Epistasis and Improved Prediction of Performance in Corn

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Abstract

To be useful, adding epistatis to a prediction model must increase predictive power. The objectives of this study were to determine: 1) using partial least squares techniques, whether the ability to predict performance can be increased by including epistasis in a prediction model; 2) whether relaxing the probability level for inclusion of a marker or interaction in a model from 0.001 to 0.01 to 0.05 would increase predictive power; 3) whether the proportion of variability accounted for could be raised to a level useful in breeding; 4) whether molecular marker models based on one year's phenotypic data could be used to predict performance in a second year; and 5) whether models based on per se data would be useful in predicting testcross performance. Data for protein, oil, and starch, were obtained from 500 S_2 lines and their testcrosses from the crosses of Illinois High Oil (IHO) x Illinois Low Oil (ILO) and of Illinois High Protein (IHP) x Illinois Low Protein (ILP) corn (*Zea mays* L.) strains. Increasing the probability level for detection of significant markers and epistatic effects from 0.001 to 0.01 to 0.05 significantly increased predictive power. Adding epistasis to a model significantly increased predictive power when models were based on data over all year and locations, when models based on one year were used to predict a second year, or when per se data were used to predict testcross performance. With epistasis in the model and P=0.05, correlations of predicted and observed means were high enough to suggest they might be useful in breeding. Marker models based on one year's data produced correlations nearly as high as phenotypic correlations between years. While epistasis significantly improved performance of models used to predict testcross performance from per se performance, the proportion of variability accounted for was somewhat lower than when predicting performance in different years.

Introduction

Epistasis is defined as the interaction between alleles at different loci. Epistasis was shown to be present for oil, protein, and starch in crosses of Illinois High Oil (IHO) x Illinois Low Oil (ILO) and of Illinois High Protein (IHP) x Illinois Low Protein (ILP) strains of corn (*Zea mays* L.) (Dudley, 2008). There have been a number of other studies demonstrating the presence of epistasis in a number of species for many different traits (e.g., Xu and Jia, 2007; Radoev et al., 2008; Rouzic et al. 2008; Melchinger et al. 2008; Melchinger et al., 2007). Thus, epistasis is important. However, there is no clear method for utilizing information from epistatic interactions to predict performance in a breeding program and no studies exist where epistasis was included in models used for prediction of performance. For knowledge of presence of epistasis to be useful in breeding for quantitative traits, adding epistasis to a model used to predict performance should increase the accuracy of prediction. In

addition, useful predictive models should be effective in predicting performance in years other than those in which the models were developed. For certain traits, prediction of testcross performance from per se performance would be useful.

In any study involving large numbers of molecular markers, collinearity among markers and over-parameterization of models become problems. These problems are amplified when all possible two-way interactions are considered. In searching for a way to reduce this problem and to develop a prediction model, we used partial least squares. Partial least squares is a method for analyzing data in which there are more predictor variables than observations and/or where there is collinearity in the predictor variable matrix. The method was first developed in the 1960's and 1970's to address problems in econometrics. It was subsequently widely used in chemometric and spectrometric modeling (Rosipal and Kramer, 2006). More recently, partial least squares has been successfully applied to problems in genomics and proteomics (Boulesteix and Strimmer, 2007; Perez-Enciso and Tenenhaus, 2003; Perez-Enciso et al., 2003). Although the method would seem to be well adapted to problems of prediction of performance in a marker assisted breeding program, particularly where large numbers of markers are used, no reports of such use were found in the literature. However, Bjornstad et. al. (2004a, 2004b) used partial least squares to relate marker and phenotypic information in QTL analysis and Mateo, et al. (2006) used it in analyzing QTL x environment interactions.

Another question of importance in a marker breeding program is the number of markers to use and whether to use all possible markers. Based on simulation results, Bernardo and Yu (2007) suggested that using all markers in a set would lead to greater gain than using only those identified as significant. In a simulation study, Hospital et al. (1997) found that reducing the type I error probability level for inclusion of a marker in a model increased relative efficiency of marker assisted selection.

