is a vector of β-glucan values for each line described above, \( b \) is the mean, \( j \) is a vector of random environmental effects, \( u \) is a vector of random polygenic effects, and \( e \) is a vector of residual errors. Observations from four long-term checks were also used to provide overlap across environments. The X, E, and Z terms are the incidence matrices relating \( y \) to \( b, j \) and \( u \), respectively. The variance of \( u = KV_A \), where K is the marker-based relationship matrix and \( V_A \) is the additive variance due to polygenic effects, was derived using the REML option in PROC MIXED.

Cycle 0 lines were sorted based on GEBV from the mixed model and the highest 40 lines were selected. The marker-based relationship matrix of those 40 lines was then subjected to cluster analysis using Ward’s linkage with 12 clusters in SAS PROC CLUSTER (SAS Institute, 2008). The line with the highest β-glucan concentration per cluster was selected for use in the final set, thus 12 parents were selected by this method. This approach was adopted to avoid excessive loss of diversity by coselection of close relatives.

**MAS of 12 Parents for Cycle 1**

To implement MAS, significant markers were first identified through association mapping. A two-stage association mapping was conducted because it was less computationally demanding and has produced results similar to a one-stage analysis (Stitch et al., 2008). First, a similar analysis to genomic selection described above was conducted except that the 450 lines (446 lines plus four checks) were assumed to be independently and identically distributed (i.e., no relationship matrix among lines was included in the model). The solution for random effects of the 446 lines plus the grand mean was treated as the new observation, \( y^* \), for association mapping (Zhang et al., 2009). Second, the association test for β-glucan concentration was conducted using the TASSEL software (Bradbury et al., 2007) with the following model:

\[
 y^* = Xb + Ma + Ps + Zu + e
\]

where \( b \) represents the mean, \( a \) is a vector of marker effects, \( s \) is a vector of population structure effects, \( u \) is a vector of random polygenic effects, and \( e \) is a vector of residual error. The \( X, M, \) and \( Z \) are incidence matrices relating \( y \) to \( b, a, \) and \( u \), respectively, while \( P \) is the matrix from principal component analysis (PCA) computed above relating \( s \) to \( y \). The variance of \( u = KV_A \), where K is the marker-based relationship matrix, and \( V_A \) is the additive variance due to polygenic effects. Using the \( P \) values for each marker, six markers potentially controlling β-glucan concentration were identified using a false discovery rate (FDR) of 0.33 (Benjamini and Hochberg, 1995).

To estimate genetic effects, the six markers were included in a model analyzed jointly using PROC MIXED in SAS, with the population structure and polygenic effects described above and response variable replaced by the original set of observations. The resulting marker and population structure effects plus the phenotypic values were then used to calculate an index (Lande and Thompson, 1990):

\[
 Index \ value = Ma + Ps + Phenotypic \ values,
\]

where M is the genotype data matrix for the six markers and \( a \) is their corresponding estimated marker effect, \( P \) is the principal component eigenvectors matrix and \( s \) consisted of the corresponding population structure effects. The phenotypic values were the \( y^* \) values used in the association analysis model. The index values were then used to rank the 446 lines. A
marker-based relationship matrix of the top 40 lines was then subjected to cluster analysis, and a set of 12 Cycle 1 parents was chosen using the approach described for genomic selection.

**BLUP Phenotypic Selection of 12 Parents for Cycle 1**

The pedigree of each line was obtained from breeders, and validated with the online Pedigree of Oat Lines (POOL) database (Tinker and Deyl, 2005). Pedigree depth was expanded based on POOL and/or POOL was updated to reflect new data from lines included in this work. Next, the pedigree data of 450 lines were analyzed using an updated version of KIN software (Tinker and Mather, 1993; available by request from NT) to derive the coancestry matrix. The coancestry matrix ranged from 0 (unrelated) to 1 (identical by descent). A mixed model in SAS was used to determine the pedigree-based BLUP values of lines (Henderson, 1984). The model in this analysis was similar to genomic selection methodology except that the covariance matrix among lines was defined by the pedigree-based coancestry. The BLUP values were also sorted and the highest 40 lines were selected. The coancestry matrix of those 40 lines was subjected to cluster analysis using PROC CLUSTER in SAS (SAS Institute, 2008) with Ward’s linkage and 12 clusters as options. The line with the highest β-glucan per cluster was selected to identify 12 parents for Cycle 1.

