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MAPPING QUANTITATIVE TRAITS IN SOYBEANS WITH ISOZYME AND MORPHOLOGICAL GENE MARKERS

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Dapartment of Crop and Soil Sciences

1988

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1988

ABSTRACT

MAPPING QUANTITATIVE TRAITS IN SOYBEANS WITH ISOZYME AND MORPHOLOGICAL GENE MARKERS

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The objectives of this study were to see if associations could be detected between quantitative traits of soybean (Glycine max (L.) Merr.) and isozyme and morphological loci. Associations between qualitative and quantitative traits may be very useful for improving breeding efficiency through indirect selection procedures. In the first part of the study, 101 northern cultivars (maturity groups 0-4) were grouped into five breeding cycles and evaluated for changes in allozyme frequency over breeding cycles. At three isozyme loci there were linear, directional changes in allozyme frequency over all breeding cycles. Allozymes at these loci may have been subjected to indirect selection due to linkages with quantitative trait loci. In the second part of the study, two F_2-derived soybean populations were developed to evaluate associations between isozyme and morphological loci and quantitative traits. A population derived from a cross of Grant by Mandarin Ottawa (GM) had ten segregating marker loci and

that from the cross of Amcor x Protana (AP) had six. Forty-seven and 59 F_2-derived lines in the F_3 generation from the AP and GM populations, respectively, were grown in replicated plots, scored for isozyme profile, and evaluated for nine agronomic traits and seven seed composition traits. Multiple regression analysis showed that there were many significant associations between marker loci and agronomic and seed composition traits. The percent of agronomic trait variation attributable to marker loci varied from 19% for yield (GM) to more than 40% for plant height (AP). The percent of seed composition trait variation accounted for by gene markers varied from 24% for percent protein (GM) to 52% for linolenic acid content (GM). In the GM population, individual marker loci accounted for more than 25% of the variation for seed size and linolenic acid content. Six marker loci showed significant inverse relationships among oleic, linoleic, and linolenic acids that was consistent with the hypothesis of fatty acid synthesis via a consecutive desaturation pathway. Additive effects accounted for more than half of the agronomic trait variation in both populations and more than half the seed composition trait variation in the GM population. There were also a number of significant non-additive effects associated with agronomic and seed composition trait variation in both populations.

TO MY WIFE, ELLEN MARIE

ACKNOWLEDGEMENTS

The author wishes to express his thanks and appreciation to Drs. Joseph W. Saunders, Everett H. Everson, and James F. Hancock for their help and guidance and especial thanks to Dr. Thomas G. Isleib, dissertation director, for his generous support and enthusiasm for this research project.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

Plant breeders improve plants through the manipulation of observable plant traits. Many traits important to plant breeders exhibit continuous variation. For such traits, variation among genotypes results in a continuous distribution of phenotypes rather than in discrete classes of phenotypes. The lack of discrete classes hinders a straightforward mendelian analysis of quantitative traits. As a consequence, the specific genes governing the expression of a quantitative trait are usually not known.

Development of an improved variety depends on the production and identification of a superior genotype, an individual with a set of superior alleles. Selection for these superior alleles is indirect and is based on phenotypic performance. Phenotype-based selection for quantitative traits is generally slow and inefficient due to large environmental influences and low heritabilities which necessitates replicate testing of genotypes over environments. After an improved genotype is developed, the set of loci and alleles conferring the superior performance is still not known. Knowledge of which loci and alleles result in superior performance could greatly improve plant breeding efficiency.

The methodology for mapping quantitative traits is not new (Thoday, 1961). In the past, the major drawback has been the lack of sufficient numbers of gene markers,

especially for poorly mapped species. With advances made in isozyme techniques and especially in restriction fragment length polymorphism (RFLP) methodology, a large number of gene markers are potentially available for most species (Helentjaris et al, 1985).

The mapping of quantitative traits could have major consequences for plant breeding progress. A system of selection at the locus level augmenting current methodologies could markedly improve heritabilities of quantitative traits. This would have great potential to increase the efficiency of current selection systems and thereby accelerate cultivar development.

II. LITERATURE REVIEW

Quantitative traits

Genetically-determined traits are generally classified into two categories, qualitative and quantitative traits. Genotypes exhibiting qualitative traits can be readily classified into discrete categories according to their phenotype. Allelic substitution at a qualitative locus results in such a major change in phenotype that differences due to genotype are readily apparent. A classic example of a qualitative trait is flower color in soybeans (Glycine max (L.) Merr.), where the W1 (dominant) allele produces purple flowers and w1 (recessive) allele, when homozygous, produces white flowers (Palmer and Kilen, 1987). In this case, an allelic substitution produces a classification difference, a readily identifiable difference in color. Progeny testing of the dominant phenotype distinguishes heterozygotes from the dominant homozygotes. Thus, all genotypic classes can be identified through phenotypic differences. Differences of degree such as short versus tall can also be considered qualitative traits, as long as genotypes can be unambiguously placed into phenotypic classes. For either type of qualitative trait, a difference in classification or difference of degree, the genotypes must be separable by phenotypic classes and exhibit mendelian or maternal inheritance. If all plant traits were qualitative in

nature, then plant breeding would be relatively simple and straightforward.

However, many traits of interest to a plant breeder are not qualitative traits, but quantitative traits. In soybeans, quantitative traits of interest include seed yield, maturity, plant height, lodging resistance, and protein and oil composition (Poehlman, 1979; Fehr, 1987a; Fehr, 1987b). Unlike qualitative traits, in which phenotypes fall into discrete classes, quantitative traits vary continuously over a wide range. Phenotypes do not fall into discrete classes, and genotypes are therefore not readily distinguished by phenotypic classes.

Quantitative variation is often thought to be due to the effects of polygenes, many genes each with small effect acting upon the trait. However, quantitative traits are defined as traits in which the magnitude of phenotypic differences between genotypes is small relative to the magnitude of phenotypic differences within genotypes (Suzuki et al., 1981). According to this definition, it is not the number of genes involved that determines the quantitative nature of a trait, but the relative size of the genetic effects compared to the size of the environmental effects. A large environmental effect will obscure small but real genetic effects and make it difficult to distinguish genotypic classes on the basis of phenotype.

Even though a large number of loci is not a prerequisite for a quantitative trait, many traits

exhibiting quantitative variation such as yield and other agronomic traits are probably determined by many genes. The number of loci affecting a quantitative trait plus the large environmental effects contributing to trait expression make expression of a trait highly complex. The basic model for phenotypic effect is:

$$P = G + E + I_{GE} ,$$

where

P is the deviation of the phenotype from the population mean;

G is the effect of the genotype;

E is the effect of the environment; and

I_{GE} is the effect of the interaction of genotype and environment.

G can be further subdivided into:

$$G = A + D + I ,$$

where

A is the sum of the additive effects of individual genes;

D is the sum of dominance interactions, i.e. interactions between alleles within a locus; and

I is the sum of epistatic interactions, i.e. interactions among genes at different loci (Falconer, 1981).

The effect that each gene has on the phenotype is modified not only by environmental effects (E) but also by

within-locus interactions (D), between-locus interactions (I), and genotype-by-environmental interactions (I_{GE}). The complications that arise because of the environmental effects, number of genes, and various interactions affecting the expression of a quantitative trait make it difficult or impossible to determine which genes affect a given trait and which alleles of those loci are associated with a positive expression of the trait.

The field of quantitative genetics was developed in part because of the inability of geneticists to resolve quantitative traits into effects attributable to one or more genes. In quantitative genetics, the genetic effects are determined on the genotype as a whole, a summation over all loci. With appropriate mating design, genetic effects can be partitioned into variation due to additive, dominant, and epistatic effects. This permits the measurement of heritability, a key concept of quantitative genetics. Heritability in the narrow sense is the ratio of additive variance to phenotypic variance and estimates the degree to which a trait is passed from parent to offspring. These estimates can then be used to determine the best breeding approach and best generation in which to make selections. The concepts developed in quantitative genetics have been extremely useful for plant breeders. However, selection methodology is based on the phenotypic value of the individual and its ability to pass on a proportion of its positive traits to its offspring. It is still not known

which loci are contributing to the positive expression of a trait and what combination of alleles would produce the best genotype for a given environment. The resolution of quantitative traits into effects due to individual loci could not only increase selection efficiency but could also greatly increase knowledge of the relation between genes and phenotype.

Quantitative Trait Loci

Quantitative traits, though not easily analyzed by mendelian genetics, are still determined by genes which behave in regular mendelian fashion. Because these genes, called quantitative trait loci (QTLs), are located on chromosomes and follow the laws of independent assortment and segregation, it should be possible in theory to map them. There are several methods by which QTLs can be mapped.

The most widely used method is the use of qualitative genes (markers) to map QTLs. The basic concept of the marker-based method is that linkages can be found between QTLs and gene marker loci. Individuals can then be grouped according to marker classes (i.e. homozygote AA, heterozygote Aa, homozygote aa) and differences in quantitative trait means of these classes should reflect allelic differences at QTLs linked to the marker loci.

This method requires a large number of independent marker loci, allelic differences at QTLs, and tight linkage between at least some of the marker loci and QTLs. The gene

markers used can be any qualitative genes which segregate in a mendelian fashion and can be readily assayed. Those gene markers which can be assayed quickly and whose heterozygote is distiguishable from either homozygote are preferable.

A large set of independent markers must be available because the location of the QTLs are unknown. The ideal situation is to have the entire genome as fully covered as possible with markers, with at least one marker per linkage group. This increases the probability of finding linkages between marker and QTLs.

Tight linkage between the marker locus and QTL is necessary for detecting differences in value between marker classes. Without tight linkage, recombination reverses the pairing between specific marker alleles and specific QT alleles resulting in a lowered mean difference between marker homozygotes. This can be seen from the following formula:

$$D = (1-2r)(d_1-d_2) \ ,$$

where

D = difference in mean value between two homozygous marker classes in the F_2 generation;

r = recombination frequency between marker locus and QTL;

d_1 = value of first homozygous line; and

d_2 = value of second homozygous line (Soller et al, 1979).

As the recombination frequency between the marker locus

and QTL approaches 50 percent, the difference between homozygous marker classes approaches zero. Likewise, the ability to discriminate between marker class groups on the basis of quantitative trait value drops to zero as the recombination frequency approaches 50 percent.

Morphological markers, allozymes, and restriction fragment length polymorphisms (RFLPs) have been used as markers to map QTLs. Allozymes and RFLPs are the more useful markers because they usually show codominant expression and have no known deleterious effects on plant structure. RFLPs are especially useful as markers because large numbers can be generated quickly allowing near-complete coverage of all linkage groups (Helentjaris et al., 1985).

Mapping QTLs with genetic markers was pioneered by Thoday (1961). Loci that were associated with high and low sternopleural chaeta number of Drosophila melanogaster were mapped to regions bracketed by qualitative genes. Tanksley et al. (1981) mapped QTLs in a backcross derived from a cross between Lycopersicon esculentum x Solanum pennellii. Using isozyme markers, they found several QTLs each for stigma exertion, leaf ratio, fruit weight, and seed weight of tomatoes. RFLPs have also been successfully used to map quantitative traits (Osborn et al., 1987).

A second method of mapping QTLs is to use chromosome manipulation techniques to analyze quantitative differences at the whole chromosome or chromosome arm level. One

procedure is to use chromosomal substitution lines to transfer whole chromosomes from one genotype to another. Comparisons can then be made between homologous chromosomes within the same genetic background. If differences between homologous chromosomes exist, then QTLs are assumed to be located on the substituted chromosome. This procedure is useful for mapping one or more QTLs to specific chromosomes but less useful for determining exact position and number of QTLs. One advantage of the method is that the effect of the QTLs in different genetic backgrounds can be easily assessed. Thoday (1961) used a chromosome method as a preliminary step in mapping QTLs with qualitative gene markers in Drosophila. Law (1966) used this method to locate at least two factors which control time to ear emergence to chromosome 7B in wheat. Robertson et al. (1981), used B-A translocations in corn to produce modified inbreds with chromosome arm substitutions. They analyzed the effects of the substitutued segments on eleven ear and whole plant traits.