The objectives of this paper are to determine: 1) using partial least squares techniques, whether the ability to predict performance can be increased by including epistasis in a prediction model; 2) whether increasing the numbers of markers and marker x marker epistatic interactions by relaxing the probability level (P) for inclusion of a marker or interaction in a model would increase predictive power; 3) whether the proportion of variability accounted for could be raised to a level useful in breeding; 4) whether a model based on results from one year can be effectively used to predict a second year's performance; and 5) whether models based on per se data would be useful in predicting testcross performance.

Materials and Methods

Details of the development of progenies and markers used in this study have been reported as have the experimental designs used (Laurie et al., 2004; Clark et al. 2006; Dudley et al., 2007). To summarize, crosses were made between plants (5-7 plants from each parental population) from generation 70 of the IHO and ILO corn strains and between plants from generation 70 of the IHP and ILP strains. The IHOxILO cross was random-mated 10 times while the IHPxILP cross was random-mated 7 times. Five hundred S2 lines were developed from each cross. For protein, oil, and starch, each line was evaluated as a line per se (PS) and in a testcross (TC) to a Monsanto inbred line tester. The IHOxILO lines per se and testcross progenies were evaluated at three locations for two years for oil, protein, and starch. The IHPxILP lines were evaluated as lines per se for two years at three

locations while the testcrosses were evaluated for only one year in three locations for oil, protein, and starch. In each year location combination, an α (0, 1) design with two replications and 50 blocks of 10 lines per replication was used. Plots consisted of 15 plants in a row 5.3 m long with 0.76 m between rows. Starch, protein, and oil concentrations were measured on a sample of grain from each plot using near-infrared transmittance.

For each S2 line, DNA was extracted from seedling tissue germinated from a bulk of about 50 seeds and SNP genotypes were obtained using procedures described by Laurie et al. (2004). Markers were chosen largely on the basis of allele frequency difference between the parents of the cross. The frequencies were estimated by genotyping a random sample of 24 individuals each from the IHP, ILP, IHO, and ILO populations as described by Laurie et al. (2004) for IHO and ILO. For the IHOxILO line, 479 SNP markers were used while 499 SNP markers were used for the IHPxILP progenies.

As in Dudley (2008), PROC MIXED in SAS software (version 9.1, SAS Institute Cary, NC), was used to calculate PS or TC line BLUPs of all phenotypic traits. Then, SAS PROC GLM was used to estimate single-locus and pair-wise marker effects. The model for each single locus analysis was

$$\text{Trait} = Mi + \text{Residual} \quad (i = 1, 2, \ldots, m) \quad (1)$$

where Trait is a line BLUP value, Mi is the ith marker genotype, and m is the total number of markers. For pair-wise marker analysis the model was

$$\text{Trait} = Mi + Mj + Mi * Mj + \text{Residual} \quad (i = 1, 2, \ldots, m-1; j = i+1, \ldots.., m) \quad (2)$$

where Mi * Mj indicates the interaction effect of the Mi and Mj marker genotypes. In the above models, marker and marker interaction effects were assumed fixed. Model (1) was used to identify significant markers; whereas model (2) was used to identify significant interaction effects, irrespective of the significance of the main effects. That is, in model (1), the single-locus marker effect is unadjusted for any interaction effect; while, in model (2), the interaction effect is adjusted for the single-locus marker main effects. For both models (1) and (2), significant markers and interactions were identified at three probability levels: P=0.001, 0.01, and 0.05.

Markers and interactions significant at each of these levels were then entered into two types of prediction models comprised of: (1) significant single-locus marker predictors (NOEP, no epistasis models); or (2) significant single-locus plus significant pair-wise marker interaction predictors (EP, epistasis models). Because of sampling, some pair-wise marker interaction genotypic arrays had empty cells. These interactions were not included in the EP prediction models.

