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Allozyme marker loci associated with favorable alleles for grain yield in maize *

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Summary. The evaluation of germplasm to identify its potential as a source of new favorable alleles is a time-consuming phase of maize hybrid breeding programs. The objective of this paper was to study the relationship between allozyme diversity and quantitative estimators of the relative number of new favorable alleles for grain yield, present in donor lines but not present in the elite hybrid. Twenty-two maize inbred lines representing heterotic groups from the United States (US) and Yugoslavia (YU) were used as donors to estimate the presence of new favorable alleles for grain yield improvement for the hybrid B73 × Mo17. In a second experiment, a 15-line diallel was grown, and 13 single crosses differing in allozyme relatedness measure (ARM) and heterotic grouping were considered as targets to be improved by the remaining 13 lines. Minimally biased estimates of new favorable alleles for grain yield (μG) and ARM values were made for all donor lines within each target hybrid. Donor lines were grouped in four allozyme-pedigree classes for each target hybrid to compare the effect of allozyme diversity with pedigree diversity. Pedigree dissimilarities had significant effects on μG estimates. Dissimilar pedigree classes had higher μG estimates than similar pedigree classes. Allozyme differences between donor inbred lines and target hybrids had inconsistent effects on μG estimates. Significant differences in μG estimates among allozyme classes were found for 31% of the target hybrids. Classes with similar allozymes had higher μG estimates more frequently than classes with disimilar allozymes. Correlation coefficients between μG estimates and ARM values were low and not significant for 12 of the 14 target hybrids.

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Introduction

Identification of germplasm with new favorable dominant alleles not present in parents of elite single crosses is an essential part of maize inbred improvement programs. Statistical procedures for estimation of the relative number of loci with new dominant favorable alleles for quantitative traits present in donor germplasm but absent in the elite hybrids have been proposed by Dudley (1984a, b; 1987a, b) and by Gerloff and Smith (1988). These statistics were found useful for discriminating among populations (Dudley 1988) and inbreds (Zanoni and Dudley 1989; Mišević 1989a, b), having new favorable alleles for the improvement of an elite maize single cross. All proposed estimators require evaluation of crosses of donor inbreds with the parents of the elite single cross, as well as of the elite single cross. Most estimators also require evaluation of the parents of elite single cross. For quantitative traits where favorable alleles are recessive, evaluation of donor inbreds is also necessary.

The relationship between allozyme dissimilarity of maize inbred and hybrid traits has been studied to establish a procedure for predicting hybrid performance (Hunter and Kannenberg 1971; Hadjinov et al. 1982; Frei et al. 1986; Lamkey et al. 1987). Results have been inconclusive. Correlations between the enzyme diversity index (EDI) and hybrid yield were poor (Hadjinov et al. 1982; Hunter and Kannenberg 1971). No association between the modified Roger's distance (MRD) of allelic

frequencies of high and low yielding inbred lines from Iowa Stiff Stalk Synthetic and the hybrid yield was found (Lamkey et al. 1987).

Goodman and Stuber (1983) concluded that the inherent difficulty associated with the effect of allozyme diversity on grain yield in maize occurs because pedigree diversity is often correlated with allozyme diversity. Pursuing this idea, Frei et al. (1986) studied grain yield in single crosses between lines with similar and dissimilar pedigrees. They found significant associations between allozyme diversity and higher yields. However, the predictive value of allozyme was most useful for lines with similar backgrounds.

In previous studies there were no attempts to use allozymes for the identification of donor germplasm carrying new favorable alleles for quantitative traits not present in the target single cross. Discovering such a pattern could be helpful in increasing the efficiency and thereby decreasing the cost of maize inbred improvement programs. The aim of this paper was to study the relationship between allozyme diversity and estimators of the relative number of new favorable alleles for grain yield, present in donor inbreds, but not present in elite single cross.

Materials and methods

The relative number of new favorable alleles for grain yield was estimated in two groups of inbred lines and several elite hybrids. In Experiment 1, 22 elite inbred lines representing heterotic groups from the United States (US) and Yugoslavia (YU) were considered as possible donors of new favorable alleles for improvement of the hybrid B73 × Mo17 (Table 1). Details of field trials and statistical analysis were given by Mišević (1989a). Briefly, grain yield of the target single cross B73 × Mo17 and crosses of donor lines to B73 and Mo17 were evaluated in 1986 at four locations in Yugoslavia. Donor lines and parents of target hybrids were also evaluated at the same locations adjacent to the hybrid trials. Experiment 2 included 15 inbred lines of US and YU origin (Table 1) and all possible hybrids produced by their diallel mating (Mišević 1989b). Some inbreds were common to both experiments. Hybrids and inbreds were evaluated at four and three locations in 1986 and 1987, respectively. The diallel mating scheme allowed each single cross in Experiment 2 to be considered as a target for improvement by the remaining 13 donor lines.