A third method to map QTLs is the inbred backcross method (Wehrhahn and Allard, 1965). In this method, an F_1 derived from a cross of two inbreds is backcrossed to one of the parents. A large number of backcross progeny are backcrossed several times to the recurrent parent and then selfed to near homozygosity. In theory, a set of near-isogenic lines should result, each carrying different quantitative trait alleles from the donor parent.

A fourth method only recently proposed for mapping QTLs but not yet tried is the use of insertional mutagenesis (Soller and Beckman, 1987). A novel DNA sequence inserted into the genome would act as a mutagen and knock out gene function at the site of insertion. A unique advantage of this method is that if a QTL is detected, it can then be located using the insert as a probe and the gene itself cloned. In practice this method would require large numbers of individuals with inserts since the number of insertion sites in a genome is very much larger than the number of target sites.

The first step in utilizing a marker-based selection system is to develop a large set of qualitative markers. In a few crop species, particularly maize (Coe, personal communication) and tomatoes (Bernatsky and Tanksley, 1986), this has already been accomplished. The methodologies for developing isozyme (Tanksley and Orton, 1986) and RFLP markers (Helentjaris et al., 1985) are well characterized.

The second step is to find potential linkages by detecting associations between gene markers and QTLs. One approach has been to monitor changes in marker allele frequency in populations undergoing selection. Stuber et al. (1980) used this approach in maize (Zea mays L.) and found that several isozyme loci showed change in allelic frequencies in populations undergoing long term selection for yield.

A second approach is to look for correlations between

marker alleles and quantitative traits among segregating progeny. The first report of an association between a quantitative character and a qualitative gene was in Phaseolis vulgaris (Sax, 1923). Pigmented beans (F_2 segregates) were found to have a greater seed weight than white beans. Everson and Schaller (1955) found an association between awn barbing and yield in a backcross population of barley (Hordeum vulgare L.). In tomato (Lycopersicon esculentum) and related species, linkages have been found between isozyme loci and cold tolerance (Vallejos and Tanksley, 1983), between an RFLP locus and soluble solid content, (Osborn et al., 1987), and between isozyme loci and fruit weight (Tanksley et al., 1982). An association was found between an incompatibility locus and an isozyme locus in apples (Malus sylvestris Mill) (Manganaris and Alston, 1987). In maize, many significant associations were found between isozyme loci and yield and yield-related traits in two F_2 populations (Stuber et al., 1987).

Associations between marker loci and quantitative traits have been reported for a number a diverse organisms. Koehn et al. (in press) found a relationship between allozyme heterozygosity and growth rate of clams. Andersson et al. (1987) analyzed six blood group loci and nine electrophoretic loci in race horses and found a significant association between markers and race performance. Associations between gene markers and quantitative traits have also been found in oats (Avena sativa L.) (Hamrick and

Allard, 1975; Kahler et al., 1980), barley (Nevo et al., 1979) and in fruit fly (Jayakar et al., 1977)

The last step is to test the purported linkages between markers and QTLs through selection experiments. Stuber et al. (1982) based a selection experiment on previous findings of allozyme frequency changes associated with yield selection in maize. Selection at eight isozyme loci showed that yield and ear number could be increased through allozyme selection. In mice, correlations of alkaline phosphatase levels with body weight led to successful two-way selection for large and small body size (Yamaki and Mizuma, 1982).

Once QTLs are extensively mapped with markers, there are several ways the information could be used in a plant breeding program. One use of the marker-QTL linkage information is to plan appropriate crosses. For different goals, selections at different sets of alleles would be required. To fix a trait at a given level of expression, parents should have near-identical alleles at loci affecting the trait of interest. If the breeder wishes to maximize variability in a segregating population, then the number of marker differences between parents should be maximized. For this approach to work, a large proportion of the phenotypic variability must be accounted for by marker-QTL linkages. To obtain transgressive segregates both parents should have plus alleles at different loci. If many marker loci are detected which show dominance or overdominance for linked

quantitative traits then F_1 hybrid production could be based on these loci.

A second use of marker-QTL linkages is for early generation screening. For quantitative traits with low heritabilities, direct selection in early generations is generally inefficient. Indirect selection based on marker-QTL linkages may be a much more effective procedure. An early generation such as an F_2 could include directional selection for a quantitative trait based on a set of marker alleles along with selection for homozygosity at marker loci. This may have the effect not only of decreasing the time to homozygosity, but also of eliminating prior to field-testing a large portion of undesirable genotypes.

A third use for QTL-marker linkages is to assist backcrossing programs. QTLs from exotic germplasm can be introgressed into adapted cultivars via backcrossing and selection for exotic markers (Soller and Plotkin-Hazan, 1977). Backcrossing efficiency can be increased and duration decreased by selecting for homozygous recurrent parent markers (Tanksley et al, 1981).

Soybean Traits

In soybeans, the inheritance of and interrelationships among quantitative traits are well-characterized (Anand and Torrie, 1963; Burton, 1987). For agronomic traits important to plant breeders, most of the genotypic variance is due to additive gene effects (Gates et al., 1960; Brim and Cockerham, 1961). Some agronomic traits such as seed size,

plant height, and lodging, also exhibit significant dominance effects (Croissant and Torrie, 1971). There are significant correlations between seed yield and plant height, lodging, and maturity (Burton, 1987) and negative correlations between yield and protein content (Kwon and Torrie, 1964).

Protein and oil are the most important constituents of soybean seed (Fehr, 1987a). There is a negative correlation between percent seed protein and percent seed oil (Brim and Burton, 1979). Soybean oil is composed of five fatty acids (Dutton and Mounts, 1966), one of which, linolenic acid, is associated with poor flavor (Moll et al., 1979). The effects of selection for altered oil and fatty acid content have been characterized in several studies (Wilson et al., 1981; Hawkins et al., 1983; Brossman and Wilcox, 1984; Wilcox and Cavins, 1985).

There is some concern that the soybean is genetically vulnerable due to a narrow germplasm base (Committee on Genetic Vulnerability of Major Crops, 1972; St. Martin, 1982; Specht and Williams, 1983). Breeding progress in soybeans may have led to a further decrease in genetic variability (Delannay et al., 1983) In other plant species, artificial selection has been associated with a decrease in the amount of allozyme variation (Levin, 1976; Ellstrand and Marshall, 1985). If isozyme loci are linked to quantitative trait loci then selection for quantitative traits may lead to a decrease of some allozymes (Hedrick and Holden, 1979).

Recent advances in genetics include molecular studies of plant DNA (Goldberg, 1978; Goldberg et al, 1978) and inheritance studies of more than a dozen enzyme loci of soybeans (Buttery and Buzzel; Gorman et al., 1982; Kiang and Gorman, 1983; Gorman, 1983; Gorman et al, 1984; Griffin and Palmer, 1987). The following studies used the electrophoretic procedures for soybeans developed by Cardy and Beversdorf (1984), the pedigree data from Hymowitz et al. (1977), and SAS statistical procedures (SAS, 1985) to determine associations between gene markers and QTLs in soybeans.

III. SOYBEAN ALLOZYME FREQUENCY

Introduction

The first extensive introduction of soybeans into the the U.S. took place in the 1920's with the importation of varieties from Manchuria (Poehlman, 1979). At first, cultivar improvement consisted of selection within these plant introductions. Later, cultivar improvement resulted from cycles of selection, hybridization among the best varieties and subsequent evaluation of progeny. (Fehr, 1987). The first cycle of improvement of soybeans in the U.S. was selection of improved cultivars derived from crosses between plant introductions. Hydridizations among these improved cultivars initiated the second cycle of selection. This process, in effect, became a recurrent selection procedure with a lengthy cycle (St. Martin, 1982). During these cycles of cultivar improvement, breeding effort centered on increasing yield and improving agronomic traits such as disease resistance, maturity, lodging, height, and shattering resistance (Fehr, 1987).

There is some concern that the germplasm base of soybeans is too narrow and renders the cultivar genetically vulnerable (Committee on Genetic Vulnerability of Major Crops, 1972). Some reasons for this concern include a limited number of introductions constituting the original U.S. soybean germplasm base (Specht and Williams, 1983), a disproportionate genetic contribution of a handful of

introductions to the current population of soybean cultivars, (Delannay et al, 1983) and a possible loss of variability associated with small effective size of the breeding population (St. Martin, 1982).

It is difficult to assess the total amount of genetic variability present in the U.S. soybean germplasm base because the genome is large and relatively unmapped. The total genome size (1N) of the soybean is estimated at 1.8×10^9 nucleotide pairs (NTP) with 40% of the total being single copy DNA (Goldberg, 1978). Based on the average size of a single copy fragment, there are an estimated 2.5×10^5 unique nucleotide sequences in the soybean genome. If the estimate of 20% (Goldberg et al., 1978) for the proportion of tobacco leaf RNA to DNA is similar in soybean, this would translate into an estimate of 5.0×10^4 structural soybean genes. With only 35 genes mapped and fewer than 200 soybean genes identified (Palmer and Kilen, 1987) estimates of total genetic diversity based on present knowledge may not be very accurate. Better estimates of total genetic diversity may be obtained when a comprehensive RFLP map of soybeans is developed.

A loss of genetic diversity in soybeans due to breeding effort should result in either a change in allele frequency or loss of alleles. By measuring allelic frequencies in different breeding cycles, changes associated with breeding effort can be determined. Isozyme loci are currently the best class of genes to use for such a study. They are

be selectively neutral; there has been no conscious selection for isozyme loci. In addition, they can be assessed at early stages, such as the seed or seedling stage, and are usually codominant, thereby eliminating progeny testing.

Methods

One hundred and one U.S. and Canadian soybean cultivars (maturity groups 0-IV) for which pedigree and isozyme data were available were classified into five groups based on breeding cycles. Pedigree information for these cultivars was obtained from Hymowitz et al. (1977) and from release notices from more recent cultivars. Classification of cultivars into cycles was determined in a stepwise fashion. The first breeding cycle was composed of cultivars derived from crosses between plant introductions. Subsequent breeding cycles were composed of cultivars derived from crosses in which at least one parent was from the previous breeding cycle and all parents were from lower-numbered breeding cycles. Cultivars classified into five breeding cycles and the ancestral lines from which these cultivars were derived are shown in Table 1.

The rationale of this approach is that breeding progress in soybeans has been accomplished through a series of cycles of selection (Fehr, 1987). From each cycle superior genotypes are intermated to produce lines for evaluation in the next cycle. Selection among these new lines will presumably result in cultivars that have

Table 1. Northern soybean varieties (maturity groups 0-IV) grouped according to breeding cycles.

Cycle	Cultivars		
Ancestral lines	A.K. (Harrow)	Mandarin Ottawa	PI 54610
	Dunfield	Mukden	Tokyo
	Illini	Richland	CNS
	Manchu	Strain 171	
1	Acme	Harly	Monroe
	Adams	Harosoy	Pagoda
	Altona	Hawkeye	Perry
	Blackhawk	Henry	Verde
	Capitol	Kanrich	Wabash
	Hardome	Lincoln	
2	Amsoy	Grant	Norchief
	Anoka	Hardin	Norman
	Bethel	Hark	Prize
	Chippewa	Harlon	Renville
	Clark	Kent	Ross
	Comet	Lindarin	Shelby
	Corsoy	Madison	Steele
	Custer	Magma	Traverse
	Disoy	Merit	Vickery
	Ford	Morsoy	
3	Adelphia	Evans	Portage
	Ada	Grande	Protana
	Amcor	Harcor	Provar
	Beeson	Hodgson	Rampage
	Bonus	Kahala	Scott
	Calland	Kailua	Vansoy
	Clay	Maple Arrow	Wayne
	Cutler 71	McCall	Wilkin
	Cutler	Mokapu Summer	Will
	Delmar	Oksoy	Wirth
	Dunn		
4	BSR 301	Franklin	Swift
	BSR 302	Maple Presto	Union
	Century	Marion	Wells
	Coles	Nebsoy	Williams
	Desoto	Pomona	
5	Crawford	Oakland	Sloan
	Cumberland	Pella	Sprite
	Elf	Pixie	Weber
	Gnome		

superiority over their parents for at least some agronomic traits. By classifying soybean cultivars according to parentage, these cycles of selection can be approximated. Although this is an imperfect reconstruction of the actual rate of breeding progress, it is deemed to be a more accurate estimate than a strictly temporal classification as used by other workers (Delannay et al., 1985).

