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Molecular marker analysis of iron efficiency in soybean (*Glycine max* (L.) Merr.)

by

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ABSTRACT

The objectives of the studies were to map genes affecting iron deficiency chlorosis in soybean, to test the hypothesis that two inheritance mechanisms control iron deficiency chlorosis in soybean, and to determine the effectiveness of nutrient solution evaluation for iron deficiency chlorosis in soybean. Chlorosis symptoms of one hundred and twenty $F_{2:4}$ lines from a Pride B216 x A15 population, and 92 $F_{2:4}$ lines from an Anoka x A7 population grown in a field of calcareous soil in 1993 and 1994 were evaluated by visual scores and spectrometric chlorophyll determinations. Each population also was evaluated separately with two cycles of nutrient solution tests in the greenhouse. Eighty-nine RFLP and ten SSR markers in the Pride B216 x A15 population, and 82 RFLP, 14 SSR and *I* (hilum color) markers in the Anoka x A7 population were used to construct linkage maps and to locate quantitative trait loci (QTL).

In the field studies, one major gene and two QTL in the Anoka x A7 population, and seven QTL in the Pride B216 x A15 population were mapped. The results demonstrated the validity of the previous hypothesis of two separate genetic mechanisms controlling iron deficiency chlorosis in soybean.

Because most QTL detected in field tests also were detected in the nutrient solution tests, these results verified that similar genetic mechanisms control iron deficiency chlorosis of soybean in field and in nutrient solution

conditions. The results confirmed that nutrient solution tests are effective predictors of iron deficiency chlorosis response in field-grown soybean.

GENERAL INTRODUCTION

Introduction

Iron deficiency chlorosis in soybean is a common problem on calcareous soils. A yield reduction occurs whenever chlorosis is observed, even when the chlorosis symptoms are slight (Niebur and Fehr, 1981). At present, most high-yielding cultivars of soybean in the north central United States can not completely resist iron deficiency chlorosis (Mason et al., 1985). Genetic improvement is a feasible approach to overcome the problem of iron deficiency chlorosis in soybean (Hinz et al., 1987).

Genetic studies of iron deficiency chlorosis can provide basic information for effective improvement of this trait. The hypothesis that a single major gene with modifiying genes (Cianzio and Fehr, 1980) or polygene (Cianzio and Fehr, 1982) genetic mechanisms control iron deficiency chlorosis was proposed. There is however no direct evidence to support this hypothesis.

Nutrient solution tests have been used to evaluate iron deficiency chlorosis in soybean (Coulombe, 1984). If nutrient solution and field evaluations test the same or similar genetic mechanisms, the combined use of nutrient solution and field tests should increase the efficiency of breeding for this trait. Dragonuk et al (1989b) compared the genetic improvement (genetic gains) for iron deficiency chlorosis between nutrient solution and field tests in a soybean recurrent selection program and suggested that nutrient solution and field

evaluations may partially select for different mechanisms of response to iron stress.

The lack of discrete phenotypic segregation for iron deficiency chlorosis (Cianzio and Fehr, 1982) has prevented the use of convential Mendelian genetic analysis (Thompson and Thoday, 1979). This problem can be overcome by using molecular markers to identify quantitative trait loci (QTL) and to characterize individual QTL (Tanksley, 1993).

The purposes of this study were to: (1) map gene(s) controlling soybean iron deficiency chlorosis in two $F_{2:4}$ populations using molecular markers; (2) test the hypothesis that a major gene with modifying genes and/or polygene inheritance mechanisms control iron deficiency chlorosis in soybean; and (3) determine the degree of similarity in genetic mechanisms controlling soybean iron deficiency chlorosis between nutrient solution and field tests.

Dissertation Organization

This dissertation is begun with a general background review followed by two research papers. The background review includes physiological studies, genetic studies, and nutrient solution tests of soybean iron deficiency chlorosis. It also covers molecular markers, gene mapping, and molecular markers applied to soybean gene mapping. Each of the two papers is written in journal article form and will be submitted for publication.

The first paper of this dissertation deals with molecular marker detection of two genetic mechanisms responsible for soybean iron deficiency chlorosis. This paper will be submitted for publication in *Molecular Breeding*. Dr. Silvia Cianzio is a co-author of this paper. She provided the two $F_{2:4}$ populations for this study, participated in the development of this research project, and contributed many valuable discussions of the results.

The second paper of this dissertation describes the molecular marker determination of the efficiency of nutrient solution test for soybean iron deficiency chlorosis. This paper will be submitted for publication in *Crop Science*. Dr. Silvia Cianzio is also a co-author of this paper. Dr. James S. Baumer and Dr. Drew Ivers at Land O'Lakes Inc., Iowa, who contributed the facilities and technology for nutrient solution tests and assisted in conducting the experiments, are co-authors of this paper as well.

Following the two research papers are General Conclusions and References sections. Literature cited in the General Introduction and Conclusions are listed in the References section.