Minimally biased estimates of new favorable alleles for grain yield (μG) present in donor inbreds but not present in elite hybrids (μG) were estimated from the hybrid and inbred means (Dudley 1987a). A statistical procedure for calculating μG estimate was given by Mišević (1989a). Error variances were calculated using the usual expression for the variance of the linear function for μG estimate. However, the resulting standard error for μG may be underestimated (Zanoni and Dudley 1989). Least significant difference values were calculated by multiplying the standard error of difference by two.

Genotypes at 27 enzyme loci (Table 2) were determined by starch gel electrophoresis for all parents of hybrids considered as targets for improvement, and for all donors of new favorable alleles for grain yield lacking in target hybrids; this was done

Table 1. Pedigree of inbreds

Line	Source	Experiment
Stiff Stalk Synthetic lines		
B73	BSSS(R)C5	1, 2
B84	BS13(S2)C0	1, 2
N7A	Oh7 × SSS	1, 2
Lancaster Surecrop related lines		
Mo17	C103 × 187-2	1, 2
Pa91	Wf9 × Oh40B)S_4 × (38-11 × L317) × 38-11S_4	1, 2
Other USA lines		
N152	Nebr. BIV syn.	1, 2
B77	BS11 (Pioneer two-ear synthetic)	1, 2
B79	BS10 (Iowa two-ear synthetic)	1, 2
Va26	Oh43 × K155	1
YU lines		
V395	Vukovar Yellow Dent	1, 2
V158	Vukovar Yellow Dent	1, 2
R455	Ruma Yellow Dent	1, 2
R59	Ruma Yellow Dent	1, 2
S144	Sid Yellow Dent	1, 2
L105	Italian Long-ear Flint	1, 2
P37-2	Unknown	1
T768	Timok Yellow Flint	1
Do37-2	Yellow Dent from Dolovo	1
Lancaster Surecrop related and YU related lines		
70/9	C103 × NS796	1, 2
HC439	Hybrid Arizona 439[a] × C103	1
LTC68	C103 selection for prolificacy[b]	1
Other lines		
LD230	Prolific A × R588 × Calqueno	1

[a] Mexican June × YU varieties
[b] C103 × Ladyfinger popcorn

Table 2. Enzyme systems used to characterize inbred lines of maize

Enzyme system	Locus	Chromosome location
Acid phosphatase	Acp1	9
Aconitase	Aco1	4
Aconitase	Aco4	?
Alcohol dehydrogenase	Adh1	1
Arginine aminopeptidase	Amp1	1
Catalase	Cat3	?
Diaphorase	Dia1	2
– glucosidase	Glu1	10
Glutamate-oxaloacetate transaminase	Got2	5
Hexokinase	Hex2	6
Isocitrate dehydrogenase	Idh1	8
Isocitrate dehydrogenase	Idh2	6
Malate dehydrogenase	Mdh1	8
Malate dehydrogenase	Mdh2	6
Malate dehydrogenase	Mdh5	5
Phosphoglucomutase	Pgm2	5
6-phosphogluconate dehydrogenase	Pgd1	6
6-phosphogluconate dehydrogenase	Pgd2	3
Phosphohexose isomerase	Phi1	1
Shikimic acid dehydrogenase	Sad1	10

Table 3. Estimate of relative number of new favorable alleles (μG) and value of allozyme relatedness measure (ARM) for four allozyme-pedigree classes and different target hybrids[a]

Target hybrid	SA-SP		SA-DP		DA-SP		DA-DP	
	ARM[b]	μG	ARM	μG	ARM	μG	ARM	μG
Parental ARM 1–3								
B73 × Mo17	3.0	1,283	1.2	2,176**	8.0	1,214	5.5	1,690 # ∓
B84 × Pa91	2.0	629	3.0	1,338**	6.0	1,014*	7.5	1,255
Mo17 × V158	2.0	−5	1.0	1,574**	–	–	7.0	1,255 ∓
B79 × R59	–	–	1.0	1,918	7.0	778	8.0	2,020 ∓
Mo17 × 70/9	2.0	2,076	2.0	3,009**	6.0	1,639*	6.5	3,057 ∓
B73 × B84	–	–	1.7	2,746	7.0	1,350	6.3	1,919 # ∓
Parental ARM 5–6								
V158 × B79	–	–	1.5	2,224	–	–	5.3	2,058
V395 × 70/9	1.5	576	2.0	2,076**	7.0	196*	7.0	2,025 ∓
B84 × Mo17	1.0	917	1.0	1,485**	6.0	617	5.2	1,247 ∓
Parental ARM 9–11								
N7A × S144	3.0	1,863	2.8	2,021	–	–	5.5	1,890
S144 × L105	–	–	1.0	2,091	–	–	5.3	1,441 #
B77 × L105	–	–	2.0	1,750	–	–	5.7	1,746
V395 × R59	2.0	1,417	2.0	2,266**	–	–	5.0	1,862
B77 × V395	2.3	1,041	2.7	1,089	–	–	5.7	1,261

