

Genomewide Selection versus Marker-assisted Recurrent Selection to Improve Grain Yield and Stover-quality Traits for Cellulosic Ethanol in Maize

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ABSTRACT

Genomewide selection (GWS) is marker-assisted selection without identifying markers with significant effects. Our previous work with the intermated B73 × Mo17 maize (*Zea mays* L.) population revealed significant variation for grain yield and stover-quality traits important for cellulosic ethanol production. Our objectives were to determine (i) if realized gains from selection are larger with GWS than with marker-assisted recurrent selection (MARS), which involves selection for markers with significant effects; and (ii) how multiple traits respond to multiple cycles of GWS and MARS. In 2007, testcrosses of 223 recombinant inbreds developed from B73 × Mo17 (Cycle 0) were evaluated at four Minnesota locations and genotyped with 287 single nucleotide polymorphism markers. Individuals with the best performance for a Stover Index and a Yield + Stover Index were intermated to form Cycle 1. Both GWS and MARS were then conducted until Cycle 3. Multilocation trials in 2010 indicated that gains for the Stover Index and Yield + Stover Index were 14 to 50% larger (significant at $P = 0.05$) with GWS than with MARS. Gains in individual traits were mostly nonsignificant. Inbreeding coefficients ranged from 0.28 to 0.38 by Cycle 3 of GWS and MARS. For stover-quality traits, correlations between wet chemistry and near-infrared reflectance spectroscopy predictions decreased after selection. We believe this is the first published report of a GWS experiment in crops, and our results indicate that using all available markers for predicting genotypic value leads to greater gain than using a subset of markers with significant effects.

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Abbreviations: GWS, genomewide selection; MARS, marker-assisted recurrent selection; NIRS, near-infrared reflectance spectroscopy; QTL, quantitative trait loci; RR-BLUP, ridge regression–best linear unbiased prediction; SNP, single nucleotide polymorphism.

MARKER-ASSISTED SELECTION for quantitative traits has traditionally relied on first identifying markers linked to quantitative trait loci (QTL). A specific form of marker-assisted selection in maize (*Zea mays* L.) is marker-assisted recurrent selection (MARS) in which (i) one generation of phenotypic selection in the target environment is conducted, (ii) markers with significant effects are used to predict the performance of individual plants, and (iii) several generations of marker-only selection are performed in a year-round nursery or greenhouse. Empirical results from private breeding programs have shown MARS to be effective at improving quantitative traits in maize, soybean [*Glycine max* (L.) Merr.], and sunflower (*Helianthus annuus* L.) (Johnson, 2004; Eathington et al., 2007). Specifically, gains from selection in 248 maize breeding populations were more than twice as large with MARS than with standard phenotypic selection methods (Eathington et al., 2007).

In contrast to previous MARS or other QTL-based selection strategies, genomewide selection (GWS, also called genomic selection; Meuwissen et al., 2001) does not involve tests of significance and uses all available markers to predict performance. Simulation

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and empirical cross-validation studies in plants have shown GWS to be more effective than strategies that use only a subset of markers with significant effects. In simulation studies for maize and oil palm (*Elaeis guineensis* Jacq.), responses to selection were 18 to 175% larger with GWS than with MARS across different numbers of QTL and levels of heritability (Bernardo and Yu, 2007; Wong and Bernardo, 2008). In empirical cross-validation studies in maize (Lorenzana and Bernardo, 2009; Guo et al., 2012), barley (*Hordeum vulgare* L.; Lorenzana and Bernardo, 2009), oat (*Avena sativa* L.; Asoro et al., 2011), and wheat (*Triticum aestivum* L.; Hefner et al., 2011), GWS predictions of observed performance were consistently more accurate than multiple-regression predictions based on significant markers only.

While these results from simulation and cross-validation studies are promising, we are unaware of published reports comparing the effectiveness of GWS and MARS in an actual selection experiment in which the best plants are selected via GWS and MARS, the selected plants are recombined to form the next cycle, several cycles of selection are conducted, and the observed responses to selection are evaluated in field experiments. In previous experiments with the intermated B73 × Mo17 population (Lee et al., 2002) at the University of Minnesota and USDA-ARS, we investigated the prospects of breeding maize for both grain yield and stover-quality traits for cellulosic ethanol (Lewis et al., 2010; Lorenzana et al., 2010). In particular, we focused on three stover-quality traits important for cellulosic ethanol: concentration of cell wall glucose in dry stover (referred to as “Glucose”); cell wall glucose released from the stover by thermochemical pretreatment and enzymatic saccharification (“Glucose Release”); and concentration of lignin as a proportion of the cell wall (“Lignin”). We found significant genetic variation and favorable or neutral genetic correlations for these traits in testcrosses of the intermated B73 × Mo17 population (Lewis et al., 2010). While we detected 7 QTL for Glucose, 10 QTL for Glucose Release, 8 QTL for Lignin, and a total of 127 QTL for other stover-quality traits we studied, none of the QTL had a major effect ($r^2 \leq 12\%$; Lorenzana et al., 2010).

We have since built on this previous research (Lewis et al., 2010; Lorenzana et al., 2010) by conducting multiple cycles of GWS and MARS for grain yield, agronomic traits, and stover-quality traits in the same B73 × Mo17 population, and we report our results herein. Our objectives in this study were to determine (i) if realized gains from selection in maize were larger with GWS than with MARS; and (ii) how multiple traits related to grain yield and stover quality respond to multiple cycles of GWS and MARS.