Literature Review

Physiological studies of iron deficiency chlorosis

Iron deficiency chlorosis (IDC) is a widespread problem on calcareous soil. Iron (Fe) deficiency in plants is reflected in a decreased ability to synthesize chlorophyll, thus resulting in yellowing of the young leaves. A characteristic feature of IDC is that the veins of the young leaves remain green (Bienfait, 1986). Interveinal chlorosis is sometimes followed by chlorosis of the veins, so that the entire leaf becomes yellow. In severe cases, the young leaves become white with necrotic lesions. The iron nutritional problem that many soil-based organisms have is not one of abundance, but rather one of availability in aerobic environments at biological pH values (Guerinot and Yi, 1994). The solubility of iron in the soil can be expressed as:

$$Fe(OH)_3 + 3H^+ \leftrightarrow Fe^{3+} + 3\,H_2O$$

Hydrolysis complexes of Fe^{3+} raise total soluble iron to approximately $10^{-10.4}$ M in calcareous soils, but plants growing in soils require approximately 10^{-8} M soluble iron. Without some modifying mechanisms, most plants would show iron deficiencies when growing in media above pH 5.0 (Lindsay, 1995).

The iron deficiency stress environment is characterized by a number of soil factors. These factors include indigenous soil composition (e.g., carbonate and iron oxide contents and reactivities); environmental variables (e. g., soil

water relations, and temperature); soil solution composition (e. g., bicarbonate concentration); and nutritional status (e.g., phosphate availability) (Loeppert et al., 1994).

The plant kingdom was classified into two groups by Marschner et al. (1986), depending on the response of plants to iron deficiency stress. Strategy I-type plants, including dicots and nongraminaceous monocots, respond to iron deprivation with both morphological and physiological changes (Guerinot and Yi, 1994; Romheld, 1987). Morphological modifications include enhanced development of lateral roots and differentiation of specialized transfer cells. Both of these changes increase the surface area available for reduction and transport of iron. Physiological responses include a) increased acidification of the rhizosphere via enhanced proton extrusion, b) secretion of phenolics and organic acids to chelate iron, and c) reduction of Fe^{3+} to Fe^{2+} via an inducible plasma membrane bound Fe^{3+} reductase. Strategy II- type plants, including graminaceous monocots, are characterized by release of phytosiderophores and by the induction of a high-affinity uptake system for Fe^{+3} phytosiderophores (Guerinot and Yi, 1994).

Reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) iron is essential for iron absorption in strategy I plants. Young lateral roots are the principal sites of reduction (Brown and Ambler, 1974; Ambler et al., 1971). Reduced iron seems to move through the protoxylem of the young lateral root to the metaxylem of the

primary root, where it is oxidized to ferric iron, chelated by citrate, and transported to plant tops (Brown, 1978; Jolley and Brown, 1994). In strategy II plants, the phytosiderophore system could involve a reduction step, i.e., the phytosiderophore Fe^{3+} complex crosses the membrane and is reduced by NADPH. As in strategy I plants, the iron is oxidized to citrate iron and transported in the xylem from the root to the leaf (Terry and Abadia, 1986).

Most of the iron found in leaves is located in the chloroplasts (Seckback, 1982) and most of the iron in the leaves is in the ferric form (Goodman and Dekock, 1982). The oxidation state of iron in leaves has been reviewed by Terry and Abadia (1986). Some of the iron in the leaves must be present in the soluble ferrous form since several enzymatic processes require Fe^{2+}, e.g., heme synthesis (Rebeiz and Lascelles, 1982). The mechanism for reduction of Fe^{3+} to Fe^{2+} ions in plant cells is unknown. One might speculate that photosynthetic electron transport could provide a suitable reductant for this purpose but there is no evidence for this (Terry and Abadia, 1986).

One of the most striking aspects of iron deficiency is the reduction in the amount of thylakoid membranes, which is accompanied by decreases in all light harvesting pigments, chlorophyll a, chlorophyll b, and carotene (Terry and Abadia, 1986). Xanthophyll also decreases but to a lesser extent so that iron deficient leaves are characteristically richer in xanthophyll. Thus, iron

deficiency chlorosis is associated with a loss of all thylakoid constituents, not just chlorophyll (Terry and Abadia, 1986).

In addition to the existence of iron-dependent steps in the pathway of chlorophyll biosynthesis, iron is part of several components in the electron transfer chain of photosystem II (PS II) and photosystem I (PS I) (Abadia and Abadia, 1993). Nodulation and symbiotic nitrogen fixation also have been considered as potentially important physiological processes inhibited by iron deficiency (O'Hara et al., 1988). Competition for iron among soil microorganisms exerts a profound effect on the interactions among saprophytic microbes, pathogens, and plants (Barash et al., 1993).

Although mechanisms describing the function of iron in the biosynthesis or maintenance of chlorophyll and other biochemical reactions are not completely clear (Miller et al., 1995; Nielands, 1981), physiological studies of iron deficiency have provided basic information for iron deficiency correction by genetic improvement in crops.