The isozyme data for the U.S. and Canadian northern soybean varieties was obtained from Gorman (1983). For each of the fourteen isozyme loci surveyed (Table 2), the allelic frequencies within a breeding cycle was determined from the proportion of varieties in the cycle which carried each allele. In cases where the variety was polymorphic, each allele was counted as one half instead of one for determining allelic frequency. The frequency of polymorphic loci over all varieties and loci was 1.7%. A simple linear regression of allozyme frequency on breeding cycle was calculated for each isozyme locus using a least squares estimation procedure (SAS, 1987).

Results

Soybean varieties from the early maturity groups, 0 - IV, were classified into five breeding cycles plus the group of ancestral lines (original introductions) (Table 1). Allozyme frequency changes over breeding cycles were computed for each enzyme. One group of isozymes (Figures 1 and 2) had erratic, non-directional changes in allozyme frequencies over breeding cycles. A second group of

Table 2. Enzymes and associated loci and alleles.

Enzyme	Locus	-------Alleles-------		
Acid phosphatase	Ap	Ap-a	Ap-b	Ap-c
Alcohol dehydrogenase	Adh1	Adh1	adh1	
Amylase	Sp1	Sp1-a	Sp1-b	
Diaphorase	Dia1	Dia1-a	Dia1-b	
Glucose-6-phosphate dehydrogenase	Gpd	Gpd	gpd	
Isocitrate dehydrogenase	Idh1 Idh2 Idh3	Idh1-a Idh2-a Idh3-a	Idh1-b Idh2-b Idh3-b	
Leucine aminopeptidase	Lap1	Lap1-a	Lap1-b	
Mannose-6-phosphate isomerase	Mpi	Mpi-a	Mpi-b	Mpi-c
Phosphogluconate dehydrogenase	Pgd	Pgd-a	Pgd-b	
Phosphoglucomutase	Pgm1	Pgm1-a	Pgm1-b	
Peroxidase	Ep	Ep	ep	
Superoxide dismutase	Sod	Sod	sod	

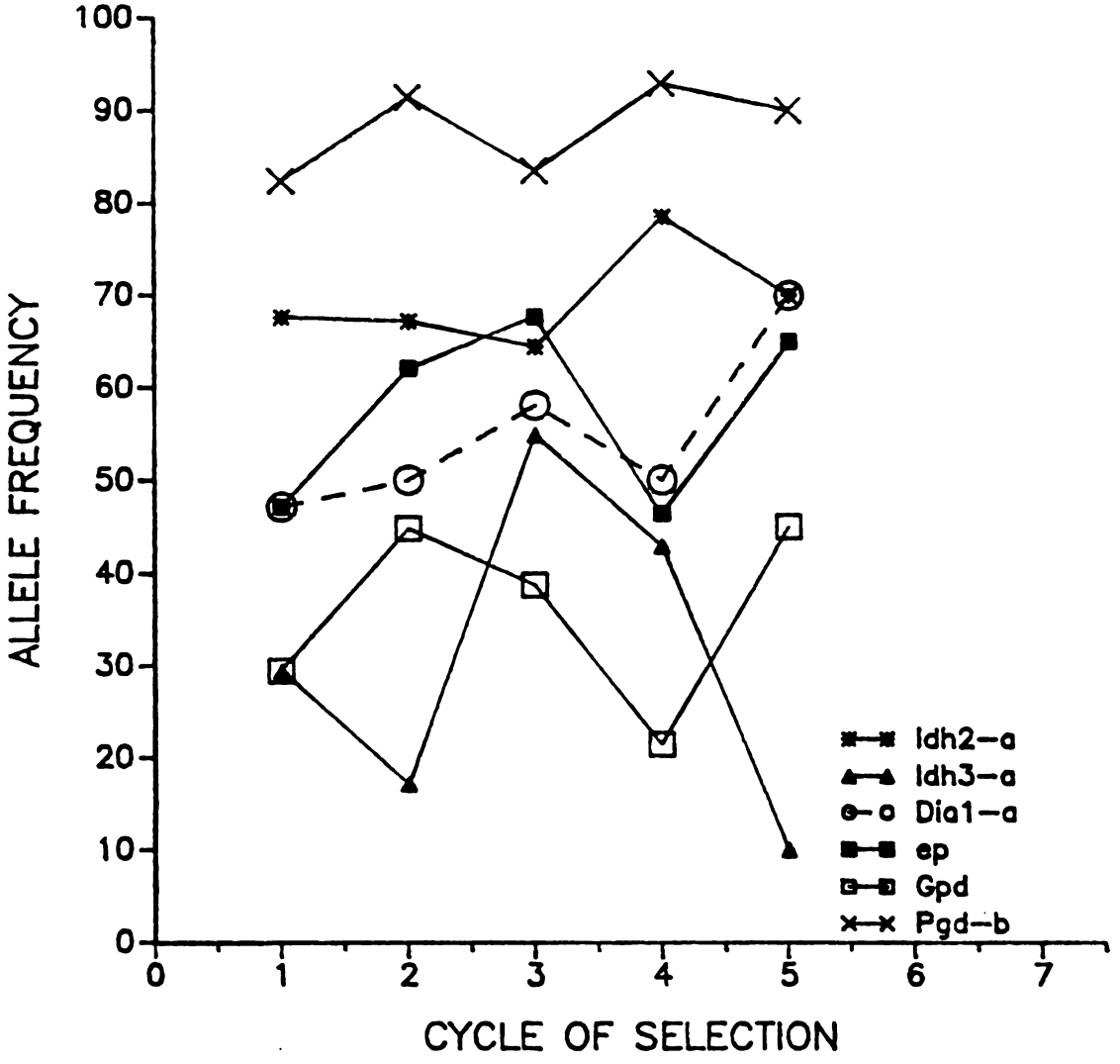

Figure 1. Allele frequency versus cycle of selection for those allozymes with an erratic response over cycles.

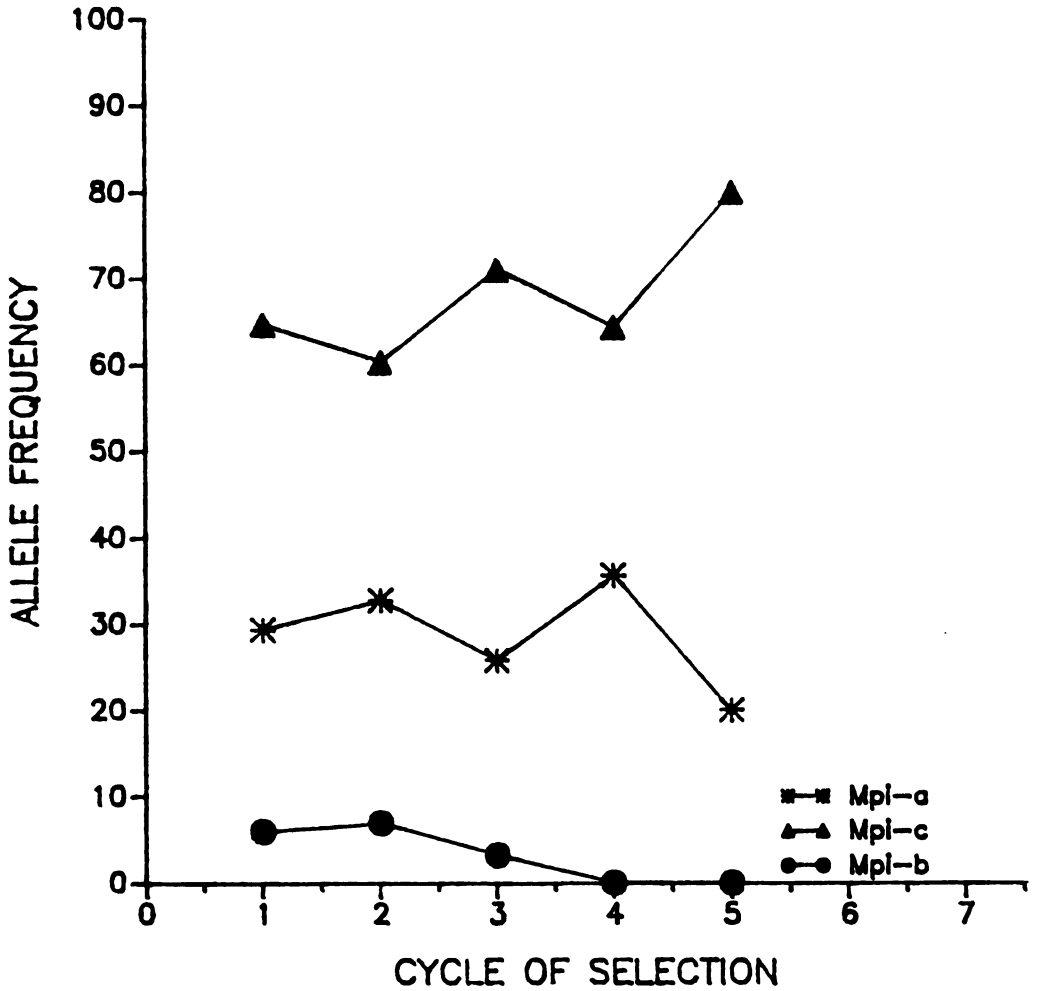

Figure 2. Allele frequencies versus cycle of selection for the three alleles of MPI which show an erratic response over cycles.

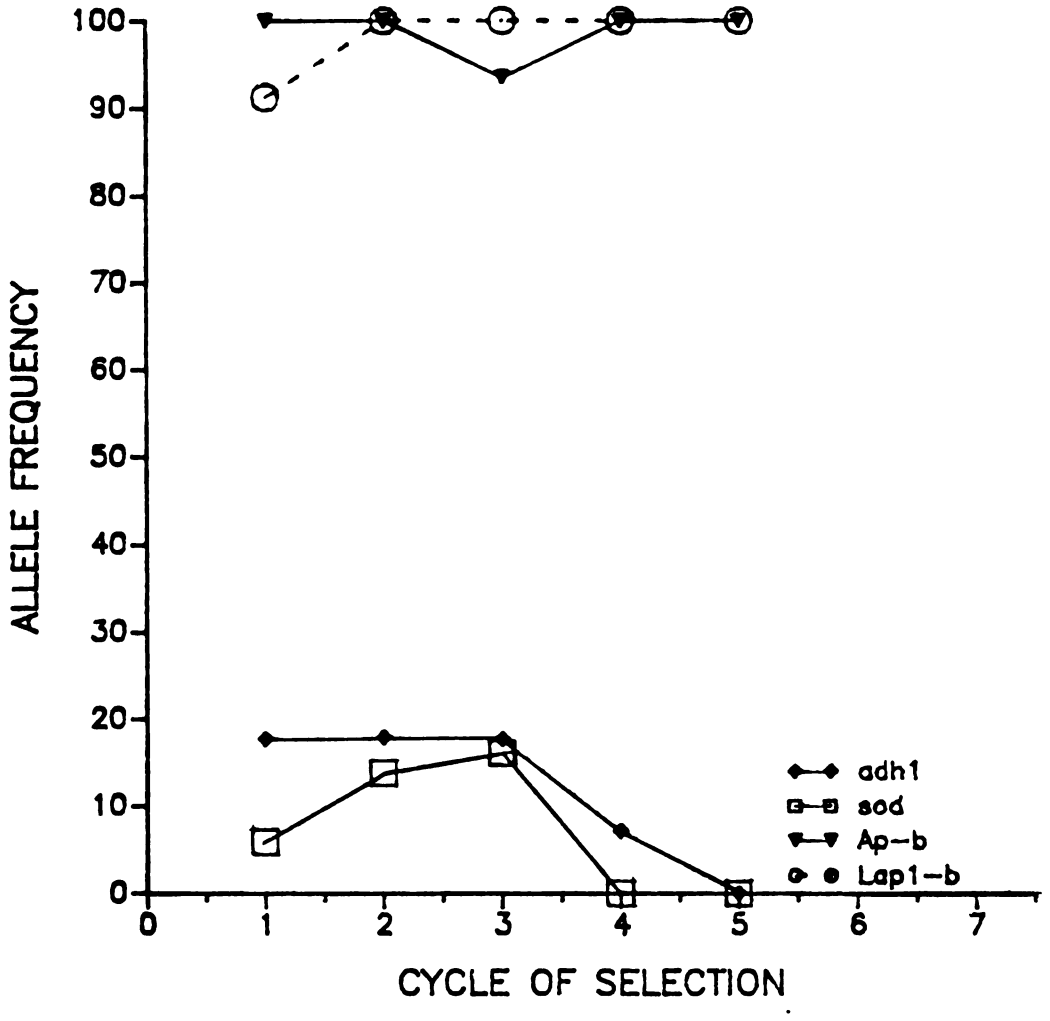

Figure 3. Allele frequency versus cycle of selection for those allozymes which become fixed over cycles.