MATERIALS AND METHODS

Overview

In this study, GWS and MARS for maize grain yield, agronomic traits, and stover quality comprised the following steps (Fig. 1): (i) an initial population (Cycle 0) was evaluated for

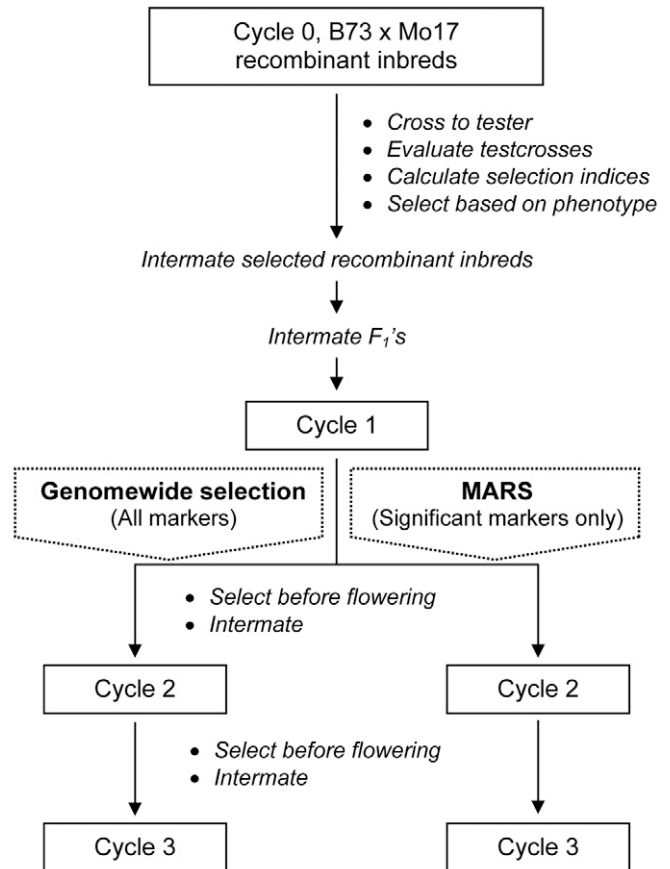


Figure 1. Genomewide selection and marker-assisted recurrent selection (MARS) in the intermated B73 × Mo17 maize recombinant inbreds.

molecular markers and trait phenotypes; (ii) multitrait selection indices were constructed to combine phenotypic data for different traits; (iii) Cycle 0 individuals were ranked according to their selection-index values (i.e., phenotypic selection) and the best individuals were selected and intermated to form Cycle 1; (iv) Cycle 1 individuals were genotyped for molecular markers; (v) for GWS and MARS and for each selection index, Cycle 1 individuals were ranked according to their marker-predicted values for the selection index, and the best Cycle 1 individuals were selected and intermated to form Cycle 2; (vi) procedures used in Cycle 1 were repeated in Cycle 2 to produce Cycle 3; and (vii) performances of Cycle 0, 1, 2, and 3 populations were compared in field trials to evaluate the progress from selection.

Phenotyping of Cycle 0 Population

The intermated B73 × Mo17 population (Lee et al., 2002) served as Cycle 0. Procedures and results of phenotypic analysis of Cycle 0 were reported by Lewis et al. (2010) and are summarized here. A total of 223 randomly chosen B73 × Mo17 recombinant inbreds were testcrossed to an elite Monsanto (St. Louis, MO) inbred. The Cycle 0 testcrosses were evaluated at two locations at the University of Minnesota Southern Research and Outreach Center at Waseca and at two locations at the University of Minnesota Southwestern Research and Outreach Center at Lamberton, for a total of four locations. The two locations within each Research and Outreach Center were planted 5 to 7 d apart and differed in soil type (Webster and

Nicollet clay loam in Waseca and Normania and Ves loam in Lamberton). Data were recorded for the following agronomic traits: grain yield (Mg ha^{-1} , adjusted to 155 g kg^{-1} moisture); grain moisture (g kg^{-1}); stalk lodging (percentage of plants with stalks broken below the ear); and root lodging (percentage of plants leaning at more than a 45° angle from the vertical).

The testcrosses were also evaluated for Glucose, Glucose Release, and Lignin. In a set of 154 calibration samples, cell wall concentration and composition were determined using the Uppsala dietary fiber method (Theander et al., 1995), whereas sugar release, after dilute acid/high-temperature pretreatment and enzymatic saccharification, was measured using a modification of the method of Dien et al. (2006). Lignin was measured by the Klason lignin procedure (Theander et al., 1995). Near-infrared reflectance spectroscopy (NIRS) calibration equations were developed from the wet chemistry data for the 154 calibration samples and their associated NIRS spectral data as described by Shenk and Westerhaus (1991). The NIRS calibration equations were then used to predict Glucose, Glucose Release, and Lignin in the entire set of stover samples.