Genetic studies of iron deficiency chlorosis in soybean

IDC is a common problem in soybeans grown on calcareous soil in the midwest United States. It results in decreased seed yield, reduced plant height, early maturity and plant mortality (Froehlich and Fehr, 1981). At present, most high-yielding cultivars of soybeans in the north central USA show some

degree of iron deficiency chlorosis (Mason et al., 1985). Phenotypic correlations between yield of lines grown on noncalcareous soil and their chlorosis resistant counterparts on calcareous soil were negative or not significant, indicating either that there was no physiological association between the traits or that the association was favorable for the selection of high-yielding lines with chlorosis resistance (Hinz et al., 1987). Therefore, genetic improvement is a feasible approach to overcome the problem of iron deficiency chlorosis in soybean.

The first genetic study of iron deficiency chlorosis in soybean was reported by Weiss (1943). He evaluated iron deficiency phenotypes in nutrient solution in pots and determined the threshold value for the visual score that could distinguish most of the efficient and inefficient cultivars. He concluded that the trait was affected by a single recessive gene with no maternal effect. Brown et al. (1967) stated that in field conditions, the iron efficient allele (*Fe*) was dominant to the iron inefficient allele (*fe*), but might be modified by other factors. Cianzio and Fehr (1980) observed that the segregation ratio of F2:4 lines fit a model involving a single major gene with codominant inheritance, and that the distribution of backcross lines (BC_1F_1) chlorosis ratings was skewed toward the recurrent parent. This suggested that modifying genes were present. Later, Cianzio and Fehr (1982) observed the segregation of iron deficiency chlorosis in another population, and found that no F_2-derived lines or BC_1F_2-derived lines were as resistant as the efficient parent. They concluded that the inheritance of

IDC in this population was typical of a quantitative character controlled by additive gene action, and that the inheritance of resistance to iron chlorosis in soybean could vary, depending on the parents used to evaluate the character. Since that time the hypotheses that two separate genetic mechanisms, i.e., a single major gene with modifying genes, and/or polygenes, control iron deficiency chlorosis in soybean were proposed. In plant breeding programs, the trait has been treated as a quantitative trait (Cianzio and Fehr, 1980).

Diers et al. (1992c) first used RFLP markers to study iron deficiency chlorosis in soybean. They identified three markers that were significantly associated with iron deficiency in an interspecific-cross population, however, no consistent result was found in a tester population which was developed from the same cross. They concluded that a) larger population sizes may be needed; and b) because the iron efficiency evaluation of the tester set was conducted only during one year, this may have increased the difficulty of estimating the iron efficiency variation present in the population and thus the detection of association between the markers and the trait.

Nutrient solution studies of iron deficiency chlorosis in soybean

Progress in breeding high-yielding cultivars with improved iron chlorosis resistance has been made (Jessen et al., 1986). Alternative methods to field evaluation on calcareous soils have been proposed, i.e., potted calcareous soils

(Byron and Lambert, 1983; Fairbanks et al., 1987), however, the decreased sensitivity of iron deficiency chlorosis detection in pots resulted in the lack of identification of some moderately inefficient genotypes. Chlorosis symptoms also were less severe in the growth chamber than in the field. Detopping seedlings was suggested as a means to increase the severity of chlorosis (Jessen et al., 1986; Piper et al., 1986). Tissue culture also was reported to be a useful technique to evaluate IDC in soybean (Stephens et al., 1990).

Nutrient solution has been a widely used technique to evaluate iron deficiency in soybean (Chaney et al., 1992). The method was developed by identifying soil factors which induced iron deficiency chlorosis (Brown and Jones, 1976). Coulombe et al. (1984) found that chlorosis of plants was closely related to the amount of HCO_3^- in the nutrient solution. Recently, modifications have been tested to reduce the costs and increase the sensitivity of screening soybean genotypes in nutrient solution (Chaney, 1988; Chaney et al., 1992; Dragonuk et al., 1989a). Advantages to this system are that; a) it can be done during the winter, b) each cycle of evaluation can be completed in only about one month, c) higher severity of chlorosis can be detected, and d) the problems associated with heterogeneity of calcareous soils and varying environment conditions can be avoided (Jessen et al., 1988).

The effectiveness of using nutrient solution for the selection of genotypes resistant to IDC was evaluated by Jessen et al. (1988). The authors obtained

high rank correlation between field and nutrient solution evaluations using eight soybean cultivars with a wide range of chlorosis ratings. Diers and Fehr (1989), evaluating the iron efficiency of genotypes in nutrient solution and field tests, concluded that replicated tests would be most appropriate in breeding for the trait. Dragonuk et al. (1989b) also tested the effectiveness of nutrient solution evaluation for iron deficiency chlorosis in a soybean recurrent selection program, and suggested that nutrient solution and field evaluations may partially select for different mechanisms of response to iron stress. Therefore, the genetic factors at the molecular level (QTL) or physiological mechanisms controlling IDC need to be identified, in order to rigorously compare the genetic mechanisms conferring iron efficiency in field and nutrient solution tests.