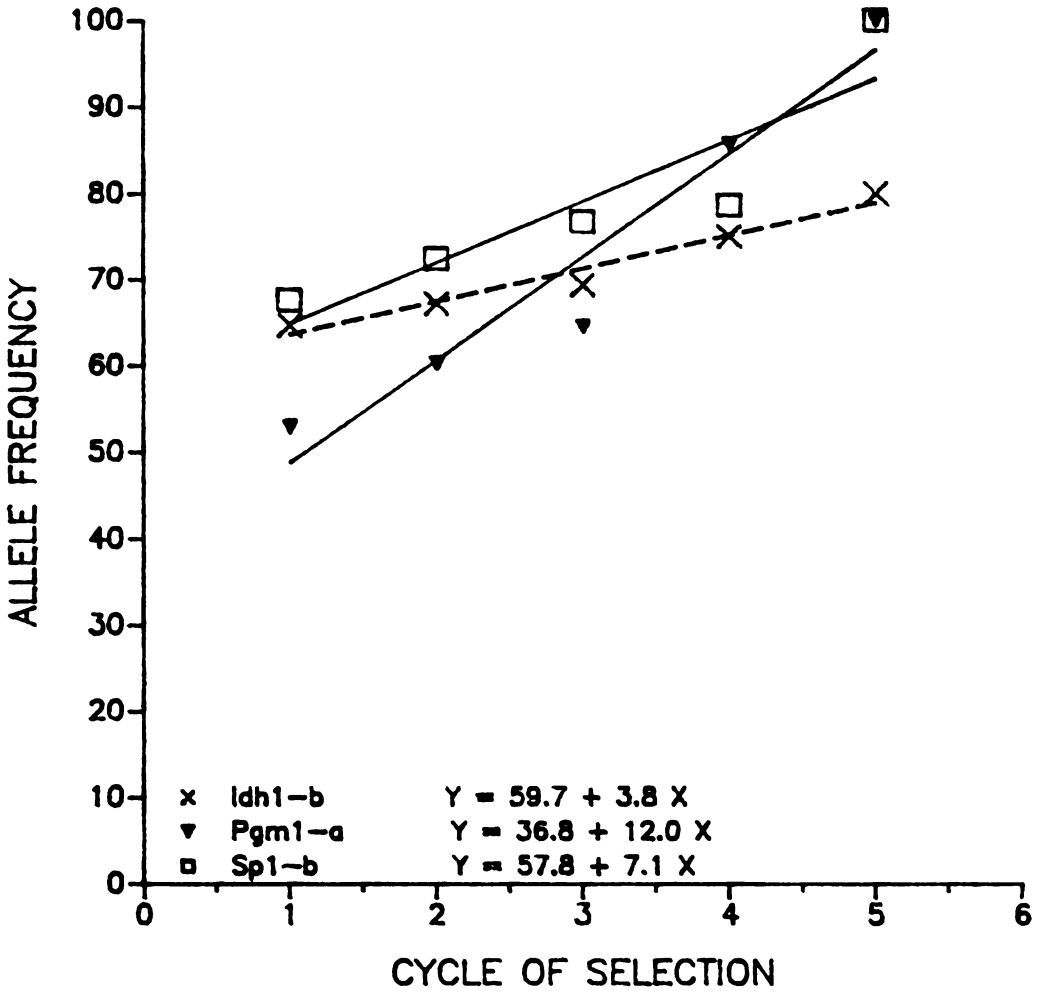

Figure 4. Linear regression of frequency of allozyme on cycle of selection.

isozymes, (Figure 3) exhibited very high initial frequency of one allele followed by fixation of that allele over breeding cycles. A third group (Figure 4) had monotonic directional changes in allozyme frequency over breeding cycles with generally small increments per cycle. All of the isozymes in the third group also had significant linear regressions of allelic frequency on breeding cycle (Figure 4). Six of the fourteen enzymes surveyed had reached fixation by the fifth cycle.

Discussion

Under Hardy-Weinberg equilibrium conditions, allelic frequencies should remain the same from generation to generation (Falconer, 1981). Plant populations subjected to domestication and artificial selection procedures do not fit the assumptions of Hardy-Weinberg equilibrium conditions, i.e. a large random mating population with no selection, migration or mutation. Gene frequencies in breeding populations are affected by selection (natural and artificial), migration (introduction of new germplasm), and random drift (due to small population size) and therefore are expected to change from generation to generation.

A comparison of allozyme diversity in cultivated soybeans versus wild species (Glycine soja) shows a loss of diversity in the cultigen (Gorman, 1983). There are fewer polymorphic enzyme loci in the cultivated species than in the wild species. A similar result was observed in Raphanus sativus L. (Ellstrand and Marshall, 1985) and in Phlox

drummondii Hook. (Levin, 1976) in which wild populations showed a greater degree of isozyme polymorphism than did cultivated populations.

Domesticated cultivars of soybean also show changes in allelic frequencies over breeding cycles (Figures 1-4). The cause of these allelic frequency changes is not known, but the most likely forces responsible are either random drift, artificial selection, or a combination of the two.

Unfortunately, the effects of these two forces cannot be readily distinguished. However, certain patterns of allelic frequency change are expected if either random drift or artificial selection is operating. Under random drift, allelic frequencies change erratically from generation to generation and do not revert back to initial frequencies (Falconer, 1981). If one allele has a very low initial frequency, that allele may be lost due simply to sampling errors. With artificial selection operating, allelic frequencies are expected to show directional change over time (Stuber et al, 1980).

The seven isozymes shown in Figure 1 and 2 have allozyme frequency fluctuations that would be expected if random drift were operating. The frequency changes are erratic and show no evidence that either allele is favored. The four enzymes shown in Figure 3 have alleles which became fixed over breeding cycles. The most probable cause is sampling error; one allele with a low initial frequency is not retained in the selected population due to the small

sample size of each cycle. The loss of one allele is independent of any selection favoring one allele over the other. The three isozymes listed in Figure 4 show a directional, linear change in allozyme fequency, a pattern expected if selection were operating on these loci. Similar results were obtained by Stuber et al (1980) in which eight of twenty maize isozyme loci surveyed showed directional changes in frequency associated with selection for yield. The allozyme frequency changes were attributed to selection at or near the enzyme loci.

In order for artificial selection to cause changes in allozyme frequencies, the enzyme locus itself must affect important plant traits or be linked to other loci which affect important plant traits. In the latter case the enzyme loci would be selectively neutral and the change in allelic frequency due to a hitch-hiking effect (Hedrick and Holden, 1979).

These results must be viewed with caution because, unlike the maize populations studies by Stuber et al., (1980), the soybean populations were not closed nor were they randomly intermated. There was non-random mating, introduction of new P.I.s at intermediate cycles, and intermating across cycles. In spite of these factors, there were consistent trends for three isozyme loci which showed results expected if the allozyme loci were undergoing selection.

If these four isozyme loci were indirectly selected

during breeding improvement, then gene(s) linked to these loci must affect agronomic traits. In this case, the isozyme loci are potentially useful as selection aids. Before isozyme loci can be used in this manner, linkage between isozyme loci and genes affecting agronomic traits must be confirmed and the association of specific allozymes with positive and negative effects on agronomic traits must be determined.

Conclusions

The results show that changes in allozyme frequency have taken place over breeding cycles. Some of these frequency changes have resulted in loss of alleles. Six of the fourteen allozyme loci were fixed by the last cycle of selection. Seven allozyme loci exhibited erratic changes in allele frequencies; selection apparently had no effect on these loci. Three loci exhibited directional, linear changes in allozyme frequencies. This effect is consistent with expectations if selection were operating on these loci.

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IV. MAPPING QUANTITATIVE TRAITS OF SOYBEAN

Introduction

Genetic improvement of yield in soybeans has been relatively modest; the average yield increase per year has been about 0.6% (Fehr, 1987). The heritability of yield and other agronomic traits is low to moderate (Fehr, 1987) so that genetic improvement based on phenotype selection is generally slow and inefficient. Selection for traits correlated with yield is usually no better than direct selection for yield (Burton, 1987).

Yield and other agronomic traits are called quantitative traits because they exhibit continous variation. Different genotypes do not produce discrete phenotypic classes, but instead, produce a continuous range of phenotypes. These quantitative traits are still determined by mendelian genes. Therefore, it should be possible to identify and map these genes. Genes which regulate quantitative traits are termed quantitative trait loci (QTLs). The difficulty in resolving genetic control of a quantitative trait into effects due to individual loci is due to the small effects of each locus relative to environmental effects and to the interactions and numbers of loci involved.

In soybeans the most readily available method for mapping QTLs is to detect linkages between QTLs and marker genes. Allozymes are currently the most useful set of

soybean gene markers available for mapping QTLs. They have no known deleterious effects on agronomic traits, are easily detected at seed or seedling stage, and usually show codominant expression. Several hundred soybean lines have been characterized for isozyme phenotype (Gorman et al, 1982, Gorman et al, 1984) and the inheritance has been reported for at least twenty isozyme loci (Gorman, 1983, Kiang and Gorman, 1983, Griffin and Palmer, 1987) including those used in this study.

In several plant species there have been reports of associations between marker genes and quantitative traits (Tanksley et al., 1982; Everson and Schaller, 1955; Hamrick and Allard, 1975) In maize (Zea mays L.), frequency of allozymes showed directional change in response to selection for yield (Stuber et al., 1980). Associations have also been detected in maize between isozyme loci and QTLs for yield and yield-related traits (Stuber et al, 1987).

This study is a preliminary investigation to determine if linkages can be detected in soybeans between isozyme and morphological marker loci and quantitative trait loci in soybeans. The quantitative traits evaluated include yield and related agronomic traits and seed composition traits.

Materials and Methods

The two experimental soybean populations used in this study were derived from crosses between Amcor and Protana (maturity group II lines), and Grant and Mandarin Ottawa (maturity group 0 lines). Parental alleles for isozyme and

morphological loci are shown in Table 3. Crosses were made in the field and in the greenhouse in the summer of 1985. In October, 1985 approximately 25 F_1 seeds from each cross were planted in the greenhouse in separate nine inch clay pots filled with sterilized soil. Thirty-six pots were placed per 2.3 m^2 section of greenhouse bench. Plants were watered as needed and fertilized with Peters 20-20-20 water soluble fertilizer. Extra lighting was provided with high-pressure sodium lamps suspended 1.8 m above the benches and spaced at 1.5 m intervals. Plants were tied to 1.2 m bamboo stakes and tops of plants were cut off at a height of 1.2 m. About 120 F_2 seeds from each cross were planted in the greenhouse in January, 1986 and were grown in the same fashion as the F_1 plants.