Multitrait Selection Indices

The Cycle 0 phenotypic data were used to construct two multiple-trait selection indices. First, a Stover Index was constructed as a nonparametric rank-sum index (Kang, 1988) that gave equal weights to high Glucose Release, low Lignin, and high Glucose. The sum of the ranks for the three traits was calculated, for example, a testcross ranked 5th for Glucose Release, 10th for Lignin, and 15th for Glucose had a Stover Index value of $5 + 10 + 15 = 30$.

Second, a Yield + Stover Index was constructed as follows. A Yield Index was constructed as $I = (\text{grain yield in t ha}^{-1}) - 0.028(\text{grain moisture in g H}_2\text{O kg}^{-1}) - 0.059(\text{stalk lodging percentage}) - 0.036(\text{root lodging percentage})$, where the weights were the retrospective-index weights that have been used by a team of experienced commercial maize breeders (Bernardo, 1991). The 223 testcrosses were ranked according to their values for the Yield Index. The Yield + Stover Index was then calculated as the sum of ranks for I and for the Stover Index. The Yield + Stover Index therefore gave equal weights to grain yield and agronomic traits as a whole and to stover-quality traits as a whole.

Selection of Cycle 0 Individuals to Form Cycle 1

Selection in Cycle 0 was based on phenotypic data only as recommended by Bernardo and Yu (2007). Testcrosses of the 223 recombinant inbreds were ranked according to their Stover Index scores and Yield + Stover Index scores. The 10 recombinant inbreds with the highest Stover Index scores were intermated for two generations to form Stover Index Cycle 1 (Fig. 1). Likewise, the 10 recombinant inbreds with the highest Yield + Stover Index scores were intermated for two generations to form Yield + Stover Index Cycle 1. The two generations of intermating were conducted in an off-season nursery on Molokai, Hawaii, in September 2008 to May 2009. In the first generation of intermating, each selected recombinant inbred was crossed to a balanced bulk of the other nine selected recombinant inbreds to obtain F_1 seeds with a one known parent. This procedure resulted in 10 half-sib bulks. In the second generation of intermating, each half-sib bulk

was crossed with a balanced bulk of the other nine half-sib bulks in a paired-row crossing system. Equal amounts of seeds from each paired row were then bulked to form a segregating population that served as Cycle 1.

Models for Genomewide Selection and Marker-assisted Recurrent Selection

The 223 recombinant inbreds were genotyped by DNA LandMarks (Saint-Jean-sur-Richelieu, QC) for 287 polymorphic, well-spaced single nucleotide polymorphism (SNP) markers. Marker effects in GWS were calculated by ridge regression–best linear unbiased prediction (RR-BLUP; Meuwissen et al., 2001). Specifically, all 287 markers were used to model the mean (across locations) index values of testcrosses as $\mathbf{y} = \boldsymbol{\mu}\mathbf{1} + \mathbf{X}\mathbf{g} + \mathbf{e}$ (Meuwissen et al., 2001), where \mathbf{y} was a 223×1 vector of index values (Stover Index or Yield + Stover Index) of the 223 testcrosses in Cycle 0; $\boldsymbol{\mu}$ was the overall mean of the index used; $\mathbf{1}$ was a 223×1 vector with all elements equal to 1; \mathbf{X} was a 223×287 design matrix, with elements equal to 1 if the recombinant inbred was homozygous for the marker allele from B73, -1 if the recombinant inbred was homozygous for the marker allele from Mo17, and zero if the marker was heterozygous; \mathbf{g} was a 287×1 vector of breeding values associated with the marker allele from B73 at each of the marker loci; and \mathbf{e} was a 223×1 vector of residual effects.

The testcross genetic variance (V_G) and residual variance (V_R) were estimated by analysis of variance of the Stover Index and Yield + Stover Index values in Cycle 0 (Lewis et al., 2010). The variance of breeding values at each of the 287 marker loci was assumed equal to $V_G/287$ (Meuwissen et al., 2001). The solution \mathbf{g} was obtained by solving the mixed-model equations (Henderson, 1984) with $\boldsymbol{\mu}$ as a fixed effect and \mathbf{g} as a random effect.

In MARS, multiple regression of Stover Index and Yield + Stover Index values on the number of marker alleles (0 or 2) from B73 was performed on a chromosome-by-chromosome basis (Bernardo and Charcosset, 2006). Significant markers on each chromosome were identified by backward elimination. A relaxed significance level ($P = 0.10$), which has been found to maximize the response to MARS (Hospital et al., 1997; Johnson, 2001), was used. Final multiple-regression coefficients were obtained by jointly analyzing all the markers found significant in the per-chromosome analyses.

Cycles 1 and 2

Selection in Cycles 1 and 2 was based only on markers. The two marker-based selection schemes and the two selection indices led to four different selection programs: GWS for the Stover Index; GWS for the Yield + Stover Index; MARS for the Stover Index; and MARS for the Yield + Stover Index. In MARS, the same set of significant markers (chosen in Cycle 0) was used in Cycles 1 and 2. Cycles 1 and 2 were grown in an off-season nursery on Molokai, Hawaii, between October 2009 and May 2010.

In each selection program, a total of 192 plants were grown in each of Cycles 1 and 2. Leaf samples were collected at the seedling stage and were sent to DNA LandMarks for SNP genotyping. In GWS, the performance of the 192 plants in each cycle was predicted as $\mathbf{M}\mathbf{g}$, where \mathbf{M} was a 192×287 matrix with elements of 1, 0, and -1 (i.e., as in \mathbf{X}), and \mathbf{g} was obtained from mixed-model analysis of the Cycle 0 testcrosses.