Molecular markers

The basic concept of 'genetic markers' can be traced back to the chromosome theory of heredity by Morgan in 1909 (c.f. Dunn, 1991). The concept is based on the use of the marker locus as a point (or region) of reference for the chromosomal segment in the marker's vicinity which permits that segment to be followed through genetic manipulations (Stuber, 1992). Genetic markers include morphological, cytogenetic, biochemical, and molecular markers. An important application of genetic markers is the identification of genetic factors (genes) that control traits of economic importance.

Morphological markers that provide easily recognizable polymorphisms, such as difference in flower color and leaf shape, etc., were the first type of

markers used in genetic studies (Landry et al., 1987). A major difficulty with this method for gene mapping is that the genetic marker itself may affect the expression of the character under study. Another difficulty is that genetic markers may not be available for certain chromosome arms (Burnham, 1966).

Cytogenetic markers, including deficiencies, chromosomal interchanges, chromosomal inversions, and trisomics, are also useful for locating genes. The main limitation of this approach is the difficulty in collecting and maintaining the marker stocks (Burnham, 1966).

Biochemical markers are based on biochemical reactions of gene products. Isozymes refer to any two distinguishable proteins that catalyze the same biochemical reaction, and are the most common protein markers (Hunter and Markert, 1957). Isozyme methods are fairly simple and inexpensive (Tanksley, 1983) which is an advantage in conducting genetic studies. However, there are two important limitations of protein markers. One is that detection of variation is limited to coding regions, and the other is that not all categories of mutational events can be detected (Vega, 1993).

Many types of DNA markers have been developed and applied in genetic studies. Botstein et al. (1980) were pioneers in the use of molecular genetic markers by introducing a technique to evaluate RFLPs (Restriction Fragment Length Polymorphisms). This technology uses restriction enzymes to cut DNA into small fragments of different lengths. When two or more individuals are compared using the same RFLP probe, there often is a difference in the length of the fragments produced by restriction enzyme digestion. The different sizes of fragments can be separated by gel electrophoresis (Southern, 1975). This size difference is called a polymorphism (Kochert, 1994). The DNA fragments are

usually inherited in a codominant fashion (Kochert, 1994). Variation in the size of allelic DNA fragment(s) results from loss or gain of a restriction site, due to point mutations, deletions, insertions, translocations, insertions, unequal crossing over, or replication slippage (Kochert, 1994; Lavi et al., 1993). RFLP analysis first was used in genetic mapping of temperature-sensitive mutants of adenovirus serotypes (Grodzicker et al., 1974). Later, a similar application was suggested for the detection of human diseases (Botstein et al., 1980; Goodbourn et al., 1983; Orkin et al., 1982), genes in plants (Beckmann and Soller, 1983), and in animals (Beckmann et al., 1986). There are many advantages of RFLP makers; such as they possess codominant expression; are detectable in all tissues and at all developmental stages; the markers are not restricted to coding sequences, and so on. An excellent review of advantages and disadvantages were discussed by Beckmann and Soller (1986).

Williams et al. (1990) proposed the use of single short random primers (usually 10-mers) in a polymerase chain reaction (PCR) as a method of generating polymorphic markers. These DNA polymorphisms result either from differences in the DNA sequence at primer binding sites or from chromosome changes affecting the length of the amplified fragment regions. After electrophoresis the amplified fragments can be visualized by ethidium bromide staining and the resulting pattern of bands can be interpreted genetically. Because RAPD (Random Amplified Polymorphic DNA) profiles are scored for the presence or absence of a single allele (DNA fragment), RAPDs are usually dominant markers. This procedure has the advantage of being technically simple, quick to perform, requires only small amounts of DNA and involves no radioactivity (Waugh and Powell, 1992).

SCARs (Sequence Characterized Amplified Regions) were introduced by Paran et al. (1993). SCAR markers are generated by cloning and sequencing RAPD fragments or other DNA fragments of interest, and designing specific primers that are complementary to the ends of original RAPD or other DNA fragments. When these markers are used in a PCR with the original template DNA, single loci are specifically amplified (Paran et al., 1993; Weising et al., 1995). SCARs may be dominant polymorphisms (one fragment amplified) or may show codominant polymorphisms (size polymorphism of amplified fragments). Therefore, DNA polymorphisms result either from differences in the DNA sequence at the primer binding sites or by chromosome changes affecting the amplified regions.

SSR (Simple Sequence Repeat) DNAs, or microsatellite DNAs, are short segments of DNA consisting of a small number of repeated nucleotide sequences such as $(CA)_n$, $(AAT)_n$, and $(AGAT)_n$ (Tautz and Renz, 1984). There are indications that variation in the number of repeats (n) results from unequal crossing over between sister chromatids or slippage of the DNA polymerase during the replication process. SSR is a PCR-based technology. The initial requirement of the procedure is to identify the repeated sequences. Two approaches have been used for identifying SSR loci: a) identification of microsatellites in GeneBank database, and generation of primers to these loci; and b) isolation of positive clones from a genomic library after hybridization to microsatellite probes (Lavi et al., 1993). After a sequence is identified the next step is to sequence the DNA on either side of the repeated region and develop PCR primers for the regions flanking the SSR. Polymorphism in this system results from variable numbers of tandem repeats of a core DNA sequence.