F_2-derived families in the F_3 ($F_{2,3}$ lines), parents, and an F_2 random sample were planted in a Capac loam soil (aeric ochraqualf) on June 11, 1986. The experimental design for each cross was a randomized complete block experiments with two replications. There were 59 $F_{2,3}$ lines and 47 $F_{2,3}$ lines from the GM and AP crosses, respectively, plus parental lines and an F_2 plot consisting of a random sample of F_2 seed. 1.2 m rows were planted with a Winter Steiger 4-row planter at 8 seeds per foot with a 50 cm row spacing. Weed control consisted of mechanical cultivation and chemical weed control (chloramben and trifluralin at recommended rates) supplemented with hand weeding as needed.

Table 3. Isozyme and morphological trait alleles of the parents of two crosses, Grant and Mandarin Ottawa, and Amcor and Protana.

	Crosses			
	GM		AP	
Locus	Grant	Mandarin Ottawa	Amcor	Protana
Aco4	a	b	.	.
Dia1	b	a	a	b
Idh1	b	a	a	b
Idh2	a	b	.	.
Me	b	a	.	.
Mpi	b	c	.	.
Pgm1	a	b	a	b
Ep	ep	Ep	Ep	ep
W1	w1	W1	.	.
T	T	t	.	.
I	i	I	I	i
R	R	r	r	R

To examine associations between isozyme loci and quantitative trait loci of soybeans, data was recorded on a per plot basis for the following traits:

Seed yield (SDYD) - Weight in grams of threshed, air-dried seed converted to kilograms per hectare.

Plant weight (PLWT)- Weight in grams of unthreshed, air-dried plants harvested at ground level and expressed in kilograms per hectare.

Seed size (SDSZ)- Weight in grams of 100 air-dried seeds .

Maturity (MAT)- recorded in days relative to 10-7-86, when 95% of the pods had reached their mature color. At this date the group 0 (Simpson), I (Hardin), II (Elgin), and III (Zane) maturity checks grown adjacent to the experimental plots were given maturity ratings of 0, +4, +10, and +14, respectively.

Lodging (LOD)- Scored at maturity on a scale of 1 to 5 with 1 being erect and 5 being prostrate.

Harvest Index (HIDX)- Ratio of seed yield to unthreshed plant weight.

Plant height - Recorded in centimeters from the ground to the top of the main stem at two dates, midseason, 8-11-86 (HTMD), and end of season, 10-11-86 (HTED).

Height differential (HTDF)- Difference between height at end of season (HTED) and height at midseason (HTMD)

Data for seed protein and oil traits were obtained from the Northern Regional Research Center, ARS, USDA, Peoria, Ill. The measurements included protein (PRO) and oil

content expressed as percentages of total seed weight, and palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) fatty acids expressed as percentages of total fatty acid content.

Flower color, purple (W1) or white (w1), and pubescence color, tawny (T) or grey (t) were recorded at flowering time and end of season, respectively. Hilum color genes (I and R) were scored on threshed seed samples. Plots were harvested with a Jari mower on October 17, 1985 (GM cross) and October 18, 1985 (AP cross). Plants were bulked within plots, placed in large paper bags, and air-dried in a barn attic for 2 months. Plants from the AP cross were placed in a hot air dryer after harvest for 3 days and then air-dried. Random plot samples from each cross were tested for moisture content.

Electrophoretic starch gel techniques used were essentially those of Cardy and Beversdorf (1984) with the following modifications in methodology or procedure. Seedlings were grown in the greenhouse under black shadecloths and sampled after 4 days. One cotyledon per seedling was sliced into two pieces and each piece (approximately 70 mg) placed into a 1.5 ml microcentrifuge tube with 0.22 ml of homogenation buffer. All isozymes were run on the "D" buffer system (Cardy and Beversdorf, 1984). Wicks were cut from Beckman filter paper (1 mm thickness) and were removed from the gel after 15 minutes of loading. Twenty-four samples were run per gel plus parental samples

which were placed on both edges and in the middle of the gel. Six F_4 seeds were sampled per harvested $F_{2,3}$ plot (rep 1 only) to determine the isozyme profile of the originating F_2 plant. Duplicate sample sets were run to provide enough gel slices to correctly score all isozyme systems. The isozyme loci analyzed in this study are listed in Table 3. Peroxidase activity was scored on six F_4 seeds per plot using the spot test procedure (Buttery and Buzzell, 1968).

The data were analyzed using the Statistical Analysis System (Statistical Analysis Institute, 1985) for personal computers. For each plant trait, an analysis of variance was used to determine variation among $F_{2,3}$ families. Plant number was used as a covariate for traits in which the partial sum of squares for plant number was significant. For each locus, two gene marker variables were created, an additive component with genotype values of $A_1A_1 = 1$, $A_1A_2 = 0$, and $A_2A_2 = -1$ and a dominant component with genotype values of $A_1A_1 = 0$, $A_1A_2 = 1$, and $A_2A_2 = 0$. Forward addition regression procedures using additive and dominant components of all loci were used to determine which gene marker components accounted for a significant proportion of the variation among $F_{2,3}$ families. The degree of dominance at each marker locus was determined by a t-test comparing the magnitudes of the additive and dominant components. The marker loci used in this study are not linked (Gorman et al, 1984; Hedges, personal communication) and therefore should all be marking different chromosomal segments.

Results and Discussion

Agronomic traits

Agronomic trait means and contrasts for the parental, $F_{2,3}$ and F_2 generations are shown in Table 4 for the Grant x Mandarin Ottawa population (GM) and in Table 5 for the Amcor x Protana population (AP). In the GM population, the parents and F_2 generation differed for end of season height (HTED) and the parents and $F_{2,3}$ generation differed for harvest index (HIDX). In the AP population, the parents differed for midseason height (HTMD).

Most of the agronomic traits were highly interrelated as seen from the correlations (based on family means) in Table 6 (GM population) and Table 7 (AP population). Yield was significantly correlated with seed weight, maturity, and height in both populations and also correlated with lodging in the GM population. Anand and Torrie (1963) found a similar relationship in soybeans; yield was positively correlated with height, maturity, and lodging susceptibility. Harvest index showed a positive correlation with yield and seed weight in the AP population but had a negative correlation with plant weight, lodging, maturity, and height in both populations. Correlations were not always consistent across populations. For example, seed weight was positively correlated with lodging in the GM population but negatively correlated with lodging in the AP population. As noted by Burton (1987), the level of

Table 4. Means of agronomic traits and contrasts for Grant (P_1), Mandarin Ottawa (P_2), and the F_2 and $F_{2,3}$ generations derived from a cross of Grant by Mandarin Ottawa.

	Means				Contrasts[1]			
Trait	P_1	P_2	$F_{2,3}$	F_2	P_1 v P_2	$F_{2,3}$ v P	$F_{2,3}$ v F_2	F_2 v P
SDYD	456.0	477.6	474.6	446.3	ns	ns	ns	ns
PLWT	766.5	791.8	839.4	772.5	ns	ns	ns	ns
SDSZ	17.07	18.46	18.03	17.89	+	ns	ns	ns
LOD	2.64	1.86	2.46	2.12	ns	ns	ns	ns
MAT	+1.31	-0.79	+1.12	+1.37	+	ns	ns	ns
HTMD	53.65	53.15	51.39	48.65	ns	ns	ns	ns
HTED	64.36	67.02	61.66	57.57	ns	+	ns	*
HTDF	10.71	13.88	10.28	08.93	ns	ns	ns	ns
HIDX	0.596	0.609	0.567	0.577	ns	**	ns	ns

+,*,** denote significance at the .1, .05, and .01 probability levels, respectively.

[1] P = Mean of the parents, P_1 = Grant, P_2 = Mandarin Ottawa.

Table 5. Means of agronomic traits and contrasts between the means for Amcor (P_1), Protana (P_2), and F_2 and $F_{2,3}$ generations derived from a cross of Amcor by Protana.

	Means				Contrasts[1]			
Trait	P_1	P_2	$F_{2,3}$	F_2	P_1 v P_2	$F_{2,3}$ v P	$F_{2,3}$ v F_2	F_2 v P
SDYD	392.5	362.5	388.7	480.0	ns	ns	ns	ns
PLWT	792.5	701.8	820.9	940.0	ns	ns	ns	ns
SDSZ	14.70	14.95	14.72	14.90	ns	ns	ns	ns
LOD	3.50	2.75	2.93	3.00	ns	ns	ns	ns
MAT	+4.84	+3.39	+3.90	+4.31	ns	ns	ns	ns
HTMD	61.0	39.5	51.96	60.0	**	ns	ns	ns
HTED	84.4	67.0	77.1	82.2	*	ns	ns	ns
HTDF	22.0	26.5	25.12	25.0	ns	ns	ns	ns
HIDX	0.492	0.519	0.479	0.503	ns	ns	ns	ns

*,** denote significance at the .05 and .01 probability levels, respectively.

[1] P = Mean of parents, P_1 = Amcor, P_2 = Protana.

Table 6. Correlations between agronomic trait means in an $F_{2,3}$ population derived from a cross of Grant by Mandarin Ottawa.

	PLWT	SDSZ	LOD	MAT	HTMD	HTED	HTDF	HIDX
SDYD	0.915 ***	0.400 ***	0.382 **	0.300 *	0.332 **	0.453 ***	0.226 ns	-0.016 ns
PLWT	.	0.428 ***	0.523 ***	0.473 ***	0.311 *	0.597 **	0.407 **	-0.405 **
SDSZ	.	.	0.318 *	0.508 ***	0.085 ns	0.280 *	0.245 ns	-0.197 ns
LOD	.	.	.	0.485 ***	0.225 ns	0.577 ***	0.457 ***	-0.443 ***
MAT	.	.	.	.	-0.292 *	0.481 ***	0.797 ***	-0.537 ***
HTMD1	.	.	.	.	.	0.523 ***	-0.272 *	0.026 ns
HTED	.	.	.	.	.	.	0.678 ***	-0.417 ***
HTDF	.	.	.	.	.	.	.	-0.493 ***

*,**, *** denote significance at the .05, .01, and .001 probability levels, respectively.

Table 7. Correlation of agronomic trait means in an $F_{2,3}$ population derived from a cross of Amcor by Protana.

	PLWT	SDSZ	LOD	MAT	HTMD	HTED	HTDF	HIDX
SDYD	0.619 ***	0.566 ***	-0.102 ns	-0.302 *	0.665 ***	0.107 ns	-0.364 **	0.559 ***
PLWT	. .	-0.190 ns	0.496 ***	0.404 **	0.664 ***	0.727 ***	0.329 *	-0.287 *
SDSZ	. .	. .	-0.587 ***	-0.633 ***	0.176 ns	-0.551 ***	-0.732 ***	0.905 ***
LOD	. .	. .	. .	0.775 ***	0.148 ns	0.646 ***	0.598 ***	-0.697 ***
MAT	. .	. .	. .	. .	-0.031 ns	0.638 ***	0.726 ***	-0.796 ***
HTMD	. .	. .	. .	. .	. .	0.443 ***	-0.217 ns	0.115 ns
HTED	. .	. .	. .	. .	. .	. .	0.771 ***	-0.629 ***
HTDF	. .	. .	. .	. .	. .	. .	. .	-0.779 ***

*,**, *** denote significance at the .05, .01, and .001 probability levels, respectively.

significance and sign of the correlation depended on the population being investigated.