In MARS, multiple-regression predictions of the performance of the individual plants were based on the significant markers only as described by Bernardo et al. (2006). In each of the four selection programs, the 10 plants with the best predicted performance were selected in each cycle and were intermated by dividing the 10 plants into five pairs according to similarity of flowering date and making reciprocal crosses between the plants in each pair. The next cycle of selection was formed by bulking equal numbers of kernels from the five paired crosses.

Progress from Selection

To form a single entry for Cycle 0, equal amounts of remnant testcross seed were bulked from all 223 intermated B73 × Mo17 recombinant inbreds. For each of the four selection programs, 64 random plants in each cycle were testcrossed to the same Monsanto tester used in Cycle 0. Kernels from the resulting ears were bulked to form testcrosses of Cycles 1, 2, and 3. Testcrosses of 11 bulked entries were evaluated: Cycle 0; Stover Index Cycle 1; Yield + Stover Index Cycle 1; Cycles 2 and 3 of GWS for Stover Index; Cycles 2 and 3 of GWS for Yield + Stover Index; Cycles 2 and 3 of MARS for Stover Index; and Cycles 2 and 3 of MARS for Yield + Stover Index.

The 11 entries were evaluated in 2010, in a randomized complete block design with two replications, at the same four locations used in the Cycle 0 trials in 2007. The 11 entries were also evaluated, in a randomized complete block design with three replications, at St. Paul, MN. The entries were grown in four-row plots, each row 5 m long and spaced 0.76 m apart, and at a plant population density of 77,000 plants ha⁻¹. The traits evaluated and procedures for measuring the traits were the same as those used in Cycle 0 (Lewis et al., 2010). Glucose, Glucose Release, and Lignin were measured by both wet chemistry and NIRS predictions.

In accordance with standard protocol, the NIRS calibration equations used for the 2010 evaluations were updated versions of the original calibration equations used for Cycle 0 in 2007. This updating was done by choosing 25 stover samples from the 2010 field trials, adding these 25 samples to the original set of 154 calibration samples from 2007, and obtaining new NIRS calibration equations for Glucose, Glucose Release, and Lignin. The 25 samples used for updating were chosen as follows. First, 13 samples whose wet chemistry values were deemed outliers were excluded. Such outliers were identified by calculating the differences among trait values in different replications within the same location, calculating the standard deviation of the difference, and determining which samples had between-replication differences significant at $P = 0.01$. Second, the Intrasoftware International NIRS 3 version 4.0 software programs “Center” and “Select” were used to choose 25 samples that represented the spectral diversity of the entire set of non-outlier samples from 2010. The wet chemistry results should be the best estimate of the true value for the populations, but because the selection was based on the original NIRS results, both sets of results are presented.

To account for missing plots, least-squares means of the entries were calculated with SAS version 9.2 (Cary, NC). The Stover Index and the Yield + Stover Index were obtained by ranking the entries within each index. Least significant differences (at a comparison-wise significance level of $P = 0.05$) were obtained with the entry × location mean squares from analysis

of variance as the error term. Given the known variability of selection responses in MARS (Johnson, 2004), linear contrasts among combinations of means were also tested for their significance. In particular, comparisons were made among (i) the mean of Cycles 2 and 3 in GWS, (ii) the mean of Cycles 2 and 3 in MARS, and (iii) the mean of Cycle 1.

Population Genetics of Genomewide Selection and Marker-assisted Recurrent Selection Cycles

For the SNP markers with significant effects for the Stover Index and for the Yield + Stover Index, correlations between Cycle 0 marker effects in MARS and in GWS were calculated. Across all markers used in GWS and in MARS, changes in marker allele frequencies were calculated from one cycle to the next. Inbreeding coefficients within each cycle were calculated as $1 - H_{C_n}/H_{C_0}$, where H_{C_n} was the mean marker heterozygosity in Cycle n whereas H_{C_0} was the expected mean marker heterozygosity in Cycle 0. We considered Cycle 0 as a noninbred reference population and, with the SNP markers having been chosen for their polymorphism between B73 and Mo17, the value of H_{C_0} was 0.5. H_{C_n} was determined from the observed SNP genotypes in Cycles 1 and 2 and was equal to the expected heterozygosity in Cycle 3, given the genotypes of the selected Cycle 2 plants. Linkage disequilibrium in the GWS populations was estimated by calculating the r^2 between markers through Haploview version 4.0 software (Barrett et al., 2005).

RESULTS AND DISCUSSION

Response to Genomewide Selection and Marker-assisted Recurrent Selection: Selection Indices

Selection-index gains through three cycles of selection were larger with GWS than with MARS. In the Stover Index populations, the Stover Index at Cycle 3 (for both the wet chemistry and NIRS results) was 14% higher (significant at $P = 0.05$) with GWS than with MARS (Fig. 2). In the Yield + Stover Index populations, the Yield + Stover Index at Cycle 3 was 33% higher (significant at $P = 0.05$) with GWS than with MARS for the wet chemistry results, and 50% higher (significant at $P = 0.05$) with GWS than with MARS for the NIRS results. In the Yield + Stover Index populations, the Yield Index at Cycle 3 was not significantly higher with GWS than with MARS (Table 1). However, the Yield Index in both Cycles 2 and 3 was significantly higher than the Yield Index in Cycle 1 with GWS, but not with MARS.