**Recombination Scheme for Cycle 1 of each Selection Method**

The 12 parents for Cycle 1 of each selection method were planted in December 2008 in the greenhouse. Two replicates of a partial diallel (Kempthorne and Curnow, 1961) were made for each selection method. In each replicate each parent was crossed to four other parents without reciprocals to generate 24 F₁ seeds. In this cycle, the two replicates of partial diallel per selection method served as two populations representing each method. In Cycle 2, the two populations per selection method served also as replications of each selection method. For brevity, the two populations per selection method were denoted GR1 or GR2 for first and second population of GS, MR1 or MR2 for MAS, and PR1 or PR2 for BLUP phenotypic selection (Fig. 1). The resulting F₂ seeds from 24 crosses per population were planted in September 2009 in the greenhouse. Two random F₂ seeds from each F₁ plant were grown from January to April 2010. Simultaneously, each F₂ plant from the populations undergoing MAS and GS methods was genotyped using the protocol described above. Seeds from self-pollination of each plant (F₂:3 progenies) were harvested, and evaluated in field studies in the summer of 2010 (Supplementary Method 2).

**GS, MAS and PS for 12 Parents of Cycle 2**

The entry effect for each line was computed from the phenotypes described above. For GS, the training population consisted of both Cycle 0 and Cycle 1 data, with marker effects estimated using RR-BLUP (Meuwissen et al., 2001; Lorenz et al., 2011). The genomic estimated breeding value for each individual in Cycle 1 (GR1 and GR2) was estimated as the sum of effects from all markers.

For MAS, estimates of six marker effects were computed in a mixed model using the F₂:3 phenotypes as the response variable, marker identities as fixed effects, and the covariance matrix of F₂:3 lines defined by the pedigree-based coancestry. Each marker effect was multiplied by the corresponding marker allele states and summed across markers to compute the total marker scores (Lande and Thompson, 1990). An index containing both phenotypic and marker scores of F₂:3 lines was developed, where the former had weight of 1.00 and the latter had a weight of 1.35 as described by Lande and Thompson (1990). The weight of 1.35 for marker score was derived using the formula \( b = \left( \frac{1}{h^2} - 1 \right) / \left( 1 - p \right) \), where the estimated \( h^2 \) for β-glucan was equal to 0.44 and \( p \) was the proportion of genetic variance explained by the markers which was 0.06 based on the original association test. The resulting estimates of index of breeding values were then ranked.
For each BLUP phenotypic selection population (PR1 and PR2), the F$_{1:3}$ line effects were refitted as response variables in a mixed model where the pedigree-based coancestry of lines was used as a covariance matrix among lines. The resulting estimates of breeding values were ranked.

Because there were only 34 to 45 lines from the GS and MS populations with high quality marker data, a random selection of 36 lines was taken for each population, including the PS populations. Estimated breeding values of the 36 lines were ranked within each population and the top 12 parents were determined as parents for Cycle 2. Finally, the 12 parents of each population were planted in the greenhouse in September 2010. A recombination scheme similar to Cycle 1 was conducted and two seeds from each cross were selected randomly at maturity to form 48 S$_0$ lines for each population (we use the S$_0$ notation here because these lines are progeny of outcrossed individuals rather than selfs from a heterozygote; Fig. 1). S$_0$ seeds from each population of Cycle 1 were planted in the greenhouse in January 2011 for advancement from S$_0$ to S$_1$ (see Supplementary Method 3 for complete details).

Field Plot Design

For each year, entries were evaluated in an incomplete block design with two replications, where blocks were nested within replications (details of entries for 2010 are shown in Supplementary Method 2). Specifically, in 2011 the entries were comprised of a random sample of 24 lines from each of the six populations of Cycle 1 (total of 144), 48 random lines from Cycle 0, the 20 unique parents of Cycle 1 and four popular oat cultivars (total of 24), 288 S$_{0:1}$ lines from Cycle 2, the 72 parents of Cycle 2, and five checks (IAN9N79–5–1–22, Baker, IA002130–2–2, Excel, and CDC Pro-Fi). For each incomplete block, the entries consisted of a random sample of six lines from Cycle 0, three lines from each population of Cycle 1 (total of 18), three lines from the parents of Cycle 1, six lines from each population of Cycle 2 (total of 36), nine lines from the parents of Cycle 2, and all of the five checks. Entries were hand-planted in hillplots with 20 seeds per plot. Hillplots were 0.30 m apart from each other and the whole experiment was surrounded with two rows of borders. Each incomplete block was composed of a 7 by 11 grid of hillplots. The experiment was grown at the Iowa State University Agronomy and Agricultural Engineering Field Research Center near Ames, IA from April to July 2011. Each hillplot was harvested by hand and threshed after 1 wk of air-drying. The same set of data as Cycle 1 was gathered in this field evaluation.

