Isozyme marker loci associated with cold tolerance and maturity in maize *

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Summary. Two maize (Zea mays L.) populations, ASI(S) and ECR-A, were evaluated for allozyme frequency changes associated with selection for improved seedling emergence, early season vigor and early maturity. Eleven marker loci were examined and four loci were used for indirect selection in an attempt to modify cold tolerance and maturity. Allozyme-selected divergent subpopulations were produced by compositing selected S1 progeny from cycle one (C1) of ASI(S) and from C2 of ECR-A. These subpopulations and S1 generations from all cycles resulting from phenotypic selection, ECR-A C1 through C7 and ASI(S) C0 through C6, were tested in cold tolerance and agronomic performance trials over five environments in 1986. Seedling emergence and seedling dry weight did not improve with phenotypic selection in ECR-A, while plant height, ear height, grain yield, grain moisture, days to mid-silk and days to mid-pollen were reduced significantly. Contrasts between divergent allozyme-selected subpopulations from ECR-A were significant for grain moisture and mid-pollen date. For ASI(S), seeding emergence increased, while plant and ear height decreased with phenotypic selection. Contrasts between allozyme-selected subpopulations were significant for plant and ear height. Changes associated with marker-based selection for ASI(S) were not in the same direction as with phenotypic selection. Selection for favorable allozyme genotypes may be effective in changing certain traits in populations that have been modified by direct selection, however results may not be predictable.

Key words: Zea mays L. – Electrophoresis – Recurrent selection – Population improvement

Introduction

Cold tolerance in maize (Zea mays L.) is an aggregate of traits incorporating the ability to germinate, emerge and establish a vigorous stand in cool, early-season environments. Associated with adequate early season performance, germplasm growing in a short, cool season must flower early and the grain must dry down quickly for harvest and storage. Unfortunately, direct selection for cold tolerance traits is difficult because uncontrollable environmental factors adversely affect reliability of field trials. The efficiency of maize improvement programs might be enhanced if molecular markers, such as allozymes, could be used to manipulate quantitative trait loci.

Data supporting the notion that allozymes could be used as markers of quantitative trait loci have gradually accumulated during the past 15 years. Stuber and Moll (1972) monitored allozyme frequency changes in a selection program for grain yield. Alleles at three peroxidase loci behaved neutrally to yield selection, whereas the frequency of the Acp1-4 allele showed a correlation with grain yield of r=0.9 over selection cycles. Stuber et al. (1980) monitored frequency changes of alleles at 20 isozymes loci in 4 long-term maize selection studies. Eight isozyme loci showed significant directional allele frequency changes, and the same alleles at each locus were consistently associated with yield. Kahler (1983) examined the effect of half-sib and S1 recurrent selection for increased grain yield on allozyme polymorphisms in...
maize and concluded that directional selection for changing allozyme frequencies at five loci was feasible. Pollak et al. (1984) reported that four of nine isozyme marker loci assayed were strongly associated with particular quantitative traits of plants in the maize cultivar 'Hays Golden'. Edwards et al. (1987) investigated 2 F2 maize populations and found that, for 25 quantitatively inherited traits, the cumulative effect of marker-linked regions of the genome explained between 8%–40% of the phenotypic variation. Using the same populations, Stuber and Edwards (1987) reported that marker-locus facilitated selection was nearly as effective as phenotypic selection for grain yield, ear height and ear number.

Allozyme associations with cold tolerance and associated traits also have been documented. Vallejos and Tanksley (1983), in an interspecific cross of Lycopersicon esculentum and a high-altitude, cold-tolerant L. hirsutum, detected a minimum of three loci associated with growth at low temperatures. Kahler et al. (1980) examined 31 populations of Avena barbata from diverse habitats in Israel using 7 isozyme systems. Principal component and multiple regression analyses revealed that temperature and altitude of the collection source were significantly correlated with particular allozyme genotypes. In an examination of 34 races of maize from Mexico, Doebley et al. (1985) found that the frequencies of 22 alleles were significantly correlated with altitude. For Bolivian maize, Goodman and Stuber (1983) reported 12 alleles that were significantly correlated with altitude. Associations of allozymes with traits related to cold tolerance and maturity would be expected in such germplasm.