Corresponding DNA regions from different individuals can be amplified either as only one fragment (dominant) or as two fragments with different lengths (codominant). SSR combines the advantages of the other classes of markers, namely being highly polymorphic-single locus markers, which are very abundant (Wang et al., 1994), reproducible, and easily amenable to genetic analysis (Lavi et al., 1993; Morgante and Olivieri, 1993). A novel technique called ISSR (Inter-Simple Sequence Repeats) involves anchoring of designed primers to a subset of SSRs and amplification of the region between two closely spaced, oppositely oriented SSRs (Kantety et al., 1995; Zietkiewicz et al., 1994). The advantage of this technique is its multiplexed banding profiles, high frequency of polymorphism, and relatively low cost (Kantety et al., 1995).

The technique of AFLPs (Amplified Fragment Length Polymorphisms) is similar to that used in RFLP analysis (Lin and Kuo, 1995; Zabeau and Vos, 1992). AFLPs detect DNA polymorphism through length differences in restriction fragments. Therefore, the genetic basis of this variation results from a change of a restriction site (same as RFLP). AFLP uses a PCR-based assay in which restriction fragments are amplified and polymorphisms are detected by the difference in the length of the amplified restriction fragments. This method uses universal primers for the PCR reaction. Adaptors are used to tag the restriction fragments and to serve as the template for the PCR primers. Two adaptors are ligated to DNA fragments thus allowing synchronous amplification of the DNA fragments (Weising et al., 1995; Zabeau and Voss, 1992). Because each locus is represented by the presence or absence of one band (allele), an AFLP is expressed as a dominant marker. Keygene (Wageningen, Netherlands)

is currently working to identify heterozygous individuals by quantifying the amount of DNA contributed by each parent (Walton, 1993).

The technical difficulty, reliability, level of polymorphism, marker inheritance, amount of DNA required, development and start-up costs of the above molecular markers have been compared (Andersen and Fairbanks, 1990; Beckmann and Soller, 1986; Rafalski and Tingey, 1993; Walton, 1993; Waugh and Powell, 1992). The different types of markers can complement each other. Therefore, the combined uses of various types of markers in genetic studies are sometimes warranted.

Gene mapping

Gene mapping is the determination of the location of a gene on a chromosome. A genetic map (or linkage map) is used to describe the linkage relationships, namely the order and distance, among linked loci (Klug and Cummings, 1994).

The basic theory of linkage according to the chiasmatype theory was published by Janssens in 1911 (c.f. Dunn, 1991). The chiasmatype theory assumes that when pairs of homologous chromosomes twist about each other in synapsis they exchange parts, or 'cross over' at the chiasma. When they split apart in a single plane, the original order of regions physically close together in each homologue will likely be retained, while more distant markers have a greater chance of being separated by a chiasma.

Since 1911, researchers have been monitoring, inducing, and mapping single gene markers in higher plants. Until the last 20 to 25 years, most of the single gene markers used in higher plant genetics were those affecting morphological characters. Common examples were genes causing dwarfism, chlorophyll deficiency or altered leaf morphology (Tanksley, 1983). However, most agronomic traits of economic importance, such as grain or forage yield and protein or oil composition, are considered to be quantitative. Although numerous other investigations have been conducted on the inheritance of quantitative traits, primarily using classical biometrical procedures, plant geneticists and breeders typically have little information on: a) the number of genetic factors (loci) involved in the expression of the traits; b) the chromosomal location of these loci; and c) the relative size of the contribution of individual loci to trait expression (Stuber, 1992). The use of isozyme and DNA markers have made such investigations possible (Dudley, 1993; Paterson et al., 1991; Smith and Smith, 1992; Stuber, 1992; Tanksley, 1983). Paterson et al. (1988) reported the first use of a complete RFLP linkage map to resolve quantitative traits into discrete Mendelian factors in an interspecific backcross population of tomato. Since then, extensive sets of mapped isozyme and DNA marker loci have been developed and documented in a number of plant taxa (O'Brien, 1993).

There are two basic methods for gene mapping. Linkage mapping is used to determine the order and distance among linked genetic markers and/or

major gene(s) controlling the trait segregating with discrete variation, while QTL mapping is used to locate the gene(s) affecting the traits segregating with continuous variation.