Data Analysis for Comparing Selection Methods

The following model was used to fit the combined data from 2010 and 2011 field evaluations using PROC Mixed in SAS:

$$y = Xb + Zu + error$$

where $y$ was the data collected ($\beta$-glucan concentration, heading date, and plant height), $X$ is the design matrix for the following fixed terms: grand mean + year + replication (year) + incomplete block (replication*year) + population while $b$ is the vector of corresponding effects. $Z$ is the incidence matrix for the entries while $u$ is the entry effect. The variance of $u$ was computed as $\sigma_u^2$, where $I$ is the identity matrix and $\sigma_g^2$ is the genetic variance estimated from the data. Out of a total of 2000 plots over 2 yr, 29 had missing data due to nongermination or insufficient numbers of seeds for planting. The population term was composed of the combinations of the population and cycle (e.g., GR1-Cycle 1, GR1-Cycle 2), parental lines for each population, checks, and the random Cycle 0 lines. These analyses were conducted to estimate adjusted means for each population of entries. Two models were compared using a goodness of fit test, the first assumed homogeneous variance and the second assumed heterogeneous variance among populations (e.g., GR1-Cycle 1, GR1-Cycle 2), checks and Cycle 0. Because the goodness of fit test showed that the model with heterogeneous variance performed better, the solution for fixed and random effects from the heterogeneous variance model was used in subsequent analyses. The estimated genotypic value (EGV) of each oat line was computed as the combination of fixed and random effects:

$$EGV = \text{grand mean} + \text{population effect} + \text{oat entry effect}.$$

Significance Test for Differences among Genetic Variances

Significance of the difference of genetic variances was determined by a likelihood ratio test, which assumes that the difference between the $-2$ REML log-likelihood of the full model and the reduced model has a chi-square distribution (Saxton, 2004). Then the $P$-value associated with the chi-square distribution was reported, with degrees of freedom defined by the difference in parameters of models under comparison. To systematically compare the genetic variances, indicator variables were developed for different groups of entries, including different populations. For example, to test the hypothesis that variance of the GS method is the same as the variance of the PS method under Cycle 2, an analysis was first conducted assuming heterogeneous variance for groups of entries: Cycle 0, GS Cycle 1, MS Cycle 1, PS Cycle 1, GS Cycle 2, MS Cycle 2, PS Cycle 2, and checks. A subsequent analysis was conducted where GS Cycle 2 and PS Cycle 2 were in the same group. The difference in $-2$ REML Log Likelihood values for the two analyses was calculated and the $P$-value associated was taken with one degree of freedom because there were eight parameters in the first test and seven in the second test.

Coancestry in Cycle 1 and Cycle 2 Progenies

Because of incomplete pedigree information among lines in Cycle 0, the marker-based relationship among lines in Cycle 0 was used as the reference for assessing coancestry of succeeding cycles of progenies. Subsequently, pedigree records of Cycle 1 and Cycle 2 were used to derive the coancestry of the progenies using the tabular method (Bernardo, 2010). The average coancestry among progenies within population of each cycle was estimated as the average among all pairs of lines, where each pairwise value is equivalent to an inbreeding coefficient of hypothetical progenies derived from that pair. Then, simulations were performed to determine what the expected increase in coancestry would be between Cycle 1 and Cycle 2 under...
random mating of every population. In these simulations, the coancestry of progenies in Cycle 1 was based on the previously estimated pedigree relationships of oat lines. Then, 12 parents at random were selected and crossed in diallel method to produce Cycle 2 progenies and the average kinship was taken. The process was repeated 10,000 times and the probability that kinship value of real selection happened in random was computed.

RESULTS
Marker-Trait Associations
The association analysis conducted in Cycle 0 demonstrated that the individual phenotypic variance explained by each of the six significant markers was close to 1% (details not shown). The estimated QTL effects ranged from $-0.39$ to $0.44$ for Cycle 0, $-0.14$ to $0.24$ for Cycle 1, and $-0.39$ to $0.59$ for Cycle 2 (Table 1). The favorable allele frequencies of the six markers ranged from $0.02$ to $0.93$ with an average of $0.27$ for Cycle 0, $0.21$ to $1.00$ with an average of $0.56$ for Cycle 1, and $0.34$ to $0.96$ with an average of $0.58$ for Cycle 2.

Means of Populations for $\beta$-glucan Concentration
The grand mean of $\beta$-glucan concentration across all years and populations was $4.83$ g/100 g. The mean of Cycle 0 was $4.57$ g/100 g, which was significantly lower than the means of Cycles 1 and 2 (Table 2). The means among populations in Cycle 1 were not significantly different, and ranged from $5.89$ to $6.01$ g/100 g. These means were greater than Cycle 0 by $1.32$ to $1.44$ g/100 g (Supplementary Table 1). In addition, the mean of the pooled set of 20 parents of Cycle 1 ($5.72$ g/100 g) was not significantly different from the means of their progenies (i.e., all populations in Cycle 1).