The contribution of individual gene markers to variability in agronomic traits is shown in Table 8 (GM population) and Table 9 (AP population). In both populations there was a broad range of results with every quantitative trait having at least one significant gene marker and every marker gene having a significant effect for at least one quantitative trait. The total percent of phenotypic variation in agronomic traits ascribable to the set of significant markers varied from about 17% to more than 45% in the GM population (Table 8), and from 8% to over 46% in the AP population (Table 9). The average percent of phenotypic variation accounted for by markers (across traits and populations) is 30.6%.

The number of loci with significant effects on agronomic variability ranged from two loci to seven loci in the GM population (Table 8) and from two to six loci in the AP population (Table 9). Most of the significant effects were small. Over 80% of the marker effects accounted for less than 10% of the phenotypic variation of agronomic traits in both populations. However, a few loci, such as the Dia1 locus in the AP population and Pgm1 locus in the AP population accounted for large effects for a number of agronomic traits.

Table 8. Percent of agronomic trait variation accounted for by additive (A-) and dominant (D-) gene marker components in an $F_{2,3}$ soybean population derived from a cross of Grant by Mandarin Ottawa.

Locus	PLWT	SDYD	SDSZ	MAT	LOD	HIDX	HTMD	HTED	HTDF
A-Aco4	.	.	.	2.06 *	.	.	.		
D-Aco4	.	.	2.77 **	.	.	.	.	.	.
A-Dial	9.58 **	7.60 **	28.21 **	17.87 **	4.38 **	4.34 *	.	.	5.10 *
D-Dial	.	.	.	.	.	.	.	.	.
A-Idh1	.	.	1.11 *	1.42 *	.	.	.	.	.
D-Idh1	.	.	.	.	.	.	.	.	.
A-Idh2	9.67 **	6.99 *	1.81 **	8.48 **	.	6.79 *	.	.	6.48 **
D-Idh2	.	.	.	.	.	.	.	.	.
A-Me	5.11 **	.	1.85 **	1.20 *	7.42 **	.	.	9.58 **	3.07 *
D-Me	.	.	0.85 *	6.91 **	.	.	5.00 *	5.15 **	.
A-Mpi	.	.	1.23 *	4.97 **	.	.	.	2.75 *	.
D-Mpi	.	.	.	.	.	.	.	.	.
A-Pgm1	.	.	.	.	.	.	3.94 +	8.44 **	.
D-Pgm1	.	.	.	2.34 **	.	.	12.22 **	.	.
A-Ep	.	.	.	.	13.56 **	.	.	.	3.29 *
D-Ep	.	.	.	.	.	5.74 *	.	.	.
A-W1	.	.	3.79 ***	.	.	.	.	.	.
D-W1	5.26 *	4.58 *	.	.	9.19 **	.	.	11.40 **	2.43 *
A-T	.	.	.	.	.	.	.	2.49 *	.
D-T	.	.	.	.	.	.	.	.	.
A	24.36	14.59	38.00	36.00	25.36	11.13	16.99	24.12	17.97
D	5.26	4.58	3.61	9.25	9.19	5.74	6.22	16.55	2.43
Total	29.62	19.17	41.61	45.25	34.55	16.87	23.21	40.67	20.40

+,*,**, *** denote significance at the .1, .05, .01, and .001 probability levels, respectively.

Table 9. Percent of agronomic trait variation accounted for by additive (A-) and dominant (D-) gene marker components in an $F_{2,3}$ soybean population derived from a cross of Amcor by Protana.

Locus	PLWT	SDYD	SDSZ	MAT	LOD	HIDX	HTMD	HTED	HTDF
A-Dia1	.	.	2.52 **	3.46 **	.	4.44 **	.	3.62 **	6.05 **
D-Dia1	.	3.57 *	.	.	.	0.99 *	4.97 *	.	.
A-Idh1	.	.	1.78 **	1.72 **	.	1.13 *	.	.	.
D-Idh1	.	.	3.35 **	1.31 *	.	4.06 **	.	.4.44 **	5.38 **
A-Pgm1	8.71 **	.	19.05 **	19.45 **	13.95 **	20.54 **	.	18.59 **	20.60 **
D-Pgm1	.	.	.	2.68 **	11.03 **	.	.	.	3.46 *
A-Ep	.	.	1.72 **	1.74 **	.	1.48 *	3.71 **	.	3.28 *
D-Ep	4.60 *	.	5.01 **	3.34 **	6.45 **	3.83 **	8.76 **	17.03 **	7.58 **
A-I	.	.	1.78 **	.	.	.	.	.	.
D-I	.	4.67 *	2.82 **	.	.	3.24 **	.	.	.
A-R	.	.	1.84 **	.	.	.	.	.	.
D-R	.	.	1.17 **	.	4.35 *	.	.	.	.
A	8.71	0	28.69	26.37	13.95	27.59	3.71	22.21	29.93
D	4.60	8.24	12.35	7.33	21.83	12.12	13.73	21.47	16.42
Total	13.31	8.24	41.04	33.70	35.78	39.71	17.44	43.68	46.35

*,** denote significance at the .05, and .01 probability levels, respectively.

The magnitude of effects and alleles associated with a positive effect are shown in Table 10 (GM) and Table 11 (AP) for each significant marker. The independent variable of the regression analysis was in units of alleles. Therefore, these figures represent the change in plant trait value resulting from an allelic substitution. Negative values for additive effects have no intrinsic meaning because the values of +1 and -1 were arbitrarily assigned to alleles for the regression analysis. The allele associated with a positive effect for that trait is shown with the significant locus. The sign associated with a significant dominant component indicates direction of dominance in increasing or decreasing expression.

The extensive associations found between qualitative marker loci and quantitative trait loci is similar to the results in maize found by Stuber et al. (1987). They also found that most of the significant associations were relatively small in magnitude. This conforms to expectations for agronomic traits which are assumed to be under polygenic control.

The limited number of markers used in this study, between six and ten, means that only a portion of the genome was covered with gene markers. Assuming quantitative trait loci to have small but equal effects and to be spread randomly throughout the genome, the percent of variability detected by independent markers should be approximately proportional to the percent of the genome covered by gene

Table 10. Regression coefficients for agronomic trait additive (A-) and dominant (D-) gene marker components and allele associated with a positive effect for that trait[1] in an $F_{2,3}$ soybean population derived from a cross of Grant by Mandarin Ottawa.

	Agronomic Trait								
Locus	PLWT	SDYD	SDSZ	MAT	LOD	HIDX	HTMD	HTED	HTDF
A-Aco4	.	.	.	0.382 a	.	.			
D-Aco4	.	.	0.533	.	.	.	.	.	.
A-Dia1	-25.0 a	-11.5 a	-1.01 a	-1.20 a	-.183 a	.00675 b	.	.	-1.40 a
D-Dia1	.	.	.	.	.	.	-1.53	.	.
A-Idh1	.	.	0.235 a	-0.312 b	.	.	.	.	
D-Idh1	.	.	.	.	.	.	.	.	.
A-Idh2	17.3 a	8.54 a	0.378 a	0.665 a	.	-.0084 b	.	.	0.969 a
D-Idh2	.	.	.	.	.	.	.	.	.
A-Me	-20.7 b	.	0.262 a	-.315 a	-2.86 b	.	.	-3.03 b	-1.10 b
D-Me	.	.	0.411	.935	.	.	-1.71	-1.87	.
A-Mpi	.	.	0.208 b	.524 b	.	.	.	1.15 b	.
D-Mpi	.	.	.	.	.	.	.	.	.
A-Pgm1	.	.	.	.	.	.	-1.21 b	-2.27 b	.
D-Pgm1	.	.	.	.760	.	.	-2.41	.	.
A-Ep	.	.	.	.	0.246 ep	.	.	.	0.865 ep
D-Ep	.	.	.	.	.	-.014	.	.	.
A-W1	.	.	-.429 W	.	.	.	.	.	.
D-W1	-26.4	-12.6	.	.	-.379	.	.	-3.45	-1.60
A-T	.	.	.	.	.	.	.	1.47 t	.
D-T	.	.	.	.	.	.	.	.	.

[1] Designation for allele is shown below regression coefficient.

Table 11. Regression coefficients for agronomic trait additive (A-) and dominant (D-) gene marker components and allele associated with a positive effect for that trait[1] in an $F_{2,3}$ soybean population derived from a cross of Amcor x Protana.

	Agronomic Trait								
Locus	PLWT	SDYD	SDSZ	MAT	LOD	HIDX	HTMD	HTED	HTDF
A-Dia1	.	.	.559	-.511	.	.027	.	-3.54	-4.38
			b	a		b		a	a
D-Dia1	.	17.84	.	.	.	.016	3.36	.	.
A-Idh1	.	.	.415	-.396	.	.016	.	.	.
			a	b		a			
D-Idh1	.	.	-.086	.772	.	-.052	.	4.96	6.05
A-Pgm1	45.01	.	-1.67	1.55	.44	-.062	.	9.02	7.60
	a		b	a	a	b		a	a
D-Pgm1	.	.	.	0.968	.461	.	..	.	4.00
A-Ep	.	.	-.456	.510	.	-.015	-2.00	.	3.68
			Ep	ep		Ep	Ep		ep
D-Ep	-38.02	.	1.29	-1.46	-.502	.047	-4.13	-11.48	-8.47
A-I	.	.	.524	.	.	.	.	.	.
			i						
D-I	.	21.65	.895	.	.	.035	.	.	.
A-R	.	.	.624	.	.	.	.	.	.
			r						
D-R	.	.	.500	.	-.288	.	.	.	.

[1] Designation for allele is shown below regression coefficient.

markers. In soybeans (n = 20), 6 to 10 markers could delineate a maximum of 30% to 50% of the genome, respectively, if each marked a whole chromosome and 15% and 25% of the genome if each marker tagged a chromosome arm. These percentages would be less if recombination rates approached 50% within a chromosome arm. The results for percent of variation accounted for by marker genes falls within the range expected given the number of markers used in this study. In both populations the average percent of variability across all agronomic traits accounted for by gene markers is about 31%.

From the partitioning of gene markers into additive and dominant components, the relative importance of each type of gene action can be assessed. Summed across all agronomic traits, additive effects were more important than dominant effects; they accounted for about 77% of the significant marker effects in the GM population (Table 5) and 58% of the significant marker effects in the AP population (Table 6). These figures should be viewed as rough approximations since only a small portion of the quantitative trait variation was detected with markers.

The relatively greater importance of additive versus dominant effects in soybean agronomic traits is highly consistent with the results of previous studies (Burton, 1987). Agronomic traits such as height, maturity, lodging resistance, unthreshed weight, and yield show mostly additive variance (Brim and Cockerham, 1961; Gates et al,

1960). However, there is some evidence for dominance variance in agronomic traits of soybeans. Small but significant dominance effects have been detected for traits such as height, lodging, and seed weight (Croissant and Torrie, 1971).

The gene action at each locus for each agronomic trait is shown in Tables 12 (GM) and 13 (AP). In the GM population, additive effects were predominant while in the AP population, overdominant effects were predominant. The presence of such a large number of overdominant effects may be explained by pseudo-overdominance. If several loci affecting a given quantitative trait are in repulsion phase linkage, exhibit partial to complete dominance, and are linked to a marker locus, then the heterozygote may have a greater value than either homozygote (Falconer, 1981). In the GM and AP populations, many of the loci show negative overdominance. In this case, overdominance would be due to repulsion phase linkage between dominant alleles with unfavorable effects relative to the quantitative trait. In a similar study in maize, a substantial number of overdominance effects were also detected (Stuber et al., 1987).

Linkage of a marker gene with more than one QTL is certainly plausible. In maize (Stuber, 1987), several QTLs affecting the same quantitative trait are often located on the same chromosome arm and in Drosophilia melanogaster,

Table 12. Gene action[1,2] at marker loci with significant effects for agronomic and seed composition traits in an $F_{2,3}$ soybean population derived from a cross of Grant by Mandarin Ottawa.