The effectiveness of allozyme selection on manipulating quantitative traits has been studied by several investigators. Stuber et al. (1982) manipulated allozyme frequencies in the 'Jarvis Golden Prolific' maize cultivar to approximate those found after ten cycles of full-sib selection for increased grain yield in the same population. One cycle of allozyme-based selection was found to be equivalent to 1.5 cycles of full-sib selection. Frei et al. (1986) generated maize subpopulations from the maize synthetic 'F26' based on allozyme frequencies associated with high and low yielding S2 progeny as evaluated in testcrosses. Selection was effective when allozyme-selected progeny were evaluated in testcrosses but not when they were evaluated as populations per se.

The objectives of this study were to: (1) determine allozyme frequency changes associated with recurrent selection for increased cold tolerance and early maturity in two maize populations; (2) identify marker loci, based on observed allozyme frequency changes, that could be used for indirect selection; (3) generate subpopulations with frequencies of marker alleles expected to provide specific phenotypic responses; and (4) evaluate the effect of allozyme selection on cold tolerance, maturity and related agronomic traits.

Materials and methods

Experimental materials

Two synthetic populations, ECR-A and AS1(S), were examined. ECR-A is a composite population produced by J. H. Lonququist (University of Wisconsin-Madison) by intercrossing 16 open-pollinated varieties adapted to the northern U.S. Corn Belt. This composite was used as a base for a mass selection program to increase the ability to germinate, emerge and quickly grow to maturity in cool, wet soils in northern Wisconsin. In each cycle, approximately 7500 seeds were planted in isolation at Marshfield, Wisconsin in early spring. After emergence, slow-growing seedlings were removed. At harvest, approximately 250 ears were visually selected from the earliest, healthy plants with the driest grain. A balanced composite of seed was formed for the next cycle by compositing equal numbers of seed from each ear. Seven cycles have been completed. AS1(S) was formed by J. L. Geadeleman at the University of Minnesota by crossing the synthetic populations BS2 and ASA and allowing five generations of random mating. Recurrent selection using visual field evaluation of S1 lines for early vigor was conducted in northern Minnesota. A 10% selection intensity was applied to approximately 190 S1 progeny each cycle. Selection was based upon date of 50% emergence and 2 visual field seedling vigor evaluations recorded at approximately the 4 and 8 leaf stages of seedling growth. Selected S1 lines were recombined one time per cycle.

Allozyme investigations

In order to identify specific alleles at marker loci that were associated with selection progress, allozymes for 11 loci (Acpl, Adhl, EstS, Glul, Gotl, Got2, Mdh1, Mdh2, Pgd2, Pgm2 and Phl) were assayed on 52–120 seedlings from C1 and C7 of ECR-A, and CO and C6 of AS1(S). Linkage relationships indicate that all marker loci segregate independently with the exception of Adhl and Phl, which are located approximately 12 units apart on the long arm of chromosome one. These 11 loci were chosen based on resolution and repeatability of the enzyme system and degree of polymorphism. Starch gel electrophoresis techniques and methodology were derived from Cardy et al. (1983), Marty et al. (1984) and Stuber and Goodman (1983). The FORTRAN program 'Genesist' (Black and Krafsur 1985) was used for allelic frequency estimations and detection of significant differences between cycles of selection. Significant differences between the initial and final cycles of selection were determined by Chi-square tests for heterogeneity of allozyme frequencies between the two selection cycles (Workman and Niswander 1970).

Allozymes whose frequencies differed significantly from the initial to final cycle of selection were chosen as marker candidates for that population. Intermediate cycles of selection [C3 and C5 for ECR-A, and C2 and C4 for AS1(S)] were then assayed for the appropriate marker loci using 74–104 seedlings. Statistical procedures developed by Wilson (1980), using transformed (2sin-1√p) allozyme frequencies, were used to test whether fluctuations in frequencies from cycle to cycle could be attributed to genetic drift alone, or whether directional selection had occurred. Linear and quadratic trends were tested using Chi-square tests incorporating sampling variation due to restricted population size during selection [assumed to be a constant of 250 for ECR-A and 20 for AS1(S)] and restricted number of plants sampled for genotypic analysis. If significant deviations from random drift were detected, and significant directional changes, as represented by linear trends, were observed, then evidence was judged sufficient to suggest that the