The means of populations in Cycle 2 ranged from $6.66$ to $6.88$ g/100 g and were significantly higher than their respective population means in Cycle 1 (Table 2, Fig. 2). These values were greater than their respective Cycle 1 means by $0.72$ to $0.93$ g/100 g (Supplementary Table 1). Overall, the corresponding cumulative increase in $\beta$-glucan concentration means ranged from $2.09$ to $2.31$ g/100 g after two cycles of selection.

The individual population means in Cycle 2 were not significantly different from each other. However, the $\beta$-glucan concentration averaged over all marker-aided selection populations (i.e., GR1, GR2, MR1, and MR2) was higher than that of the populations subjected only to phenotypic selection ($P < 0.08$; Table 2). The mean of parents for each population in Cycle 2 was not significantly different from their respective progenies.

Table 1. Frequency of favorable alleles of selected markers for $\beta$-glucan concentration g/100g for two cycles of selection. Estimated genetic effects (g/100g) were computed from multiple regression models.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Cycle 0 Effect</th>
<th>Allele frequency</th>
<th>Cycle 1 Effect</th>
<th>Allele frequency</th>
<th>Cycle 2 Effect</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>oPt-11819</td>
<td>$-0.39$</td>
<td>$0.06$</td>
<td>$-0.05$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>oPt-14067</td>
<td>$0.42$</td>
<td>$0.08$</td>
<td>$0.24$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>oPt-18130</td>
<td>$-0.30$</td>
<td>$0.07$</td>
<td>$0.04$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>oPt-18282</td>
<td>$-0.23$</td>
<td>$0.45$</td>
<td>$0.13$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>oPt-8249</td>
<td>$0.44$</td>
<td>$0.02$</td>
<td>$-0.14$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>oPt-11728</td>
<td>$-0.32$</td>
<td>$0.93$</td>
<td>NA$^2$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>oPt-7232$^5$</td>
<td>$-0.11$</td>
<td>$0.58$</td>
<td>$-0.39$</td>
<td></td>
<td></td>
<td>$0.61$</td>
</tr>
</tbody>
</table>

$^1$All marker effects were significant ($P < 0.05$) in Cycle 0. The effect of oPt-14067 was significant in Cycle 1 while oPt-7232 was significant in Cycle 2.

$^2$The marker was not included in the DArT genotyping report.

$^3$oPt-7232 was significant in single marker test in Cycle 0 but was not included in multiple marker-test.

Table 2. Least squares means for $\beta$-Glucan concentration (g/100g) and their standard errors for populations and parental lines.

<table>
<thead>
<tr>
<th>Populations</th>
<th>Cycle</th>
<th>LS means</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base$^7$</td>
<td>0</td>
<td>4.57</td>
<td>0.12</td>
</tr>
<tr>
<td>GR1</td>
<td>1</td>
<td>5.90</td>
<td>0.13</td>
</tr>
<tr>
<td>GR2</td>
<td>1</td>
<td>5.95</td>
<td>0.12</td>
</tr>
<tr>
<td>MR1</td>
<td>1</td>
<td>6.01</td>
<td>0.11</td>
</tr>
<tr>
<td>MR2</td>
<td>1</td>
<td>5.95</td>
<td>0.10</td>
</tr>
<tr>
<td>PR1</td>
<td>1</td>
<td>5.89</td>
<td>0.12</td>
</tr>
<tr>
<td>PR2</td>
<td>1</td>
<td>5.95</td>
<td>0.11</td>
</tr>
<tr>
<td>GR1</td>
<td>2</td>
<td>6.73</td>
<td>0.11</td>
</tr>
<tr>
<td>GR2</td>
<td>2</td>
<td>6.87</td>
<td>0.11</td>
</tr>
<tr>
<td>MR1</td>
<td>2</td>
<td>6.75</td>
<td>0.10</td>
</tr>
<tr>
<td>MR2</td>
<td>2</td>
<td>6.88</td>
<td>0.11</td>
</tr>
<tr>
<td>PR1</td>
<td>2</td>
<td>6.66</td>
<td>0.09</td>
</tr>
<tr>
<td>PR2</td>
<td>2</td>
<td>6.68</td>
<td>0.08</td>
</tr>
<tr>
<td>Cycle 1 Parents$^1$</td>
<td>1</td>
<td>5.72</td>
<td>0.17</td>
</tr>
<tr>
<td>GR1 Parents</td>
<td>2</td>
<td>6.75</td>
<td>0.21</td>
</tr>
<tr>
<td>GR2 Parents</td>
<td>2</td>
<td>6.82</td>
<td>0.22</td>
</tr>
<tr>
<td>MR1 Parents</td>
<td>2</td>
<td>6.56</td>
<td>0.19</td>
</tr>
<tr>
<td>MR2 Parents</td>
<td>2</td>
<td>6.81</td>
<td>0.21</td>
</tr>
<tr>
<td>PR1 Parents</td>
<td>2</td>
<td>6.83</td>
<td>0.17</td>
</tr>
<tr>
<td>PR2 Parents</td>
<td>2</td>
<td>6.27</td>
<td>0.17</td>
</tr>
</tbody>
</table>