Linkage mapping (Major gene mapping) - Linkage mapping has been the most common method of major gene mapping. This method uses recombination frequency to estimate the relative genetic distance between two linked genes. The measurement of linkage is usually quite simple and fast and no complex statistical methods are necessary (Fisher and Balmukand, 1928). This principle remains the basis for genetic mapping studies today. However, many linkage mapping methods are restricted to a test cross population. In an F_2 population, which is common in plant breeding programs, the classification data can not provide the linkage information in a straightforward manner. Therefore, during the early 1900s, many different methods were developed and used to estimate linkage. Mather (1951) concluded that the method of maximum likelihood was the only method that can lead to efficient estimates for all types of problems. Morton et al. (1986) calculated likelihoods in the form of LOD scores, defined as the logarithm of relative likelihood of linkage versus nonlinkage. Since the computation of maximum likelihood method for multiple loci is complex and time consuming, computer programs for major gene mapping have been developed. LIPED was the first program to compute the likelihood of genetic linkage of human pedigrees (Ott, 1973). The LINKAGE-1 program was

designed to analyze genetic linkage for a segregating population (Suiter et al., 1983). However, both the LIPED and LINKAGE-1 programs are designed only for two-point analyses (Ott, 1973; Suiter et al., 1983) which give rough linkage estimates when a small population is studied (Lander et al., 1987). To overcome this problem, the MAPMAKER program using multipoint linkage analysis was developed (Lander et al., 1987). Recently, G-MENDEL was developed for handling nonstandard segregation and for estimating pooled and heterogeneity statistics when data are available from two or more populations segregating at common loci (Liu and Knapp, 1990). Furthermore, the *JoinMap* program was published for combining linkage data that have been collected in different experiments or populations (Stam, 1993).

QTL mapping - Thompson and Thoday (1979) point out that the history of quantitative genetics is separated from traditional Mendelian genetics by the methodology used to classify phenotypes of continuous traits into discrete groups and to make counts of the number of individuals in each group. The first report regarding the association of a simple inherited genetic marker with a quantitative trait in plants was done by Sax (1923) when he observed that segregation for seed size was associated with a seed coat color in beans. Breese and Mather (1957) further advanced these concepts by splitting a chromosome into a number of component pieces and showing each of them to contain some of the relevant factors (genes). Later, the principle was well exploited with

detailed procedures and discussions (Thoday, 1961). Accordingly, the underlying assumption of using marker loci to detect QTL (or polygenes) is that linkage disequilibrium exists between alleles at the marker and alleles of the linked QTL (Tanksley, 1993).

The strategies for detecting QTL have been extensively reviewed by researchers (Dudley, 1993; Knapp, 1994; Tanksley, 1993). There are two main approaches for detecting QTL.

(A) Identification of marker(s) linked with QTL

Single point analysis is the simplest method to detect QTL by analyzing the data with one marker at a time (Tanksley, 1993). The method does not require a complete molecular linkage map. The disadvantage of this method is the disturbance generated by crossovers between marker and QTL. Thus the further a QTL is from the marker, the less likely it is to be detected statistically and the magnitude of the QTL effect will be underestimated (Tanksley, 1993).

Two-point analysis (or interval analysis) simultaneously uses a pair of linked markers to detect QTL (Lander and Bostein, 1986a; Lander and Bostein, 1986b). The advantage of two-point analysis versus one-point analysis is that higher efficiency can be obtained when the linked markers are fairly far apart (Tanksley, 1993). The disadvantage is that the recombinant individuals with one crossover between the two flanking markers are not included in the analysis and, therefore, the population size is reduced.

Extreme phenotype analysis is also called selective genotyping or trait-based analysis. In order to use informative individuals (e.g., those not affected by incomplete penetrance or lethal genes), only individuals in the extreme tails of the distribution are analyzed with markers (Lander and Botstein, 1989; Lebowitz et al., 1987). The strategy of extreme phenotype analysis substantially increases efficiency whenever growing and phenotyping additional progeny requires less effort than completely genotyping individuals at all markers.

Bulked segregant analysis involves the comparison of two pooled DNA samples of individuals from a segregating population originating from a single cross. Within each pool, or bulk, are the individuals which are identical for the trait or gene of interest but which are randomly distributed for all other genes. Two pools contrasting for a trait are analyzed to identify markers that distinguish them. Therefore, bulked segregant analysis provides a fast and economic method to focus on regions of interest or areas sparsely populated with markers (Michelmore et al., 1991).

(B) Localization of QTL

Interval mapping with the maximum likelihood method has been widely used in determining the location(s) of QTL (Lander and Botstein, 1989). The computer program MapMaker-QTL is a convenient tool for data analysis (Lincoln et al., 1992). The LOD score in maximum likelihood method, is defined as the logarithm of the relative likelihood of the null hypothesis that a QTL is at

a specific location versus the alternative hypothesis that a QTL is not at that location within an interval. When the peak LOD score within an interval exceeds the threshold LOD value, we can accept the hypothesis that at least one QTL is located at this point (Lincoln et al., 1992). Appropriate thresholds for LOD scores are based on a number of parameters, including the particular genome, type of cross, and density of linkage map used (Dudley, 1993; Lander and Bostein, 1989). Although interval mapping with the maximum likelihood method has been criticized for failing to detect two linked QTL and to estimate QTL parameters from multiple environments evaluated during replicated experiment designs (Knapp, 1994), this method is still the most common approach for QTL mapping.