[a] Comparison between different ARM and μG estimates valid only within same target hybrid
[b] Average ARM between target hybrid and donor lines
* Difference between SA-SP and DA-SP classes are larger than 2 × standard error of difference
** Differences between SA-SP and SA-DP classes are larger than 2 × standard error of difference
# Differences between SA-DP and DA-DP classes are larger than 2 × standard error of difference
∓ Differences between DA-SP and DA-DP classes are larger than 2 × standard error of difference

according to the procedures of Stuber et al. (1986). Twenty loci were polymorphic, but enzyme genotypes were not identified for all lines at the *Aco4* and *Amp1* enzyme loci. Forty-eight alleles occurred at 18 loci across all inbreds.

A measure of genetic relatedness (ARM) among inbreds was established by determining the number of enzyme loci that had nonidentical allozymes for each possible pair of inbreds. The minimum value for this allozyme relatedness measure (Frei et al. 1986) was 1 and the maximum was 11.

Elite hybrids in Experiment 2 were selected by the criteria of heterotic grouping and ARM values. Six, three, and four hybrids with low (1–3), intermediate (5–6), and high (9–11) ARM values, respectively, were selected as targets for improvement. Hybrids developed by crossing lines with similar and dissimilar pedigrees were selected for targets within each ARM group, whenever possible.

Minimally biased estimates of new favorable alleles (μG) (Dudley 1984a, 1987a) were calculated for grain yield for all donor lines when each selected single cross was considered as a target hybrid. This statistic is a quantitative estimation of the relative, not the absolute number of new favorable alleles present in a donor line, and is thus dependent on the unit of measurement. ARM values for each donor line were calculated as the number of enzyme loci that had nonidentical allozymes with a target hybrid. Thus, two kinds of ARM values were calculated. The first, parental ARM, represents allozyme relatedness between parents of target hybrids, and the second represents allozyme relatedness between donor lines and target hybrids (Table 3).

Dudley (1984a), Zanoni and Dudley (1989), and Mišević (1989a, b) found that inbreds from heterotic groups other than parents of target hybrids usually have higher μG estimates than inbreds having the same pedigree as one of the parents. In order to separate the effect of pedigree diversity from allozyme diversity, donor inbreds were grouped into two classes: one class with allozymes similar to the target hybrid (SA) and the second class with allozymes disimilar to the target hybrid (DA) (Frei et al. 1986). Criteria for the separation of inbreds into allozyme classes were not uniform for all target hybrids; they were dependent on ARM values of donor inbred lines for each target hybrid. However, the largest ARM value in the SA classes was 3, and the smallest ARM value in the DA classes was 5. The maximum range within allozyme classes was three ARM units. Allozyme classes were further divided into subclasses of inbreds with similar (SP) and dissimilar (DP) pedigree to the parents of target hybrids. Thus, four allozyme-pedigree classes were established: SA-SP, SA-DP, DA-SP, DA-DP. Average ARM values and μG estimates were calculated for each allozyme-pedigree class from inbred estimates as the mean of inbreds belonging to the same allozyme-pedigree class. The number of inbreds in different classes was not the same within or across target hybrids.

Results and discussion

ARM values and μG estimates for four allozyme-pedigree classes and each target hybrid are given in Table 3. Within this set of inbreds it was not possible to complete the SA-SP and DA-SP classes for all target hybrids. No donor inbreds were found to have adequate ARM value for each of five and seven target hybrids within the SA-SP and DA-SP classes, respectively. However, for all target hybrids there were donor lines in both DP classes.