The prediction models were evaluated by partial least squares regression using SAS PROC PLS as described by Dudley and Johnson (2009). In partial least squares regression, the original data are decomposed into factors extracted from the dependent x predictor variables cross products matrix. With stipulation of the appropriate options, PROC PLS produces a prediction model, comprised of a minimum number of factors (not greater than 15), which is not significantly different at the 0.10 probability level from the model with the lowest mean square error of prediction. In the analysis of all models, the following PROC PLS options were stipulated: method=simpls; cv=split; cvtest. The dependent variables in the models were the testcross (TC) and per se (PS) BLUP values for protein,

oil, and starch for each of the 500 lines in the population sample from the IHPxILP cross and the 500 lines from the population sample from the IHOxILO cross.

NOEP and EP prediction models were evaluated at each probability level for each trait in the TC and PS populations. In addition, NOEP models containing all markers (P=1.0) were evaluated for each trait of the TC and PS populations. Because of software limitations on the number of variables accommodated, evaluation of EP models with all possible two-way interactions was not possible. Note that the prediction models have the form of multiple regression equations in which the independent variables entered are conditional upon significance in preliminary single-locus or pair-wise marker analyses.

For each model evaluation, 500 bootstrap (random sampling with replacement) samples were obtained in which the first 400 lines from each sample were used for calibration and the remaining 100 lines comprised the target set for prediction. Pearson correlations between predicted and observed values of the 100 lines were calculated using PROC CORR in SAS. Thus, 500 correlations of observed with predicted values were obtained for each model. Using PROC MEANS in SAS, means, 95% confidence intervals and minimum and maximum values for the 500 correlations were obtained. Differences between models, probability levels, or crosses were declared significant if the 95% confidence intervals did not overlap. For ease of presentation, correlations were squared to obtain the percentage of variability accounted for by a particular model (r^2) and comparisons between models were made using r^2 values.

As an additional indication of the importance of marker interactions, the number of significant interactions containing 0, 1, or 2 markers significant in single-locus tests was determined as well as the total number of markers involved in significant interactions.

In addition to the work just described, prediction models were developed using individual year data to predict alternate years. In this study, BLUPs were calculated for each individual year using data from all locations within a year for oil, protein, and starch. Both per se and testcross data were used for the IHOxILO cross. Only per se data were used for the IHPxILP cross because testcross data were available from only one year. Based on marker data and individual year BLUPs, both NOEP and EP models developed using PROC PLS were used to predict a second year's performance. For the IHOxILO cross, data from 2001 were used to predict performance in 2002, and data from 2002 were used to predict performance in 2001. In the IHPxILP cross 2002 data were used to predict 2003 performance and 2003 data were used to predict 2002 performance. In all cases, only markers and interactions significant at the 0.05 probability level were used. Pearson correlations between predicted and observed and between BLUPs in different years were obtained for 100 bootstrap samples in each comparison.

The importance of epistasis in predicting testcross performance based on per se data was studied. In this study, marker data and per se performance data were used to develop models which were then used to predict testcross performance for both EP and NOEP models. Phenotypic correlations between per and testcross data were also obtained. As in the study comparing years, 100 bootstrap samples were used. In all cases data were expressed as percent of the variability accounted for based on a squared Pearson correlation.

Results and Discussion

Significant genetic variance was found for all traits and heritabilities were high enough to allow identification of QTL (Table 1). Data in Table 1 were reported by Dudley (2008).