$^7$The test of contrast of population means showed that Cycle 0 is significantly different from each of the populations in Cycle 1 and Cycle 2. The Cycle 1 populations is also significantly different from Cycle 2 populations ($P < 0.0001$). A contrast between selection methods showed that the mean of the GS and MS populations are significantly different ($P < 0.08$) from PS population means.

$^1$Parents are not significantly different from their respective progenies.
Correlated Response to Selection

The responses in β-glucan concentration were accompanied by responses in days to heading and plant height, but these were significant only between Cycle 1 and 2 of the MAS method (Table 3, Fig. 2). Specifically, the increase in β-glucan concentration was accompanied by a mean increase of 3.38 d to heading date in progenies of MAS populations. However, the opposite response occurred in plant height, where selection for higher β-glucan concentration using MAS was accompanied by a mean reduction of 5.61 cm.

Progeny Performance for β-glucan Concentration

The progenies, parents, and checks in the evaluation trial displayed a large range of β-glucan concentration BLUP values (3.86 to 9.06/100 g, data not shown for all entries). The random sample of lines from Cycle 0 had β-glucan concentration ranging from 3.86 to 6.81, the progenies in Cycle 1 had values ranging from 4.38 to 8.33, and the progenies in Cycle 2 had values ranging from 6.05 to 8.11 (Supplementary Fig. 1). Of the 20 lines with the greatest β-glucan concentration, 11 of the lines were derived from genomic selection populations, eight were from the marker-assisted selection populations, and one line was from the phenotypic selection populations (Table 4). In the top 20 lines, six were Cycle 1 progenies (of which three were parents of Cycle 2) and 14 were Cycle 2 progenies.

Individual Genetic Variances of Populations in Selection Experiments

Individual comparisons of populations showed that genetic variances of populations in Cycle 1 ranged from 0.38 (PR2-Cy1) to 0.66 (GR1-Cy1) and from 0.12 (PR2-Cy2) to 0.37 (GR2-Cy2) in Cycle 2 (Supplementary Table 2; Fig. 3). Estimated genetic variances of populations from Cycle 0 to Cycle 2 were all significantly greater than zero (Supplementary Table 2). The reduction in genetic variance for every population from Cycle 1 to Cycle 2 ranged from 0.03 to 0.34, which corresponded to reductions of 9 (MR2) to 70% (PR1).

Comparison of Genetic Variances Across Selection Methods

Estimated genetic variances between Cycle 0 and Cycle 1 within each selection method indicated a nonsignificant reduction (P > 0.01, Table 5). A significant reduction was detected only between Cycle 1 and 2 of the PS populations (P < 0.01). The genetic variances within Cycle 1 were not significantly different between selection methods. For Cycle 2, only the variance between GS and PS populations were significantly different from one another (P < 0.01).

Average Coancestry

The average coancestry among progenies in the GS Cycle 1 was 0.41 (GR1 = 0.41 and GR2 = 0.41) and increased to 0.48 (GR1 = 0.49 and GR2 = 0.46) in Cycle 2 (Fig. 4). Similarly, the average coancestry in MS Cycle 1 was also 0.41 (MR1 = 0.41 and MR2 = 0.41) and increased to 0.49 (MR1 = 0.49 and MR2 = 0.48) in Cycle 2. On the other hand, the average coancestry for PS Cycle 1

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**Table 3. Estimate of differences and their standard error between cycles of each selection method.**