Mapping genes of soybean with molecular markers

Soybean genetic map development proceeded slowly relative to genetic maps of crops of similar economic importance. This has been due largely to inherent difficulties in performing sucessful sexual crosses, a lack of cytogenetic markers, and unavailability of proper genetic stocks (Shoemaker et al., 1996). In 1962, only nine morphological markers were assigned to four linkage groups (Johnson and Bernard, 1962). Even recently, just 63 morphological, pigmentation, or isozyme markers have been mapped to 21 classical linkage groups (Palmer and Hedges, 1993). Since the development of the first RFLP linkage maps in soybean (Apuya et al., 1988), many genetic factors that affect

traits of economic importance have been identified and mapped with molecular markers.

The major genes mapped by molecular markers in soybeans include phytophthora resistance (Diers et al., 1992a; Polzin et al., 1994), cyst nematode resistance (Weisemann et al., 1992), mosaic virus resistance (Yu et al., 1994), nodulation (Landau-Ellis et al., 1991; Polzin et al., 1994), mildew resistance (Polzin et al., 1994), root fluorescence (Devine et al., 1993), palmitate content (Nickell et al., 1994), and linolenic acid content (Brummer et al., 1995). Shoemaker and Specht (1995) also integrated five pigmentation, four morphological, and two isozyme loci of the classical map into molecular map using the *JoinMap* program.

QTL mapped by molecular markers in soybeans include hard seededness (Keim et al., 1990a), seed protein and oil contents (Diers et al., 1992b; Lark et al., 1994; Mansur et al., 1993a), fatty acid content (Diers and Shoemaker, 1992), reproductive traits (Keim et al., 1990b; Lark et al., 1995; Mansur et al., 1993a; Mansur et al., 1993b), morphological traits (Keim et al., 1990b; Lark et al., 1994; Lark et al., 1995; Mansur et al., 1993a; Mansur et al., 1993b), cyst nematode resistance (Concibido et al., 1994; Webb et al., 1995) and iron deficiency chlorosis (Diers et al., 1992c). This list is not an inclusive. Shoemaker (1994) and Shoemaker et al. (1996) have provided reviews of this topic.

Soybean gene mapping has provided an opportunity to understand the genetic mechanisms controlling qualitative or quantitative traits and to provide the information necessary to manipulate genes precisely and efficiently. In order to better understand the genetic mechanisms affecting quantitative traits, several suggestions have been made: i.e., use of intraspecific crosses in the

genetic analysis of agronomic traits such as yield, plant height, and lodging, which are difficult to evaluate in interspecific crosses (Lark et al., 1993); multiple populations for detecting QTL allelic polymorphisms distributed in different populations, larger population sizes; and evaluation of phenotypes with replications under multiple environments for detecting QTL with minor gene effect and interaction between QTL and environment (Diers et al., 1992c; Dudley, 1993; Shoemaker et al., 1996).

At present, although physiological studies have contributed many methods to correct iron deficiency chlorosis (IDC), genetic improvement is thought to be a less expensive and feasible strategy to overcome the problem in soybean (Chaney, 1985). Thus the improvement of IDC is a major goal of soybean breeding programs in the north central United States.

The main plant breeding activities include manipulation of genetic variability and evaluation for the trait (Kleese and Duvick, 1980). However, due to confounding effects among the number of genes, the magnitude of the gene effect, and environmental effects, conventional quantitative genetic analysis can not provide direct and extensive evidence to prove the hypothesis that two genetic mechanisms control the genetic variability of soybean IDC (Cianzio and Fehr, 1980; Cianzio and Fehr, 1982), and to determine the effectiveness of nutrient solution test for soybean IDC.

Molecular markers have provided a powerful approach for the study of quantitative traits and for locating and manipulating individual genetic factors associated with the traits (Stuber, 1990). The potential use of molecular markers will allow us to understand better the genetic mechanisms controlling

soybean IDC and to determine the effectiveness of nutrient solution evaluation for soybean IDC.

I. MOLECULAR MARKER DETECTION OF TWO GENETIC MECHANISMS RESPONSIBLE FOR IRON DEFICIENCY CHLOROSIS IN SOYBEAN

A paper to be submitted to Molecular Breeding

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Abstract

The objectives of this study were to map genes for iron deficiency chlorosis in two intraspecific [*Glycine max* (L.) Merrill] soybean populations, and to test the hypothesis that a major gene with modifiers and/or a polygene mechanism controls iron deficiency chlorosis in soybean. Chlorosis symptoms of one hundred and twenty $F_{2:4}$ lines in Pride B216 x A15, and of 92 $F_{2:4}$ lines in Anoka x A7 populations were evaluated by visual scores and spectrometric chlorophyll content determinations of leaves at the V4 stage (third trifoliolate leaf fully developed) in a field of calcareous soil in 1993, and at V2 (first trifoliolate leaf fully developed) and V4 stages in 1994. Eighty-nine RFLP and ten SSR markers in the Pride B216 x A15 population, and 82 RFLP, 14 SSR, and *I* (hilum color) markers in the Anoka x A7 population were used to construct linkage maps and to locate quantitative trait loci (QTL) affecting iron deficiency chlorosis. QTL controlling visual scores and/or chlorophyll concentrations were detected on linkage groups B2, G, H, I, L, and N of the Pride B216 x A15 population. In this population no QTL were detected with large effects suggesting a typical polygene mechanism. In contrast, one QTL contributed an average of 72.7% of