Increasing the p-value threshold (P) used to determine marker or interaction effects for inclusion in the PLS analysis from 0.001 to 0.01 significantly increased the percentage of variability accounted for in all comparisons except for the difference between the 0.001 and 0.01 probability levels for starch PS in the IHPxILP cross (Table 2). For the NOEP model, changing P from 0.01 to 0.05 significantly increased the percentage of variability accounted for in all comparisons. For starch, increasing P from 0.01 to 0.05 for the EP model significantly increased the percentage of variability accounted for in both per se and testcross models in both crosses, but for protein, a P increase from 0.01 to 0.05 did not increase variability accounted for in either testcross progeny or in the IHOxILO cross for per se progeny. Only the per se progeny in IHPxILP failed to show a significant increase for oil. For all comparisons, the increase in variability accounted for from 0.001 to 0.01 was greater than from 0.01 to 0.05. Although data were not obtained for P values >0.05 for the EP model, the small change for the EP model from 0.01 to 0.05, and the small, inconsistent change from 0.05 to 1.0 for the NOEP model, suggest that increasing P beyond 0.05 is not likely to improve prediction accuracy. Thus, while most QTL studies have stressed the importance of reducing Type 1 errors by using stringent probability levels, these data suggest that for prediction of performance, Type 2 errors, within limits, may be as important as Type 1 errors. This result agrees with the results of a simulation study by Hospital et al. (1997) who found that increasing the probabilities of allowing entry of a marker and keeping a marker in the model increased relative efficiency of selection. Bernardo and Yu (2007) suggested, based on simulation results, that increasing the number of markers included in the model to P=1.0 (using all markers) should increase gain relative to using markers selected at P=0.2, 0.3, or 0.4. They did not consider epistatic interactions. However, in this study, including all markers in the model significantly increased the percentage of variability accounted for in only six of twelve comparisons. Even when all markers were included in a NOEP model, the percentage of variability accounted for was less than for an EP model with P=0.05 except for protein per se and starch per se in the IHOxILO cross further indicating the importance of epistasis. Because at P=0.05 percentage of variability accounted for was generally maximized, P=0.05 was used in studying prediction between years and between per se and testcross performance.

Did including epistasis in the model increase the percentage of the variability accounted for? To answer this question, four different traits, two different crosses, three different probability levels and, two types of progenies were used. Thus, there was replication across traits, types of progenies, and crosses. In addition, the importance of epistasis in predicting performance in a second year using a first year's data and in predicting testcross performance from per se performance was measured. When compared to the NOEP model, the addition of epistasis significantly increased the percentage of variability accounted for in all cross-trait-generation-probability level combinations when: 1) all data were used for prediction (Table 3); when prediction of performance in a second year was measured (Table 4); and when testcross performance was predicted from per se performance (Table 5). Thus, in all cases, including epistasis in the model increased its predictive efficacy.

To compare traits when all data were used to develop models, the mean gain in percentage of variability accounted for by including epistasis in the model for each trait at each probability level was

obtained by averaging over types of progeny and crosses. Gains for oil were slightly higher than for protein and starch (Table 3). Average gain for the IHPxILP cross was approximately twice that for the IHOxILO cross regardless of P level. The reason for the greater impact of epistasis in the IHPxILP cross is not apparent. Unlike the results when all data were used, the gain due to including epistasis in the model was greater in IHOxILO than in IHPxILP when one year was used to predict a second year. The reason for the difference between all data and the year prediction data in the value of epistasis in different crosses is not apparent although it should be noted that the years involved in the IHOxILO cross were 2001 and 2002 and the years involved in the IHPxILP cross were 2002 and 2003. The gain from including epistasis in the model was generally smaller for prediction of testcross performance from per se performance than for the other two predictions (Table 5). In addition, the percent of variability accounted for was lower. This result may be due to the effect of the tester on the epistatic effects.

The importance of epistasis suggests that interacting gene networks may be important. If so, markers included in significant interactions may not be significant in single marker analysis. Nearly all the markers used (a minimum of 475/479 for IHOxILO and 497/499 for IHPxILP) were included in a significant interaction at the P=0.05 level (Table 6) in the analysis of all data even though many fewer were significant in the single marker analysis. Nearly half the significant interactions for all traits included only one significant marker (Table 6). For oil, protein, and starch, the percentage of interactions with no significant markers was higher in the IHOxILO cross than in the IHPxILP cross. Conversely the percentage of interactions with both markers significant was higher in IHPxILP than in IHOxILO. The reason for the difference between crosses is not clear. Because as many as 40 percent of the significant interactions did not contain a significant marker, nearly half contained only one significant marker, and nearly all markers were involved in a significant interaction, evaluation of all possible marker interactions rather than just those between markers found significant in single marker analysis is important. This result agrees with the conclusion of Xu and Jia (2007) that whether two loci interact does not depend on whether or not the loci have individual main effects and casts doubt on the common practice of estimating epistatic effects only for pairs of loci of which both have significant main effects. The differences between crosses in the increased predictive efficacy by including epistasis and the difference between results from using all data and predicting a second year from a first should provide a cautionary tale for systems biologists. Systems may be context dependent, i.e. results may vary depending on the cross and the environments studied.