<table>
<thead>
<tr>
<th>Selection method</th>
<th>β-Glucan concentration</th>
<th>Heading date</th>
<th>Plant height</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/100g</td>
<td>days</td>
<td>cm</td>
</tr>
<tr>
<td>Cycle 1 - Cycle 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GS</td>
<td>1.35 ± 0.15***</td>
<td>1.03 ± 0.78</td>
<td>−0.56 ± 1.41</td>
</tr>
<tr>
<td>MS</td>
<td>1.41 ± 0.14***</td>
<td>−1.11 ± 0.81</td>
<td>−2.86 ± 1.51</td>
</tr>
<tr>
<td>PS</td>
<td>1.35 ± 0.14***</td>
<td>0.50 ± 0.80</td>
<td>0.17 ± 1.43</td>
</tr>
<tr>
<td>Cycle 2 - Cycle 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GS</td>
<td>0.87 ± 0.12***</td>
<td>−0.14 ± 0.71</td>
<td>0.41 ± 0.95</td>
</tr>
<tr>
<td>MS</td>
<td>0.84 ± 0.11***</td>
<td>3.38 ± 0.63***</td>
<td>−2.75 ± 1.31</td>
</tr>
<tr>
<td>PS</td>
<td>0.75 ± 0.10***</td>
<td>−0.68 ± 0.72</td>
<td>0.32 ± 0.93</td>
</tr>
</tbody>
</table>

*** Significant at the 0.001 probability level.
was 0.43 (PR1 = 0.43 and PR2 = 0.43) and increased to 0.50 (PR1 = 0.49 and PR2 = 0.52) in Cycle 2. Under the random mating simulations conducted, the average random-selection coancestries were 0.45, 0.45, and 0.47 under GS, MS, and PS, respectively (Fig. 4). The probabilities of the observed coancestries under random mating were 0.0160, <0.0001, and <0.0001 for GS, MS, and PS, respectively. Not surprisingly, all three methods significantly increased coancestry relative to random mating (Falconer and Mackay, 1996), but the deviation was least severe under GS.

**DISCUSSION**

**Response to Selection for β-Glucan Concentration**

The mean β-glucan concentration of every population in Cycle 1 was 29 to 32% greater than the mean of Cycle 0 despite the fact that the first cycle of selection was conducted based on data from oat growing regions throughout North America. Since these gains were measured in Iowa, a stressful environment for oat (Cervantes-Martinez...
et al., 2001), this confirms earlier observations that relative rankings and additive effects of β-glucan are stable across diverse environments. The responses detected in this study were greater than the 4 to 11% reported by Cervantes-Martinez et al. (2001) after 1 cycle of selection for β-glucan concentration. This occurred despite a comparable selection intensity (12/446 Cycle 0 lines in this work compared with 40/1665 Cycle 0 lines by Cervantes-Martinez et al., 2001). A possible reason was that our base population consisted entirely of adapted breeding lines and inbred cultivars whereas the base population in the previous work consisted of S₀ progenies from only 23 breeding lines. This base population would probably have a narrower genetic variance than our base population resulting in a lower response to selection (Fehr, 1987; Bernardo, 2010).

Although the means of populations in Cycle 2 were greater than the means in Cycle 1, all populations in Cycle 2 had a lower rate of response than their counterpart in Cycle 1. One obvious reason for this was the fact that we used a lower selection intensity (i = 1.097, proportion = 0.33) for selecting parents of Cycle 2 than for selecting parents of Cycle 1 (i = 2.32, proportion = 0.027) in the previous cycle. Another reason is that the genetic variance from which to select was already reduced from the first generation of selection (Robertson, 1960). Nonetheless, the results indicate that all three selection methods were effective in increasing β-glucan concentration in both cycles of selection.

**Comparison of Responses Across Selection Methods**

The similar performance of all selection methods in Cycle 1 was partly a manifestation of selecting many of the same founding parents: 20 parents were common among selection strategies. In Cycle 2, although comparisons between individual methods were not significant, the two marker-based (i.e., GS and MAS) methods outperformed phenotypic selection (P < 0.08) when their means were combined. This suggests that the marker-aided methods produced more progenies with greater β-glucan concentration than did the PS method. Because markers were not used to accelerate the breeding cycle, the advantage attributable to using markers is due purely to their ability to identify parents with the best breeding values. For GS, this advantage is speculated to result from a higher overall accuracy of breeding value prediction. For MAS, this advantage is speculated to result from a more rapid increase in the frequency of favorable alleles at major additive loci. We note that MAS apparently improved gain despite the fact that the markers identified were not validated and explained relatively little of the variance. The low variance arose in part because of low initial favorable allele frequencies rather than because of small effects of the loci. Additionally, because the markers were not validated, it may be best to think of them as high heritability correlated traits rather than as causal factors in affecting β-glucan concentration.