The 14 to 50% superiority in gains from GWS over MARS were consistent with previous simulation and cross-validation results. In a simulation experiment very similar in design to the empirical experiments reported here, the gain from GWS was 18 to 43% larger than gain from MARS for traits controlled by 20, 40, or 100 QTL and with a heritability of 0.20, 0.50, or 0.80 (Bernardo and Yu, 2007). In a previous analysis of the Cycle 0 population, maximum

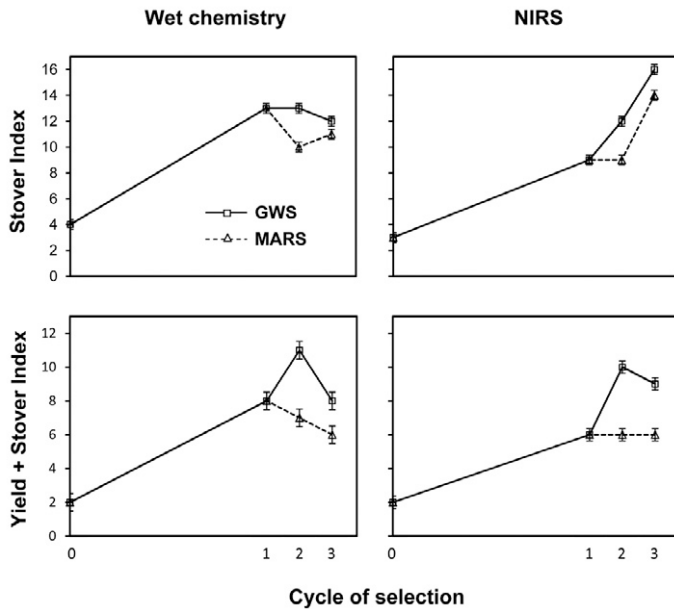


Figure 2. Response to genomewide selection (GWS) and marker-assisted recurrent selection (MARS) in testcrosses of the intermated B73 × Mo17 maize population. Selection in Cycle 0 was based on phenotypic values, whereas selection in Cycles 1 and 2 was based on marker effects. The x-axis assumes that testcross phenotypic selection in Cycle 0 requires 2 yr, whereas each cycle of marker-only selection requires 4 mo. The LSD ($P = 0.05$) values for the indices were as follows: 0.73 for Stover Index (wet chemistry) in Stover Index populations, 0.72 for Stover Index (near-infrared reflectance spectroscopy [NIRS]) in Stover Index populations; 1.04 for Yield + Stover Index (wet chemistry); and 0.62 for Yield + Stover Index (NIRS). The error bars indicate half of the LSD values.

prediction accuracies for the Yield + Stover Index were 0.70 with GWS and 0.51 with MARS (Lorenzana and Bernardo, 2009). Likewise, maximum prediction accuracies for the Stover Index were 0.65 with GWS and 0.41 with MARS.

Marker effects for the Stover Index and Yield + Stover Index were calculated from NIRS measurements of Glucose, Glucose Release, and Lignin in Cycle 0, and changes in the Stover Index and Yield + Stover Index—as measured by NIRS—were generally in the positive direction (Fig. 2). On the other hand, the wet chemistry results for the Stover Index and the Yield + Stover Index generally showed a decrease in performance in Cycles 2 and 3 compared with Cycle 1 (Fig. 2). In Cycle 0, the correlations between NIRS predictions and wet chemistry measurements were 0.96 for Glucose, 0.91 for Glucose Release, and 0.81 for Lignin on a dry matter basis (Lewis et al., 2010). After selection, the correlation between NIRS predictions and wet chemistry measurements remained high for Glucose ($r = 0.82$) but decreased substantially for Glucose Release ($r = 0.37$) and Lignin ($r = 0.31$). This inconsistency may have contributed to the discrepancy in selection responses as measured by NIRS vs. wet chemistry.

In general, we can expect selection gain primarily for the criteria used during the selection process (i.e., NIRS predictions) regardless of how predictive those criteria are of the true, underlying values of the traits. Our results seem to suggest selection for the NIRS phenotype rather than for the underlying values as measured by wet chemistry. Furthermore, in a MARS experiment with six maize populations,

Table 1. Trait means for 11 maize populations developed by genomewide selection (GWS) and by marker-assisted recurrent selection (MARS) for a Stover Index and a Yield + Stover Index.