With regard to the third objective dealing with determination of usefulness of the models in plant breeding, note again that addition of epistatic terms in the model consistently produced a gain in predictive accuracy from a non-epistatic base. Though these gains are statistically significant, two questions remain: (1) are the general magnitudes of the base correlations large enough to indicate practical prediction accuracy? and (2) does the gain in accuracy justify inclusion of epistatic terms in the model? To address these questions, we compare the results reported herein from analysis with results reported in the literature regarding the correlations between early and late generation testcross yields in corn. Jensen et al. (1983) compared S2 and S5 generation testcrosses at a number of locations and reported r^2 values that ranged from 19.4 to 67.2 percent; Hallauer and Lopez-Perez (1979) found r^2 values ranging from 2.9 to 31.4 percent in correlations of S1 and S8 testcross yield; and Rodriquez and Hallauer (1991) reported an r^2 value of 9.6 percent for the correlation of S0 and S4 testcross yield. The mean values obtained in this study at the P=0.05 level (Table 2) using the models including epistasis, whether using all phenotypic data, predicting one year from another, or predicting testcross

performance from per se performance, are as high or higher than those reported for early generation testing. Furthermore, the minimum values obtained for epistatic models at the P=0.05 level (Data not shown) are generally in the range of values reported for early generation testing for grain yield.

Based on our long familiarity with the corn breeding industry, we believe that early generation testing is a wide-spread, common practice, and, because of its pervasiveness, has been found to be useful in prediction of advanced generation testcross performance. Hence, because the r^2 values reported herein are well within the range of values reported for early generation testing, we conclude that prediction from marker models similar to those described here will be useful.

To evaluate the usefulness of epistatic models in predicting a second year's performance from the first year's performance, epistatic models were compared with the percent of variability accounted for by the phenotypic correlations between years (Table 4). The increased variability accounted for by the phenotypic correlation was higher for the IHPxILP cross than for the IHOxILO cross for all traits. For the IHOxILO cross, the predicted model accounted for from 85-99 percent of the variability accounted for by the phenotypic correlation while for the IHPxILP cross only about 80 percent of the variability accounted for by the phenotypic correlation was accounted for by the marker model. Thus, correlations for the marker based model were nearly as good as the phenotypic correlations in both crosses. The percent variability accounted for in a second year based on a first year's data for oil in the IHOxILO cross and for protein and starch in the IHPxILP cross seem high enough to be useful.

The lowest percentages of variability accounted for either by phenotypic correlations or by predictive models were obtained by the correlations between per se and testcross data (Table 5). However, the predictive models came closer to accounting for the variability accounted for by phenotypic correlations than in the comparisons between years. In fact, only half of the comparisons showed a significant increase of the phenotypic correlations over the predicted models with epistasis.

Because inclusion of epistatic terms consistently and significantly increased the correlation of observed and predicted performance regardless of the comparisons being made, we believe that consideration of epistasis in predictive models has considerable merit.

Conclusions and Implications

Adding epistasis to a model for predicting performance significantly increased predictive power for oil, protein and starch in per se and testcross progenies in two different crosses, IHOxILO and IHPxILP when all data were used to develop a model, when one year's data were used to predict a second year, and when per se progenies were used to predict testcross performance. Thus, from a plant breeding perspective, epistasis is important. Increasing the probability level for identifying significant markers and epistatic interactions from P=0.001 to P=0.01 significantly increased the predictive power for all comparisons. The increase to P=0.05 increased predictive power for all traits in the NOEP model and for half the traits in the EP model. This likely resulted from the inability to detect relatively small epistatic interactions at the 0.001 and 0.01 probability levels.

The finding that nearly all markers used were involved in a significant interaction at the 0.05 level and that as many as 75% of the significant interactions included at least one marker not found significant in single marker analysis suggests that genes controlling the traits studied may be a part of a

complex gene network involving segments of nearly all parts of all chromosomes. This further stresses the importance of evaluating all possible marker interactions rather than only those between markers significant in single marker analysis.