	Locus									
Trait	Aco4	Dia1	Idh1	Idh2	Me	Mpi	Pgm1	Ep	W1	T
PLWT	.	A	.	A	A	.	.	.	-OD	.
SDYD	.	A	.	A	.	.	.	.	-OD	.
SDSZ	OD	A	A	A	D	A	.	.	A	.
MAT	A	A	A	A	D	A	.	.	.	.
LOD	.	A	.	.	A	.	.	A	-OD	.
HIDX	.	A	.	A	.	.	.	OD	.	.
HTMD	.	-OD	.	.	-OD	.	-D	.	.	.
HTED	.	.	.	.	-D	A	A	.	-OD	A
HTDF	.	A	A	.	A	.	.	A	-OD	.
PRO	.	.	.	.	.	-OD	.	OD	.	D
OIL	A	OD	OD	.	-OD	.	.	-D	.	-OD
16:0	-OD	A	.	.	A	.	-OD	-OD	.	.
18:0	A	A	.	.	.	.	-OD	-OD	.	.
18:1	.	.	.	.	.	.	A	.	D	.
18:2	.	.	.	A	.	.	.	.	-OD	.
18:3	.	.	A	A	.	.	A	.	A	A

[1] A = additive effects, D = complete dominance, and OD = overdominance

[2] negative sign indicates gene action with respect to the negative allele.

Table 13. Gene action[1,2] at marker loci with significant effects for agronomic and seed composition traits in an $F_{2,3}$ soybean population derived from a cross of Amcor by Protana.

	Locus					
Trait	Dia1	Idh1	Pgm1	Ep	I	R
PLWT	.	.	A	-OD	.	.
SDYD	OD	.	.	.	OD	.
SDSZ	A	-OD	A	OD	D	D
MAT	A	D	PD	-OD	.	.
LOD	.	.	D	-OD	.	-OD
HIDX	D	-OD	A	D	OD	.
HTMD	OD	.	.	-D	.	.
HTED	A	OD	A	-OD	.	.
HTDF	A	OD	D	-OD	.	.
PRO	A	D	.	.	.	.
OIL	.	OD	A	-D	-OD	A
16:0	OD	OD	D	-OD	A	.
18:0	A	A	A	D	-OD	.
18:1	-OD	-D	.	-OD	-D	-OD
18:2	A	OD	.	.	D	OD
18:3	A	-D	.	.	OD	.

[1] A = additive effects, PD = partial dominance, D = complete dominance, and OD = overdominance

[2] negative sign indicates gene action with respect to the negative allele.

linkages have been detected between a QTL and marker genes located at varying distances from the QTL (Jayakar et al., 1977).

Gene action at the locus level can, in some instances, explain variation at the phenotypic level. The F_2 and $F_{2,3}$ generations showed negative heterosis for end of season height (GM population). Sixteen percent of the phenotypic variation for height was accounted for by two marker loci with dominant or overdominant gene action (Table 8). In the AP population, no heterotic effects in the F_2 or $F_{2,3}$ generation were evident even though there were significant dominant effects associated with all of the agronomic traits. This may be due to positive and negative dominance effects negating each other when summed over loci (Brim and Cockerham, 1961). Almost all of the agronomic traits in the AP population have both positive and negative dominance or overdominance effects associated with gene markers.

The effect of marker heterozygosity on agronomic trait expression was calculated by regressing agronomic trait level on the number of heterozygous marker loci. The degree of heterozygosity had little effect on agronomic trait expression, only seed size in the AP population showed a significant relationship with heterozygosity.

In surveying the range of gene marker-QTL associations, two types of effects are evident. In one scenario, some marker loci have significant associations with an array of agronomic traits. Examples of these include the Dial and

Idh2 loci in the GM population and the Pgm1 locus in the AP population. One explanation is that there are several QTLs affecting different quantitative traits linked to the same marker locus. A second explanation is that one or more QTLs linked to the marker locus have pleiotropic effects across many agronomic traits. This implies a common genetic regulation for many of these agronomic traits. Processes such as developmental timing and partitioning of assimilate flow may be regulated by a particular set of interacting genes. Structural or regulatory differences in one or more these genes may result in pleiotropic differences at the phenotypic level. A third explanation is that the QTL linked to the marker has a direct effect on only one agronomic trait but that pleiotropic effects at other loci produce a statistical correlation between the marker and other agronomic traits. For breeding uses, the latter effect is usually more desirable because there will not be any pleiotropic effects or linkages between desirable and undesirable QTLs associated with a marker locus.

The second effect noted is that some marker loci had relatively large effects for specific agronomic traits. The Dia1 locus (GM) and Pgm1 locus (AP) accounted for 28% and 19% of the phenotypic variation for for seed size, respectively. These genes may fall into an intermediate classification, between major genes, with mendelian segregation, and minor genes, whose effect can not be studied individually (Falconer, 1981) The effects of these

intermediate genes may be too small to produce discrete phenotypic classes yet may still have a noticeable impact on the agronomic trait. In the Grant x Mandarin Ottawa cross, the latter parent has the positive Dial allele for seed size and has a significantly higher mean for seed size (Table 3).

Seed composition traits

Seed composition trait means and contrasts for the parental, F_2, and $F_{2,3}$ generations are presented in Table 14 (GM population) and in Table 15 (AP population). In the GM population there were no significant differences among the means for any of the seed composition traits. In the AP population there were significant differences for protein percent between the parents and between the F_2 and $F_{2,3}$ generations. There were also significant differences between the midparent and $F_{2,3}$ generation for oleic, linoleic, and linolenic acid content and between the midparent and F_2 generation for oleic and linoleic acid content.

The correlations among means for seed composition traits are shown in Table 16 (GM) and Table 17 (AP). In general, there were few correlations among the seed composition traits. The only correlations that were significant in both populations were the correlations of oleic acid with linoleic acid and linolenic acid. Protein and oil percent were negatively correlated in the GM population but not significantly correlated in the AP population.

Table 14. Means of seed composition traits and contrasts between the means for Grant, Mandarin Ottawa, and the F_2 and $F_{2,3}$ populations derived from a cross of Grant by Mandarin Ottawa.

	Means				Contrasts[1]			
Trait	P_1	P_2	$F_{2,3}$	F_2	P_1 v P_2	$F_{2,3}$ v P	$F_{2,3}$ v F_2	F_2 v P
PRO	42.90	42.15	43.19	43.55	ns	+	ns	ns
OIL	17.65	17.30	17.32	17.00	ns	ns	ns	ns
16:0	10.70	11.20	10.89	11.00	ns	ns	ns	ns
18:0	3.10	2.95	2.95	3.05	ns	ns	ns	ns
18:1	19.80	19.30	19.88	20.05	ns	ns	ns	ns
18:2	55.20	55.25	55.15	54.95	ns	ns	ns	ns
18:3	11.20	11.30	11.09	10.95	ns	ns	ns	ns

+ denotes significance at the .1 probability level

[1] P = Midparent, P_1 = Grant, P_2 = Mandarin Ottawa.

Table 15. Means of seed composition traits and contrasts between the means for Amcor (P_1), Protana (P_2), and F_2 and $F_{2,3}$ populations derived from a cross of Amcor by Protana.

	----------Means----------				-----Contrasts[1]------			
Trait	P_1	P_2	$F_{2,3}$	F_2	P_1 v P_2	$F_{2,3}$ v P	$F_{2,3}$ v F_2	F_2 v P
PRO	40.85	44.80	42.88	41.85	***	ns	*	ns
OIL	19.00	18.95	19.34	19.10	ns	ns	ns	ns
16:0	10.30	10.10	10.41	10.20	ns	ns	ns	ns
18:0	2.70	2.95	2.87	2.85	ns	ns	ns	ns
18:1	22.44	22.18	19.43	18.58	ns	***	ns	***
18:2	53.94	54.52	56.15	57.17	ns	***	ns	***
18:3	10.70	10.30	11.15	11.05	ns	**	ns	ns

*,**, *** denote significance at the .05, .01,and .001 probability levels, respectively.
[1] P = Midparent, P_1 = Grant, P_2 = Mandarin Ottawa.

Table 16. Correlations between seed composition trait means in an $F_{2,3}$ population derived from a cross of Grant by Mandarin Ottawa.

	OIL	16:0	18:0	18:1	18:2	18:3
PRO	-0.448	0.063	-0.118	0.018	-0.020	-0.005
	***	ns	ns	ns	ns	ns
OIL	.	-0.042	0.134	0.127	-0.041	-0.157
	.	ns	ns	ns	ns	ns
16:0	.	.	0.426	-0.167	-0.261	-0.087
	.	.	***	ns	*	ns
18:0	.	.	.	0.019	-0.284	-0.136
	.	.	.	ns	*	ns
18:1	.	.	.	.	-0.756	-0.282
	.	.	.	.	***	*
18:2	.	.	.	.	.	-0.208
	.	.	.	.	.	ns

*,*** denote significance at the .05 and .001 probability levels, respectively.

Table 17. Correlations between seed composition trait means in an $F_{2,3}$ population derived from a cross of Amcor by Protana.

Trait	OIL	16:0	18:0	18:1	18:2	18:3
PRO	0.112	0.175	-0.215	0.062	-0.067	-0.144
	ns	ns	ns	ns	ns	ns
OIL	.	0.472	-0.202	0.260	-0.241	-0.235
	.	***	ns	ns	ns	ns
16:0	.	.	-0.121	-0.298	0.114	0.001
	.	.	ns	*	ns	ns
18:0	.	.	.	-0.032	-0.070	-0.038
	.	.	.	ns	ns	ns
18:1	.	.	.	.	-0.856	-0.333
	.	.	.	.	***	*
18:2	.	.	.	.	.	-0.139
	.	.	.	.	.	ns

*,*** denote significance at the .05 and .001 probability levels, respectively.

The contribution of individual gene markers to seed composition trait variability is shown in Tables 18 (GM population) and 19 (AP population). The range of effects was similar to that found for agronomic traits; every gene marker affected at least one seed quality trait and every seed quality trait had at least one significant gene marker. The total percent of phenotypic variation per seed composition trait accounted for by the set of significant markers varied from about 19% to almost 53% in the GM population (Table 18) and from about 15% to 32% in the AP population (Table 19). The magnitude of effects for each significant marker and allele associated with a positive effect is shown in Table 20 (GM population) and in Table 21 (AP population).

The number of significant associations per seed composition trait varied from two to six in the GM population and from two to five in the AP population. Most of the loci had relatively small effects; 90% or more of the significant markers accounted for 10% or less of the phenotypic variation for seed composition traits.

The average percent of seed composition trait variation accounted for by gene markers (summed across traits and populations) was 27%. This is similar to the result found for agronomic traits and within the range expected given the number of gene markers used in these populations.

Summed across all seed composition traits, additive effects were more important than dominance effects in the GM

Table 18. Percent of seed composition trait variation accounted for by additive (A-) and dominant (D-) gene marker components in an $F_{2,3}$ soybean population derived from a cross of Grant by Mandarin Ottawa.

	Trait						
Locus	PRO	OIL	16:0	18:0	18:1	18:2	18:3
A-Aco4	.	4.54 *	.	.	.	.	.
D-Aco4	.	.	3.82 *	.	.	.	.
A-Dial	.	.	5.49 *	5.07 *	.	.	.
D-Dial	.	4.89 *	.	.	.	.	.
A-Idh1	.	.	.	3.18 +	.	.	3.32 *
D-Idh1	.	6.79 **	.	.	.	.	.
A-Idh2	.	.	.	.	.	19.00 **	31.81 **
D-Idh2	.	.	.	.	.	.	.
A-Me	.	.	6.38 *	.	.	.	.
D-Me	.	5.26 **	.	.	.	.	.
A-Mpi	.	.	.	.	.	.	.
D-Mpi	6.22 *	.	.	.	.	.	.
A-Pgm1	.	.	.	.	5.46 *	.	5.53 **
D-Pgm1	.	.	9.57 **	15.37 **	.	.	.
A-Ep	.	4.71 *	.	.	.	.	.
D-Ep	6.47 *	3.05 *	4.40 *	4.06 *	.	.	.
A-W1	.	.	.	.	5.64 *	.	5.98 **
D-W1	.	.	.	.	6.39 **	3.92 *	.
A-T	5.54 *	.	.	.	.	.	6.08 **
D-T	6.46 *	3.00 *	.	.	.	.	.
A	5.54	22.99	11.87	8.25	13.10	19.00	52.72
D	19.15	9.25	17.79	19.43	6.39	3.92	0
Total	24.69	32.24	29.66	27.68	19.49	22.92	52.72

+, *, ** denote significance at the 0.1, 0.05 and 0.01 probability levels, respectively.