Population	Grain yield Mg ha ⁻¹	Moisture g kg ⁻¹ dm	Root lodging %	Stalk lodging %	Yield Index	Wet chemistry			NIRS [†]		
						Glucose g kg ⁻¹ dm	Glucose release g kg ⁻¹	Lignin g kg ⁻¹ CW [‡]	Glucose g kg ⁻¹ dm	Glucose release g kg ⁻¹	Lignin g kg ⁻¹ CW
C0	10.4	238	7.2	0.5	3.45	300	509	203	294	514	201
Stover Index											
C1	10.4	223	5.0	0.2		307	548*	195	308*	527	199
GWS C2	10.1	224	1.5*	0.0*		317*	536	196	311*	533*	198
GWS C3	10.0	222	2.3	0.4		313	506	193*	312*	537*	196
MARS C2	9.9	223	7.4	0.0*		309	545*	197	307	536*	200
MARS C3	10.4	226	3.9	0.2		309	545*	198	304	539*	195
Yield + Stover Index											
C1	10.1	230	4.2	0.3	3.53	312	517	195*	305	529	199
GWS C2	10.8	218*	8.0	0.5	4.32*	314*	517	199	311*	532	202
GWS C3	10.7	228	1.3*	0.0*	4.32*	300	549*	197	300	529	199
MARS C2	9.7	207* [§]	2.4	0.1	3.78	311	517	196	308*	524	201
MARS C3	10.4	230	2.9	0.1	3.89	305	511	197	305	521	201
Mean	10.3	229	4.2	0.2		308	527	196	305	530	199
LSD _{0.05}	0.8	17	5.4	0.4	0.78	13	35	7	13	18	6

* Cycle 1 (C1), Cycle 2 (C2), or Cycle 3 (C3) means were significantly different ($P = 0.05$) from the mean of the Cycle 0 (C0) population.

[†] Near-infrared reflectance spectroscopy.

[‡] CW, cell weight.

[§] Mean was significantly different ($P = 0.05$) from the mean of the C1 population.

grain yield decreased from Cycle 1 to Cycle 2 in two populations and grain moisture increased from Cycle 1 to Cycle 2 in three populations (Johnson, 2004). The variable rates of genetic gain, both positive and negative, we observed over multiple cycles of selection were therefore consistent with previous MARS studies (Johnson, 2004).

Most of the gains in selection were obtained in Cycle 1, which resulted from phenotypic selection in Cycle 0. Previous simulation results have shown that the eventual gains after multiple cycles of GWS are slightly larger if selection in Cycle 0 is based on phenotypic values instead of a phenotype plus marker index (Bernardo and Yu, 2007). Furthermore, a breeder might consider multiple breeding populations as candidates for GWS but would likely discard those populations that perform poorly in the Cycle 0 yield trials. Selection in Cycle 0 based on phenotypic values instead of markers therefore prevents the unneeded genotyping of Cycle 0 populations that are immediately discarded for their poor performance.

Previous cross-validation analysis of the Cycle 0 population indicated that, as we observed in the current study, the gain from one cycle of GWS is expected to be less than the gain from one cycle of phenotypic selection (Lorenzana and Bernardo, 2009). The lower expected and observed per-cycle gains with GWS than with phenotypic selection indicate that GWS is most useful (i) when genotyping is cheaper than phenotyping as was the case in our experiments, and (ii) when multiple cycles of selection are conducted when heritability is effectively zero, such as in an off-season nursery or greenhouse (Bernardo and Yu, 2007). Aggressive use of an off-season nursery or greenhouse could allow three generations of marker-based selection each year in maize, with genetic gains being made in each generation. Such gains per unit time, as well as gains per cycle, are shown in Fig. 2, assuming that one cycle of testcross phenotypic selection requires 2 yr whereas one cycle of marker-based selection requires 4 mo.

Overall, our results indicate that in a recurrent selection scheme, using all available markers for predicting genotypic value leads to greater genetic gain than using the subset of markers with significant effects. To our knowledge this is the first reported empirical comparison of GWS and MARS in crops.

Response to Genomewide Selection and Marker-assisted Recurrent Selection: Individual Traits

The different cycles of selection showed significant variation ($P = 0.05$) for each trait (Table 1). Improvement over Cycle 0 was observed for all traits except for grain yield and Lignin measured by NIRS. However, except for grain moisture in Cycle 2 of MARS, none of the GWS and MARS cycles showed any improvement over Cycle 1 (which, as previously mentioned, was obtained by

phenotypic selection in Cycle 0). The significant improvements in the NIRS-based Yield + Stover Index and Stover Index (Fig. 2) from Cycle 1 to Cycle 3 of GWS were therefore not accompanied by significant improvements in performance for individual traits. Nevertheless, linear contrasts indicated that in the Yield + Stover Index populations, mean grain yield was 0.69 Mg ha^{-1} higher (significant at $P = 0.05$) in Cycles 2 and 3 of GWS than in Cycles 2 and 3 of MARS. Likewise, in the Yield + Stover Index populations, mean Glucose Release (measured by wet chemistry) was 20 g kg^{-1} higher (significant at $P = 0.05$) in Cycles 2 and 3 of GWS than in Cycles 2 and 3 of MARS.

A selection index has been shown to be the most efficient approach to improve multiple traits, and is superior to the use of independent culling levels or tandem selection. But when selection is for t traits, the improvement for a given trait is on average only $1/\sqrt{t}$ as large as the improvement obtained from selection for only that trait (Hazel and Lush, 1942). Having overall improvements in selection indices comprising three traits (Stover Index) or seven traits (Yield + Stover Index) without detectable improvements in each component trait was therefore not unexpected.

We calculated marker effects for the Stover Index and Yield + Stover Index themselves, rather than for the component traits of each index. We used this approach to have an equal comparison between MARS and GWS. Because GWS uses all available markers, it would have been possible to calculate marker effects for each component trait independently, and then calculate the Stover Index and Yield + Stover Index based on the predicted performance for each trait. This approach, however, was not possible in MARS because different traits would have identified different markers as significant. We speculate that calculating marker effects for individual traits rather than for an index of multiple traits may lead to better control of changes in individual component traits in GWS.