The mean percentages of variability accounted for at the 0.05 level for all traits and the epistatic model, both when all data were used and when one year's data were used to predict a second year, were large enough to suggest epistatic models had predictive power as good as early testing and thus could be useful in plant breeding. However, some caution is needed. The progenies studied resulted from random-mating the IHOxILO cross 10 times and the IHPxILP cross 7 times. In most corn breeding programs, marker and performance data are collected from early generation progenies or from doubled haploid lines, neither of which have been random mated. Thus the unbroken linkage blocks are much larger than in random-mated progenies. These larger linkage blocks may contain blocks of genes for which interactions will average out and the usefulness of the epistatic model may be lower. This question merits further research. Dudley and Johnson (2009) suggested a second caution concerning effects of environments. However, correlations using data obtained in one year to predict performance in a second year were nearly as large as those obtained when all data were used in the predictive model. A third caution involves the number of progenies evaluated. In this study, 500 progenies were evaluated in each cross. This is much larger than the usual number of progenies used in a marker based corn breeding program. Thus, it may be necessary to increase the number of progenies to successfully identify epistatic interactions useful in a breeding program. Despite these limitations, the predictive power of the epistatic model when markers and interactions were identified at the P=0.05 level is high enough to justify considering inclusion of epistatic effects in marker assisted breeding programs.

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Table 1. Summary statistics from IHPxILP and IHOxILO studies.

Trait	Cross	Generation‡	Mean§	Genetic Variance	H^2†	C.V. § (%)
			g kg^{-1}			
Oil	IHOxILO	PS	70.6	119.0**	0.93	9.7
	IHOxILO	TC	49.2	10.2**	0.94	4.5
	IHPxILP	PS	44.1	9.88**	0.89	8.7
	IHPxILP	TC	45.3	3.03**	0.77	2.3
Protein	IHOxILO	PS	129.0	71.7**	0.88	6.1
	IHOxILO	TC	126.0	21.4**	0.77	6.1
	IHPxILP	PS	134.0	219.0**	0.92	6.7
	IHPxILP	TC	103.0	32.0**	0.83	6.7
Starch	IHOxILO	PS	585.0	716.0**	0.84	4.4
	IHOxILO	TC	677.0	33.2**	0.83	1.0
	IHPxILP	PS	681.0	114.0**	0.94	1.0
	IHPxILP	TC	699.0	23.2**	0.79	0.8

**=Significant at the 0.01 probability level.
† = heritability on an entry mean basis.
‡ PS=per se generation, TC=testcross generation.
§ C.V.=coefficient of variation.

Table 2. Change in % variability accounted for from P=0.001 to P=0.01 (G001-01), from P=0.01 to P=0.05 (G01-05) and from P=0.05 to P=1.0 (G05-1) for non-epistatic (NOEP) and epistatic (EP) models.

Trait	GEN	CROSS†	G00101NOEP	G00101EP	G0105NOEP	G0105EP	G051NOEP‡
Oil	PS	HOLO	13.9*	17.4*	5.2*	3.9*	-0.7ns
Oil	PS	HPLP	3.8*	20.4*	5.8*	0.2ns	0.8ns
Oil	TC	HOLO	6.7*	14.3*	6.0*	2.8*	1.2ns
Oil	TC	HPLP	3.0*	18.9*	5.4*	3.1*	3.4*
Protein	PS	HOLO	8.7*	16.4*	5.9*	1.5ns	17.6*
Protein	PS	HPLP	4.0*	13.9*	2.4*	2.2*	1.8ns
Protein	TC	HOLO	9.4*	30.9*	7.0*	-0.6ns	0.5ns
Protein	TC	HPLP	5.1*	18.1*	1.7*	0.0ns	3.0*
Starch	PS	HOLO	9.1*	12.3*	2.9*	7.0*	9.7*
Starch	PS	HPLP	0.7ns	11.4*	4.3*	1.9*	0.0ns
Starch	TC	HOLO	6.6*	21.8*	6.4*	2.3*	1.9*
Starch	TC	HPLP	13.6*	34.0*	1.8*	1.5*	2.1*
Yield	TC	HOLO	9.5*	21.1*	3.6*	1.5ns	0.5ns
Yield	TC	HPLP	7.9*	36.0*	3.7*	1.1ns	-2.0*

*=95% confidence intervals between EP and NOEP models did not overlap

† HOLO=IHOxILO; HPLP=IHPxILP.