Table 4. Estimates of the relative number of new favorable alleles (μG) for two allozyme classes, SA and DA, averaged over pedigree groups and two pedigree classes, SP and DP, averaged over allozyme groups for different target hybrids

Target hybrid	SA	DA	SP	DP
B73 × Mo17	1,730	1,452	1,248	1,933**
B84 × Pa91	984	1,134	821	1,296**
Mo17 × V158	1,569	1,255	−5	1,414**
B79 × R59	1,918	1,399*	778	1,969**
Mo17 × 70/9	2,542	2,348	1,858	3,033**
B73 × B84	2,746	1,634*	1,350	2,333**
V158 × B79	2,224	2,058	–	2,141
V395 × 70/9	1,326	1,111	386	2,051**
B84 × Mo17	1,201	932	767	1,366**
N7A × S144	1,942	1,890	1,863	1,955
S144 × L105	2,091	1,441*	–	1,766
B77 × L105	1,750	1,746	–	1,748
V395 × R59	1,842	1,862	1,417	2,064**
B77 × V395	1,065	1,261	1,041	1,175

* Differences between SA and DA classes larger than 2 × standard error of difference
** Differences between SP and DP classes larger than 2 × standard error of difference

Table 5. Correlation coefficients between estimates of relative number of favorable alleles for grain yield (μG) and allozyme relatedness measures (ARM) for different target hybrids

Target hybrid	*r*
B73 × Mo17	−0.39
B84 × Pa91	0.17
Mo17 × V158	0.30
B79 × R59	−0.06
Mo17 × 70/9	0.22
B73 × B84	−0.63*
V158 × B79	−0.03
V395 × 70/9	0.22
B84 × Mo17	−0.02
N7A × S144	−0.21
S144 × L105	−0.59*
B77 × L105	0.01
V395 × R59	0.12
B77 × V395	−0.19

* Significantly different from zero at 0.05 probability level

Significant differences in μG estimates were frequently found between the SA-SP and SA-DP classes, as well as between the DA-SP and DA-DP classes. The DP class had a higher μG estimate than the SP class in all target hybrids. These differences were significant in six of nine and six of seven target hybrids, within the SA and DA classes respectively. Differences in μG estimates between the SA-SP and DA-SP classes were inconsistent. For hybrid B84 × Pa91, the DA-SP class had a significantly higher μG estimate than the SA-SP class. However, the opposite was found for hybrids Mo17 × 70/9 and V395 × 70/9. There were no significant differences in average μG estimates between the SA-SP and DA-SP classes for the two remaining target hybrids where comparisons were possible. Significant differences in μG estimates between the SA-DP and DA-DP classes were found in only 3 of 14 target hybrids. On average, the SA-DP class had a higher μG estimate than the DA-DP class. The relationship among allozyme-pedigree classes was similar across parental ARM values and across pedigree similarities of target hybrids.

The effect of pedigree classes SP and DP on μG estimates was averaged over both allozyme classes, and the efect of allozyme classes SA and DA on μG estimates was averaged over both pedigree classes (Table 4). Significantly higher μG estimates were found for the DP than the SP groups in 9 of 11 target hybrids where comparisons were possible.

Differences in the μG estimate between the SA and DA groups of lines averaged over both pedigree classes were significant in 3 of 14 target hybrids. Higher estimates were found for the SA than for DA class.

Correlations between μG estimates and ARM values were low and not significant in 12 of 14 target hybrids. Coefficients of correlations ranged from −0.63 to +0.38 (Table 5). Hybrids S144 × L105 and B73 × B84 had a significantly negative correlation coefficient between the μG and ARM values.

The results support findings that pedigree dissimilarities have a significant effect on μG estimate. Inbreds with a dissimilar pedigree to both parents of the target hybrid had significantly higher μG estimates than lines more genetically similar to the target hybrid. In contrast, inbreds with similar allozymes tended to have higher μG estimates than inbreds with dissimilar allozymes, although this relationship was less consistent than that seen with pedigress.

The effect of pedigree dissimilarity on estimates of the relative number of new favorable alleles was reported in previous papers (Dudley 1984a; Zanoni and Dudley 1989; Mišević 1989a, b). We can only speculate on the possible reasons for poor association between allozyme relatedness measures and estimates of new favorable alleles. It is possible that some or all 18 enzyme loci are not linked to loci for grain yield responsible for the differences in μG estimate in the germ plasm studied. If enzyme and grain yield loci are linked, enzyme loci may be linked not only to grain yield loci for which particular donor lines carry favorable alleles, but also to grain yield loci for which other donor lines carry unfavorably alleles. For a particular target hybrid, one group of donor lines could carry alleles at enzyme loci linked to loci with unfavorable alleles for grain yield, and another group of lines could carry alleles at enzyme loci linked to loci with favorable alleles for grain yield. In the first group, high ARM values would be associated with high estimates of unfavorable alleles (low estimates of μG), and in the

second group high ARM values would be associated with high μG estimates. No association between the enzyme relatedness measure and the estimate of new favorable alleles could be established for such a target hybrid. It may also be that for particular donor lines, some enzyme loci are linked to loci with favorable alleles and other loci are linked to loci with unfavorable alleles for grain yield. Again, no association between ARM and μG would be found unless there are significantly more enzyme loci in one of the two groups.

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