Previous efforts to reduce the lignin content of maize stover (e.g., by brown midrib mutant genes) have often resulted in increased root or stalk lodging (Barriere and Argillier, 1993; Tesso and Ejeta, 2011). The Stover Index GWS Cycle 2 population and the Yield + Stover Index Cycle 1 population had significantly less Lignin (as estimated by wet chemistry) than the Cycle 0 population but neither population showed significant changes in stalk or root lodging. This result was consistent with the nonsignificant genetic correlations between Lignin and stalk and root lodging in Cycle 0 (Lewis et al., 2010). On the other hand, the maximum reduction in Lignin in our study (10 g kg^{-1} cell weight based on wet chemistry; Table 1) was much smaller than the 51 g kg^{-1} reduction reported for the *bmr2* mutation and 40 g kg^{-1} reduction reported for the *bmr3* mutation (Chabbert et al., 1994).

Population Genetics of Genomewide Selection and Marker-assisted Recurrent Selection Cycles

A total of 58 SNP markers were significant ($P = 0.10$) for the Stover Index and 59 SNP markers were significant for the Yield + Stover Index. The mean of the absolute values of marker effects was 4.8 to 5.1 times larger with MARS than with GWS (Fig. 3). Among the 58 SNP markers used in MARS for the Stover Index, the correlation between marker-effect estimates in MARS and in GWS was 0.62. Among the 59 SNP markers used in MARS for the Yield + Stover Index, the correlation between marker-effect estimates in MARS and in GWS was 0.68.

In MARS, changes in mean SNP allele frequency were larger from Cycle 1 to Cycle 2 (0.14 to 0.15) than from Cycle 2 to Cycle 3 (0.09 to 0.10; Table 2). In GWS, per-cycle changes in mean SNP allele frequency were fairly constant from Cycle 1 to Cycle 3. From Cycle 1 to Cycle 3, the change in mean allele frequency at the SNP markers used in selection was slightly higher with MARS (0.24) than with GWS (0.22). These results were expected because selection was concentrated at fewer marker loci in MARS than in GWS. Across the two selection indices and both selection methods, correlation coefficients between the estimated size of the marker effect and the change in marker allele frequency were moderate, ranging from 0.45 to 0.54.

The observed frequencies of heterozygotes at the SNP markers indicated that the GWS and MARS cycles became increasingly inbred as selection progressed (Table 2). Based on all 287 SNP markers, the estimated inbreeding coefficients in Cycle 3 of GWS were 0.28 for the Yield + Stover

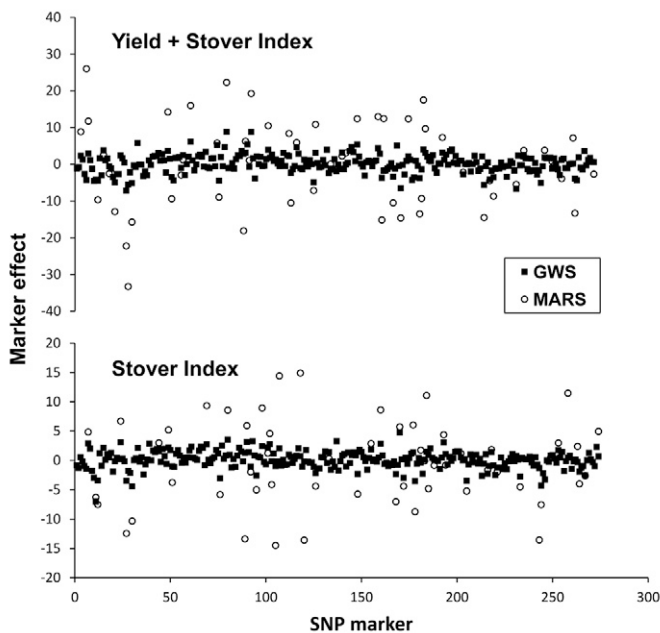


Figure 3. Single nucleotide polymorphism (SNP) marker effects for multiple-trait indices with genomewide selection (GWS) and marker-assisted recurrent selection (MARS) in testcrosses of the intermated B73 × Mo17 maize population.

Table 2. Changes in single nucleotide polymorphism (SNP) allele frequency and SNP-based inbreeding coefficients in maize populations developed by genomewide selection (GWS) and by marker-assisted recurrent selection (MARS) for a Stover Index and a Yield + Stover Index.

Population [†]	Mean change in SNP allele frequency from previous cycle [‡]	Inbreeding coefficient		Mean r^2 between adjacent SNP markers [§]
		All SNPs	SNPs used in MARS	
Stover Index				
C1	0.14	0.17	0.22	
GWS C2	0.11	0.24	0.26	0.44
GWS C3	0.12	0.36	0.39	0.40
MARS C2	0.14		0.26	
MARS C3	0.09		0.38	
Yield + Stover Index				
C1	0.14	0.16	0.14	
GWS C2	0.11	0.21	0.28	0.44
GWS C3	0.09	0.28	0.34	0.39
MARS C2	0.15		0.26	
MARS C3	0.10		0.33	

[†] C0, Cycle 0; C1, Cycle 1; C2, Cycle 2; or C3, Cycle 3.