‡ Only the NOEP model was available for the difference between P=0.05 and P=1.0

Table 3. Percentage of variability accounted for by prediction model. Markers and interactions in the model selected at three different probability levels (0.001, 0.01, 0.05). Values are averages of 500 bootstrap samples.PS and TC refer to per se and testcross progeny, respectively. NOEP, EP, and EPGAIN refer to models without epistasis, with epistasis, and EP-NOEP, respectively.

			P=0.001			P=0.01			P=0.05			P=1.0‡
Trait	Progeny	Cross†	NOEP	EP	EPGAIN	NOEP	EP	EPGAIN	NOEP	EP	EPGAIN	NOEP
Oil	PS	HOLO	28.6	36.6	8.0*	42.5	54.0	11.5*	47.7	57.9	10.2*	47.1
	PS	HPLP	32.9	47.5	14.5*	36.7	67.9	31.2*	42.5	68.1	25.6*	43.3
	TC	HOLO	38.6	45.6	7.0*	45.3	59.9	14.6*	51.3	62.7	11.5*	52.4
	TC	HPLP	29.9	45.4	15.5*	32.9	64.3	31.4*	38.3	67.4	29.1*	41.7
Protein	PS	HOLO	20.8	27.1	6.4*	29.5	43.6	14.1*	35.4	45.0	9.6*	53.0
	PS	HPLP	46.0	56.0	10.0*	50.0	69.9	19.9*	52.4	72.1	19.7*	54.2
	TC	HOLO	22.4	23.1	0.8*	31.8	54.0	22.2*	38.8	53.4	14.6*	39.3
	TC	HPLP	42.5	51.0	8.5*	47.6	69.1	21.4*	49.3	69.1	19.8*	52.3
Starch	PS	HOLO	33.1	35.0	1.9*	42.1	47.3	5.2*	45.0	54.3	9.3*	54.8
	PS	HPLP	49.6	60.2	10.7*	50.3	71.6	21.3*	54.6	73.4	18.8*	54.6
	TC	HOLO	33.2	34.9	1.8*	39.8	56.7	16.9*	46.2	59.0	12.7*	48.2
	TC	HPLP	34.5	36.7	2.3*	48.0	70.7	22.7*	49.8	72.2	22.4*	52.0

*=95% confidence intervals between EP and NOEP models did not overlap. † HOLO=IHOxILO; HPLP=IHPxILP; ‡=model with all single markers included but no epistatic interactions.

Table 4. Percent of variability account for by a marker model based on one year used to predict a second year. Means of 100 bootstrap samples. Data from the first year in the years column was used to predict performance in the second year.

				Predicted†			Phenotypic ‡		
Trait	Gen	Cross	Years	NOEP	EP	EPGAIN	PHEN	NOEPGAIN	EPGAIN
Oil	PS	HOLO	2001V2002	48.3	70.1	21.8*	75.9	27.6*	5.8*
Oil	PS	HOLO	2002V2001	49.8	70.7	20.9*	75.6	25.8*	4.9*
Oil	TC	HOLO	2001V2002	52.6	76.5	23.9*	79.7	27.1*	3.2*
Oil	TC	HOLO	2002V2001	53.8	74.9	21.1*	79.5	25.7*	4.6*
Protein	PS	HOLO	2001V2002	26.6	55.2	28.6*	64.3	37.7*	9.1*
Protein	PS	HOLO	2002V2001	34.3	56.0	21.7*	64.1	29.8*	8.1*
Protein	TC	HOLO	2001V2002	24.8	36.3	11.5*	38.2	13.4*	1.9*
Protein	TC	HOLO	2002V2001	25.3	37.6	12.3*	37.9	12.6*	0.3ns
Starch	PS	HOLO	2001V2002	39.8	49.8	10.0*	54.3	14.5*	4.5*
Starch	PS	HOLO	2002V2001	33.9	48.8	14.9*	54.2	20.3*	5.4*
Starch	TC	HOLO	2001V2002	35.5	50.2	14.7*	53.2	17.7*	3.0*
Starch	TC	HOLO	2002V2001	35.4	51.0	15.6*	53.1	17.7*	2.1*
Oil	PS	HPLP	2002V2003	42.7	51.2	8.5*	64.9	22.2*	13.7*
Oil	PS	HPLP	2003V2002	43.0	51.3	8.3*	64.9	21.9*	13.6*
Protein	PS	HPLP	2002V2003	54.4	60.9	6.5*	75.8	21.4*	14.9*
Protein	PS	HPLP	2003V2002	53.4	61.0	7.6*	75.8	22.4*	14.8*
Starch	PS	HPLP	2002V2003	57.1	62.9	5.8*	78.3	21.2*	15.4*
Starch	PS	HPLP	2003V2002	53.3	63.5	10.2*	78.3	22.5*	14.8*