**Correlated Response to Selection**

The correlated response of β-glucan concentration with other traits must be considered to avoid undesirable shifts during selection. In this study, correlated responses for heading date and plant height were detected only in the populations under the MAS method. A previous phenotypic selection experiment by Cervantes-Martinez et al. (2002) demonstrated that selection for β-glucan concentration did not affect heading date, while a reduction in plant height was detected after one cycle of selection. Conversely, β-glucan concentration was not correlated with heading date and plant height when evaluated in populations that were not undergoing selection (Holthaus et al., 1996). One potential reason for the correlated response observed in the MAS strategies lies in the relative positions of QTLs on genetic maps: one β-glucan QTL (near marker oPt.8249) was located 14 cM away from the previous cycle 1 QTL (near bcd1968B and bcd1797D).
on linkage group 24_26_34 (Tinker et al., 2009), while another $\beta$-glucan QTL (near marker oPt.7232) was located 3–6 cM away from a heading date QTL on linkage group 17 (near cdo1467A, umn370, isu1755B, and isu1364; Holland et al., 1997; Tinker et al., 2009). Similarly, marker oPt.8249 is 8 and 14 cM away from plant height QTL bcd1643A and umn220, respectively. Colocalizations of QTL for $\beta$-glucan, heading date and plant height were also observed by Kianian et al. (2000) and De Koeyer et al. (2004). The proximity of these QTL in the oat genome, and the weight that was given to $\beta$-glucan concentration markers during MAS, could have caused the observed correlated response. The lack of a correlated response in PS and GS could be due to the polygenic nature of these traits. Therefore, it is not expected that all QTL for $\beta$-glucan are associated in one direction with QTL for heading date and plant height. This suggests that alternate strategies of MAS could reduce correlated responses to selection, either through deliberate targeting of specific correlations, or through additional cycles of recombination to break undesired correlations.

**Progeny Performance for $\beta$-Glucan Concentration**

Although it is useful to measure the mean performance of a breeding strategy, one can argue that the ultimate measurement is whether it produces better varieties (Zhong and Jannink, 2007). This is particularly true in a self-pollinating crop such as oat where regional production is based on a small number of highly adapted inbred varieties. Furthermore, based only on the phenotypes of the best lines, we expect that one further cycle of selection would lead to means in the marker-based (i.e., MAS and GS) strategies that were even more superior to those from continued BLUP phenotypic selection. This follows because the genetic variance in GS and MS programs remained higher than that in the PS program, such that the selection differential of best parents in GS and MS could be higher than that of best parents under PS (Supplementary Fig. 1).

We noted that 19 progenies in the top 20 high $\beta$-glucan entries came from either GS or MS populations. This deviation in the origin of the top progenies suggested that the tails of the distributions of the methods differed even more than their means. Grouping the programs into “marker-based” (i.e., MS or GS representing four programs out of six) versus “phenotype-based” (i.e., PS), we asked whether this deviation between groups could occur by chance drift alone or was driven by differences in the effectiveness of the methods. We used the reasoning that each selection method was represented by two populations and that differences in the progeny performance across two populations within a method could be due to drift but not to differences in selection performance. Thus, differences in the tails of two populations represented the null-hypothesis of no difference between methods. We performed randomization tests within each method by bootstrap sampling progeny from each population and determined how often 19 or more of the top 20 progeny came from only one population. The probability under this simulation of the null hypothesis was less than 0.05 for each method (data not shown). This test was imperfect because it represented the groups being contrasted by only one program each whereas the observed progeny came from four and two programs for the marker-based and phenotype-based groups, respectively. Nevertheless we think these results support the alternate hypothesis that the observed deviation in the origins of the top progenies was driven in part by difference in selection effectiveness.

From the examination of pedigrees of the top 20 progenies, 11 out of 20 of the original parents of Cycle 1 are in the pedigrees of the top 20 progenies (Table 4, Supplementary Table 3). Of those 11, five were developed by the Iowa State University oat breeding program. In addition, three of the 11 lines have relationships with one another, specifically ND030288 was derived from a cross between Hifi and IAN979–5–1–22. The high occurrence of IAN979–5–1–22 either directly as parent or as part of ND030288 in the pedigrees of both Cycles 1 and 2 confirms that the former has a high proportion of favorable alleles for $\beta$-glucan concentration. Lines IA95111 and AC Antoine were also frequently present, and both could be valuable sources for $\beta$-glucan alleles that are not present in IAN979–5–1–22.