Table 19. Percent of seed composition trait variation accounted for by additive (A-) and dominant (D-) gene marker components in an $F_{2,3}$ soybean population derived from a cross of Amcor by Protana.

	Trait						
Locus	PRO	OIL	16:0	18:0	18:1	18:2	18:3
A-Dial	2.12 *	.	.	6.02 *	.	4.76 **	1.72 +
D-Dial	.	.	4.22 **	.	3.86 **	.	.
A-Idh1	9.38 **	.	2.59 **	4.61 **	1.14 *	.	5.87 **
D-Idh1	3.47 *	4.11 *	5.12 **	.	4.07 **	5.10 **	2.01 *
A-Pgm1		.4.43 *	5.30 **	10.20 **	.	.	.
D-Pgm1	.	.	3.85 **	.	.	.	.
A-Ep	.	3.93 *	.	2.59 *	.	.	.
D-Ep	.	3.55 *	5.92 **	3.10 *	1.43 *	.	.
A-I	.	.	3.52 **	.	5.02 **	2.78 **	.
D-I	.	8.37 **	.	2.51 *	9.94 **	7.57 **	1.58 +
A-R	.	3.52 *	.	.	1.15 *	.	
D-R	.	.	.	.	5.75 **	4.13 **	
A	11.50	11.88	11.41	23.42	7.31	7.54	7.59
D	3.47	16.03	19.11	5.61	25.05	16.80	2.01
Total	14.97	27.91	30.52	29.03	32.36	24.34	9.60

+,*,** denote significance at the 0.1, 0.05, and 0.01 probability levels, respectively.

Table 20. Regression coefficients for seed composition trait additive (A-) and dominant (D-) gene marker components and allele associated with a positive effect for that trait[1] in an $F_{2,3}$ soybean population derived from a cross of Grant by Mandarin Ottawa.

	Trait						
Locus	PRO	OIL	16:0	18:0	18:1	18:2	18:3
A-Aco4	.	-.149 b	.	.	.	.	.
D-Aco4	.	.	-.191	.	.	.	.
A-Dia1	.	.	.181 b	.072 b	.	.	.
D-Dia1		.278	.	.	.	.	.
A-Idh1	.	.	.	-.061 b	.	.	-.239 b
D-Idh1	.	.272	.	.	.	.	.
A-Idh2	.	.	.	.	.	.617 a	-.525 b
D-Idh2	.	.	.	.	.	.	.
A-Me	.	.	.171 a	.	.	.	.
D-Me	.	-.216	.	.	.	.	
A-Mpi	.	.	.	.	.	.	.
D-Mpi	-.434	.		.	.	.	.
A-Pgm1	.	.	.	.	.375 a	.	-.198 b
D-Pgm1	.	.	-.239	-.111	.	.	.
A-Ep	.	.176 ep	.	.	.	.	.
D-Ep	.545	-.265	-.183	-.087	.	.	.
A-W1	.	.	.	.	-.387 W	.	.249 w
D-W1	.	.	.	.	.705	-.468	.
A-T	.209 t	.	.	.	.	.	.251 t
D-T	.291	-.166	.	.	.	.	.

[1] Allele designation allele is shown below regression coefficient.

Table 21. Regression coefficients for seed composition trait additive (A-) and dominant (D-) gene marker components and allele associated with a positive effect for that trait[1] in an $F_{2,3}$ soybean population derived from a cross of Amcor by Protana.

Locus	PRO	OIL	16:0	18:0	18:1	18:2	18:3
A-Dia1	.208 b	.	.	.063 b	.	-.394 a	.157 b
D-Dia1	.	.	.158	.	-.573	.	.
A-Idh1	.412 a	.	-.080 b	.078 a	.270 a	.	-.233 b
D-Idh1	.340	.776	.278	.	-.643	.727	-.214
A-Pgm1	.	.559 a	.197 a	-.128 b	.	.	.
D-Pgm1	.	.	.177 **	.	.	.	.
A-Ep	.	.421 ep	.	-.058 Ep	.	.	.
D-Ep	.	-.585	-.266	.0824	-.359	.	.
A-I	.	.	-.084 I	.	.627 i	-.340 I	.
D-I	.	-.671	.	-.096	-.438	.324	.277
A-R	.	.331 r	.	.	.263 r	.	.
D-R	.	.	.	.	-.842	.852	.

[1] Allele designation is shown below regression coefficient.

population (Table 18) but not in the AP population (Table 19). Additive effects accounted for 64% of the total marker variation in the GM population but only 47% of the marker variation in the AP population. Brim and Cockerham (1961) found significant additive variance but no dominance variance for percent seed protein and percent seed oil.

Gene action associated with individual marker loci is shown in Tables 9 and 10 for seed quality traits. The majority of effects were either dominant effects or overdominant effects. Additive effects accounted for less than one-half of the significant locus effects in both populations. As noted in the agronomic trait results section, pseudo-overdominance may be responsible for the large number of observed overdominance effects. The other possibility is that the large number of non-additive effects found was due to sampling error because of the small number of loci evaluated.

Marker heterozygosity had few significant effects on the level of seed composition traits. The only significant effects were in the AP population in which oleic and linoleic fatty acids had a negative and positive relationship, respectively, with marker heterozygosity. The relationship of fatty acid level with marker heterozygosity can be explained in terms of individual loci with significant dominant effects, rather than simply due to the number of heterozygous marker loci. Oleic acid had five marker loci with significant negative dominant effects and

linoleic acid had three loci with significant positive dominant effects.

Two effects were particularly evident among the gene marker-seed composition linkage data. One was that a single locus had a major effect on fatty acid content. The Idh2 locus (GM) accounted for almost a third of the variation for linolenic acid. Five loci, all with additive effects accounted for more than half the variation for linolenic acid content. Wilcox and Cavins (1985) proposed a single gene with additive effects controlling the level of linolenic acid. Their results were based on a cross between a mutant line and the cultivar from which the mutant arose. Therefore other loci regulating linolenic acid content would not be segregating among the progeny. The results of this study are consistent with a hypothesis of one major gene and four minor genes regulating linolenic acid content.

The second effect noted was the occurrence of negative correlations in both populations between oleic acid and linoleic acid and between oleic and linolenic acid. This is highly consistent with the results of Wilson et al. (1981) and Hawkins et al. (1983). There were also inverse relationships at the locus level between oleic, linoleic, and linolenic acids. In the GM population (Table 20), there was an inverse relationship between linoleic and linolenic acid at the Idh2 locus and between oleic and linolenic acid at the Pgm1 and W1 loci. In the AP population (Table 21), there was an inverse relationship between linoleic and

linolenic acid at the Dial locus, between oleic and linolenic acid at the Idhl locus, and between oleic and linoleic acid at the I locus. The negative associations among these three fatty acids most likely result from a consecutive desaturation pathway (Dutton and Mounts, 1966) in which oleic acid is desaturated to linoleic acid, which is then desaturated to linolenic acid. Results of this study at the gene marker level not only support the consecutive desaturation hypothesis, but also associate specific marker alleles with a positive or negative effect on fatty acid content.

Plant breeding efficiency may be improved through indirect selection of marker genes linked to quantitative trait loci. In a self-pollinated species such as soybean, selection of marker genes may be used to fix favorable quantitative trait alleles in early generations. This procedure might be effective in eliminating many of the inferior genotypes in early generations and allow the breeder to concentrate his efforts on a smaller set of superior genotypes. Alternately, by choosing parents with the same marker alleles, a quantitative trait might be fixed at a desired level and show little or no variation among the progeny. For F_1 hybrid production, parents would be chosen to maximize the number of marker loci displaying dominant gene action.

One use of marker-QTL linkages is to manipulate specific quantitative traits. In soybeans, traits of

interest include seed yield, protein, oil, and linolenic acid. Low levels of linolenic acid are desirable because this fatty acid is associated with poor flavor (Moll et al., 1979). In the GM population, selection for low linolenic content could be based on additive effects at five marker loci which account for more than 50% of the variation for linolenic content. Selection at these five loci might be more efficient than selection based on phenotype.

A second use of marker - QTL linkages is to break correlations among quantitative traits. Many of the agronomic traits have positive correlations and protein has negative correlations with yield and oil content. By selecting for marker loci that have significant effects for only one of the correlated traits, it may be possible to independently alter the level of that trait.

A third use of marker - QTL linkages is to construct selection indices. It should be possible to select simultaneously for several traits by selecting favorable alleles at a number of complementary loci. Because the magnitude of effects per marker allele can be determined, appropriate weights can be given to each quantitative trait.

Two of the allozymes which showed a linear, directional response to breeding cycle were incorporated in the GM and AP crosses. One of these, Pgm1-a, showed significant, positive associations (AP population) with maturity, lodging, and unthreshed plant weight, traits highly correlated with yield. This may explain, in part, why this

allele increased in frequency over breeding cycles. The second allozyme, Idh1-b, had relatively small effects on maturity in both crosses but no large effects which might explain why this allele increased in frequency with breeding cycle.

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V. CONCLUSIONS

Fourteen enzyme loci were analyzed for changes in allozyme frequency over breeding cycles. Seven isozyme loci showed erratic changes in allozyme frequency over breeding cycles. Four isozyme loci had a high initial frequency of one allozyme which subsequently became fixed over breeding cycles. Three isozyme loci showed directional, linear changes in allozyme frequencies over breeding cycles. The first two groups showed no effect of artificial selection on allozyme frequency while the last group showed a directional change in allozyme frequency associated with breeding effort. From these results it is hypothesized that selection at linked loci led to the directional change in frequency of the Idh1-b, Pgm1-a, and Sp1-b allozymes.

Using isozyme and morphological loci as genetic markers, many significant associations were found between the gene markers and quantitative trait loci. Gene markers accounted for a substantial portion of the phenotypic variation for agronomic and seed composition traits. For end-of-season plant height and linolenic acid content (GM population), the percent of phenotypic variation accounted for by gene markers exceeded 40% and 50%, respectively. For some traits such as linolenic acid (GM) and seed size (GM), individual marker loci accounted for more than 25% of the phenotypic variation. The QTL linked to these marker loci

with large effects on phenotypic variation may be classified as having effects of intermediate magnitude.

Gene markers were partitioned into components associated with additive and dominant gene action. Additive effects were more important than dominant effects for agronomic traits in both populations and for seed composition traits in the GM population. In both populations, there were a number of significant overdominant effects.

The inverse relationships observed between effects of allozymes associated with oleic, linoleic, and linolenic acids support the hypothesis of synthesis via sequential desaturation from oleic, to linoleic, to linolenic acid. The large additive effect of the Idh2 locus on linolenic acid (GM population) is in agreement with the proposed major gene control for linolenic acid (Wilcox and Cavins, 1985) but is modified to include four minor genes with additive effects.

The Pgm1-a allozyme, which had a linear increase in allozyme frequency over breeding cycles, was associated with large, significant effects for a number of agronomic traits. This result supports a hypothesis that the change over breeding cycle in allozyme frequency at the Pgm1 locus was due to selection at nearby QTLs which are associated with favorable agronomic traits.

The results of this study were based on the crosses, environment, and specific set of marker loci used in this

experiment. With different genetic backgrounds, environments, or marker loci, the results may vary considerably. Genotype by environmental interactions associated with marker loci or epistatic effects between marker loci could modify the results. The presence of these kinds of interactions can only be determined by testing a larger set of crosses in multiple environments.

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