*=Gain in % variability significant as measured by non-overlapping 95% confidence intervals. Under predicted columns gain is measured as difference between EP and NOEP models. Under Phenotypic columns gain is measured as the difference between % variability accounted for by phenotypic correlation and that accounted for by the NOEP model (NOEPGAIN) or by the difference between the phenotypic correlation and the EP model (EPGAIN).

† Data from predicted correlations between years. ‡Data from phenotypic correlations.

Table 5. Percent of variability accounted for in testcrosses by predicted values based on EP and NOEP models from per se data and by phenotypic correlations. Mean of 100 bootstrap samples.

		Predicted‡			Phenotypic§		
Trait	Cross†	NOEP	EP	EPGAIN	PHEN	NOEPGAIN	EPGAIN
OIL	HOLO	41.4	51.6	10.2*	53.1	11.9*	1.4*
OIL	HPLP	30.0	39.8	9.8*	39.7	9.3*	0.3ns
PROTEIN	HOLO	16.0	23.9	7.9*	24.6	8.6*	0.7ns
PROTEIN	HPLP	45.5	51.7	6.2*	52.2	6.7*	0.5ns
STARCH	HOLO	13.7	15.9	2.2*	19.9	6.2*	4.0*
STARCH	HPLP	40.1	44.8	4.7*	48.7	8.6*	3.9*

*=significant gain based on non-overlapping 95% confidence intervals.

† HOLO=IHOxILO, HPLP=IHPxILP.‡

‡ NOEP=% variability accounted for by the non-epistatic model, EP=% accounted for by the epistatic model; EPGAIN= gain by including epistasis in the model.

§ PHEN=% variability accounted for by the phenotypic correlation between per se and testcross data; NOEPGAIN=gain of phenotypic correlation over the NOEP model, EPGAIN=gain of phenotypic correlation over the EP model.

Table 6. Number of significant interactions at 0.05 level (NI), number of markers significant at 0.05 level (NM), number of markers included in at least one significant interaction (NMI), and percentage of significant interactions with 0, 1, or 2 significant markers (NSMI) in the interaction.

			Number			NSMI		
Trait	Cross†	Generation	NI	NM	NMI	0	1	2
Oil	HOLO	PS	6303	197	476	34.5	48.9	16.6
	HPLP	PS	8008	249	498	24.1	49.1	26.8
Protein	HOLO	PS	6432	204	476	33.4	50.4	16.2
	HPLP	PS	7844	226	498	16.8	48.6	34.7
Starch	HOLO	PS	6183	193	475	34.7	46.7	18.6
	HPLP	PS	7260	295	497	17.9	48.6	33.5
Oil	HOLO	TC	6410	181	476	31.4	50.0	18.6
	HPLP	TC	6800	292	497	29.0	49.1	21.9
Protein	HOLO	TC	6142	194	476	35.4	48.4	16.2
	HPLP	TC	8282	291	497	17.7	48.8	33.5
Starch	HOLO	TC	6469	190	476	34.9	49.3	15.8
	HPLP	TC	7302	268	497	21.3	48.9	29.8

† HOLO=IHOxILO; HPLP=IHPxILP.