**Estimated Changes in Genetic Variance**

Response to selection is dependent on the genetic variance of the selected parents ( Falconer and Mackay, 1996). In our study, all selection methods resulted in a reduction of variance for $\beta$-glucan concentration from Cycle 0 to Cycle 2. This is in agreement with Cervantes-Martínez et al. (2001). Given that we conducted only two cycles of selection and assuming that $\beta$-glucan concentration is controlled by polygenic effects, changes in allele frequencies of all QTL could be too small to alter genetic variance (Falconer and Mackay, 1996, p. 201). Another reason for a reduction in genetic variance from Cycle 1 to Cycle 2 may be the “Bulmer Effect,” where selection results in negative covariance (i.e., LD) between genes controlling the trait ( Bulmer, 1971), which in turn reduces the trait variance. In other words, because Cycle 1 is a product of selection in Cycle 0, selection might have created repulsion-phase LD among high $\beta$-glucan QTL alleles. In turn this led to a lower variance for Cycle 2. Although recombination is known to breakdown LD, the limited diallel crossing conducted among parents in our study probably had little effect on LD. Therefore, the “Bulmer Effect” may still play a role in the reduction of variance ( van der Werf and de Boer, 1990).
Genetic Variance and Coancestry

Although all methods reduced genetic variance, the magnitude of decrease was not the same across the three selection methods. Greater coancestry among lines and therefore inbreeding among individuals can contribute to reduction in genetic variance (Sorensen and Kennedy, 1984). In our study, the magnitude of decrease of genetic variance might be explained by the differences of buildup of coancestry of the various methods (Supplementary Fig. 2). For instance, the higher coancestry of progenies detected in PS populations could be explained by the fact that BLUP-based PS can increase the chance of coselection of sibs as parents (Sonesson et al., 2005), and is expected to do so more than GS (Daetwyler et al., 2007). In our study, the coselection of sibs with similar breeding values for β-glucan concentration could have eventually led to lower genetic variance for β-glucan concentration in Cycle 2 of PS (Supplementary Fig. 2). Selection of sibs reduces the effective population size, which in turn increases the probability of fixing deleterious alleles (in this case, alleles conferring low β-glucan content), resulting in reduced long term gains from selection. The higher chance of coselection of sibs happens in the BLUP PS method because pedigree information does not account for Mendelian segregation (see Supplementary Fig. 3 for comparison of marker-based and pedigree-based relationships in oat) resulting in a higher correlation of estimated breeding values within families (Daetwyler et al., 2007). On the other hand, GS can account for Mendelian segregation, which can lead to a reduced correlation of estimated breeding values within families (Daetwyler et al., 2007). Therefore, the use of markers can reduce the selection of related individuals in a breeding program, resulting in less reduction of genetic variance. The similar level of coancestry between MAS and GS progenies in Cycle 1 might be explained by the fact that both methods used marker-based relationships in the final selection of diverse parents.

Breeding Implications

The single year recurrent selection cycle (Frey et al., 1988) implemented in this study is seldom used in oat breeding, perhaps because of difficulties in making a large number of crosses during the recombination stage. However, this work has demonstrated that recurrent selection is highly effective in achieving rapid gains and superior progeny for a trait such as β-glucan. The superior progenies that were developed during this experiment have been submitted to the National Small Grains Collection (Aberdeen, Idaho) for preservation and distribution (Supplementary Table 4).

In regard to different selection methods, the advantage of MAS and GS over PS was small on a per cycle basis, and these results support the cross-validation experiments of Heffner et al. (2011a, 2011b) in wheat, where MS and GS accuracies were comparable to those of PS. We conclude that any substantial advantage of GS to increase selection response would probably come from conducting at least two cycles per year, which is not possible for phenotypic selection. In this scheme, one cycle could be conducted at an off-season location and the other in the target environment with the addition of phenotypic data. The presence of top-performing progenies from GS and MAS also suggests the superiority of these methods in cultivar development. However, index-based MAS may not have the advantage of GS in accelerating selection, because phenotypic data collected during the summer season would still be required to account for a polygenic effect (Dekkers, 2007). Finally, in our study, the use of few markers in MAS for β-glucan concentration resulted in a non-favorable correlated response with heading date which would need to be addressed in applied breeding.

Although GS can provide a rapid increase in genetic gain for β-glucan concentration by factors such as multiple cycles per year (Asoro et al., 2011) and greater selection intensity, these factors may also lead to faster rate of loss of genetic diversity. The loss in genetic diversity can eventually lead to decreased genetic variance for the trait of interest and lower gain from selection (Robertson, 1960). In this case, a strategy which introgresses unrelated germplasm could be employed jointly with GS (Odegard et al., 2009; Bernardo, 2009). Another strategy would be a selection criterion that weights the low-frequency favorable alleles more heavily to avoid losing them. This approach could sustain gains from selection and limit the loss of genetic variance (Jannink, 2010). Therefore, implementing GS in large breeding programs will require strategies that will balance rapid genetic gain and preserve genetic variation in elite breeding populations.

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