[‡] Mean of absolute values of the change in frequency of the SNP alleles from Mo17.

[§] Mean r^2 between adjacent SNP markers was 0.45 in C0.

Index population and 0.36 for the Stover Index population. Based on the 58 or 59 SNP markers used in MARS, the inbreeding coefficients in Cycle 3 of MARS were 0.33 for the Yield + Stover Index population and 0.38 for the Stover Index population. When estimation of inbreeding in the GWS populations was restricted to the 58 or 59 SNP markers used in MARS, the inbreeding coefficients became very close to those observed in the MARS cycles.

Inbreeding in the GWS and MARS populations did not cause inbreeding depression because the performance of each cycle was evaluated based on testcross performance (to an unrelated inbred) rather than per se performance. Inbreeding in the GWS and MARS populations was expected to restrict long-term selection response. However, the objective in our study was to maximize short-term response and we therefore selected and intermated only 10 recombinant inbreds or individual plants in each cycle. In the absence of selection, intermating 10 recombinant inbreds in Cycle 0 would have led to an inbreeding coefficient of 0.10, whereas intermating 10 plants in each succeeding cycle would have increased inbreeding by 0.05. The higher rates of loss of heterozygosity in the GWS and MARS populations (Table 2) therefore also reflected the effect of selection.

The GWS approach exploits linkage disequilibrium among markers, and a minimum r^2 between adjacent markers of 0.10 to 0.20 has been recommended for GWS (Calus et al., 2008; Hayes et al., 2009). With 287 SNP markers used in GWS in the current study, the mean r^2

between adjacent markers was 0.45 in Cycle 0. This high level of initial linkage disequilibrium explains why high prediction accuracies were previously found in Cycle 0 across a wide range of numbers of markers. In particular, for a population size of $N = 178$ recombinant inbreds in Cycle 0, prediction accuracies for the Yield + Stover Index were 0.66 with 256 markers, 0.68 with 512 markers, and 0.68 with 768 markers (Lorenzana and Bernardo, 2009). For the Stover Index, prediction accuracies were 0.56 with 256 markers, 0.63 with 512 markers, and 0.64 with 768 markers. Given these previous results for the Cycle 0 population (Lorenzana and Bernardo, 2009), we considered 287 SNP markers and $N = 223$ recombinant inbreds as adequate for our experiment. The mean r^2 between adjacent markers decreased only very slightly to 0.44 in the Cycle 1 populations and to 0.39 to 0.40 in the Cycle 2 populations (Table 2). In addition to a slow approach to linkage equilibrium for closely linked marker loci, inbreeding and selection could also have helped maintain a high r^2 between adjacent markers.

The RR-BLUP approach we used to model SNP effects involved the biologically inaccurate but computationally convenient assumptions that the SNP effects had equal distributions and that epistasis was absent (Meuwissen et al., 2001). The RR-BLUP approach is in contrast to a Bayesian estimation framework, which allows for unique variances for each marker locus (Meuwissen et al., 2001). Compared to the RR-BLUP approach used here, previous work in maize (Lorenzana and Bernardo, 2009; Guo et al., 2012), barley (Lorenzana and Bernardo, 2009; Iwata and Jannink, 2011; Heslot et al., 2012), wheat (Heffner et al., 2011; Heslot et al., 2012), and oat (Asoro et al., 2011) have shown little to no improvement from Bayesian estimation. Using the same B73 \times Mo17 testcross population we studied, Lorenzana and Bernardo (2009) found that RR-BLUP performed as well or better than an empirical Bayes method that allowed epistasis and unequal marker variances (Xu, 2007). Bayesian procedures for GWS have been found useful for traits with major QTL (Hayes et al., 2010; Resende et al., 2012), but no major QTL for yield, agronomic traits, or stover-quality traits were detected in the Cycle 0 population (Lorenzana et al., 2010).

Wet Chemistry versus Near-infrared Reflectance Spectroscopy for Measuring Stover Quality

The NIRS technology enables many samples to be assayed very efficiently, whereas the wet chemistry process is time-consuming and more expensive (Hames et al., 2003). In particular, the cost of phenotyping for Glucose, Glucose Release, and Lignin in our study was \$153 per sample with wet chemistry and was less than \$5 per sample with NIRS. Strictly speaking, however, the NIRS predictions are valid only for populations that have the same spectral and compositional

characteristics as the training population used to develop the NIRS prediction equations. If the spectral and compositional characteristics change through selection, the original NIRS prediction equations may no longer be valid. In accordance with commonly accepted procedures, we attempted to account for such changes by choosing 25 stover samples from the 2010 field trials, adding these 25 samples to the original set of 154 calibration samples from Cycle 0 (evaluated in 2007), and obtaining new NIRS calibration equations for Glucose, Glucose Release, and Lignin. As previously mentioned, the correlation between NIRS predictions and wet chemistry measurements remained high for Glucose ($r = 0.82$) but decreased substantially for Glucose Release ($r = 0.37$) and Lignin ($r = 0.31$) even with the updated NIRS calibration equations. Further research is needed on the use of NIRS when spectral and compositional characteristics of plant biomass change through selection.

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