the visual score variation and 68.8% of the chlorophyll concentration variation and was mapped on linkage group N in the Anoka x A7 population. One other QTL for visual score variation, and one for chlorophyll concentration variation were detected on linkage groups A1 and I, respectively. Due to the large LOD score and gene effect, and stable expression of the QTL on linkage group N, we reclassified the quantitative data into qualitative data fitting a one major gene model according to the means of the QTL genotypic classes in the Anoka x A7 population. The putative major gene was mapped in the same interval of linkage group N using both visual scores and chlorophyll concentrations, thus verifying that one major gene is involved in segregation for iron chlorosis deficiency in the Anoka x A7 population. This study has demonstrated the validity of a previous hypothesis that two separate genetic mechanisms control iron deficiency in soybean.

Introduction

Iron deficiency chlorosis (IDC) occurs in interveinal tissue of young leaves when a plant is unable to utilize available iron from the soil. IDC in soybean is a common problem on calcareous soil in the midwestern United States (Froehlich and Fehr, 1981), causing yield reduction whenever yellowing is observed, even if the symptoms are slight (Niebur and Fehr, 1981). At present, no soybean cultivar can completely resist iron deficiency chlorosis (Mason et al., 1985).

Mather (1943) developed the concept of gene expression systems by defining polygene action as a 'buffering' mechanism and major gene action as a

'switch' mechanism. The genetic control of soybean iron deficiency first was reported by Weiss (1943). He concluded the trait was affected by a single recessive gene with no maternal effect. He also found variation in iron efficiency among efficient varieties and among inefficient varieties. Significant negative correlation between chlorophyll concentrations and visual scores for iron deficiency chlorosis (IDC) in soybeans was reported by Cianzio et al. (1979). Cianzio and Fehr (1980) assessed IDC in a field of calcareous soils and reported that chlorosis was controlled by a major gene with modifying genes. Later, however, Cianzio and Fehr (1982) used a different population and concluded that iron deficiency chlorosis was controlled quantitatively with additive gene action. They inferred that the inheritance of resistance to iron deficiency chlorosis in soybean can vary, depending on the parents used in developing the populations and the test conditions used to evaluate the character. Since then, IDC has been proposed to be controlled by either one of two genetic mechanisms; a single major gene with modifying genes, or a polygene mechanism, i.e., many genes, each with small effect.

Molecular markers such as randomly amplified polymorphic DNA (RAPD), restriction fragment length polymorphisms (RFLP), and simple sequence repeats (SSR) have proven to be useful tools to study inheritance of genes of economic importance. Soybean gene mapping has been reviewed by Shoemaker (1994) and Shoemaker et al. (1996). Traits that have been mapped include reproductive and morphological traits (Keim et al., 1990b; Lark et al., 1994; Lark et al., 1995; Mansur et al., 1993a; Mansur et al., 1993b), nodulation (Landau-Ellis et al., 1991; Polzin et al., 1994), root fluorescence (Devine et al., 1993), hard seededness (Keim et al., 1990a), seed protein and oil content (Diers

et al., 1992b; Lark et al., 1994; Mansur et al., 1993a), fatty acid content (Diers and Shoemaker, 1992), palmitate content (Nickell et al., 1994), linolenic acid (Brummer et al., 1995), phytophthora resistance (Diers et al., 1992a; Polzin et al., 1994), cyst nematode resistance (Concibido et al., 1994; Webb et al., 1995; Weisemann et al., 1992) mildew resistance (Polzin et al., 1994) and mosaic virus resistance (Yu et al., 1994). Shoemaker and Specht (1995) also integrated five pigmentation, four morphological, and two isozyme loci of the classical map into molecular map using the *JoinMap* program (Stam, 1993). In addition, Diers et al. (1992c) used RFLP markers to evaluate segregation for iron deficiency chlorosis in an interspecific cross population (*Glycine max* x *Glycine soja*). The authors detected three markers that were significantly associated with iron deficiency chlorosis, but the result obtained with the study population was not in accordance with that of a tester population. The lines for the study and tester populations were obtained from the same cross.

The objectives of our study were to map gene(s) for iron deficiency chlorosis in two *Glycine max* x *Glycine max* populations and to test the proposal that iron deficiency chlorosis in soybean is controlled by two genetic mechanisms; a major gene with modifying genes, or by classical polygenic inheritance.

Materials and Methods

Plant materials

One hundred and twenty $F_{2:4}$ lines from Pride B216 x A15, and 92 $F_{2:4}$ lines from Anoka x A7 were used in this study. The efficient parents, A7 and

A15, were selected from recurrent selection programs (Fehr et al., 1984; Jessen et al., 1988). Cianzio and Fehr (1980) previously used the inefficient parent Anoka as genetic material to evaluate IDC and concluded that a single major gene and modifying genes controlled iron deficiency. Cianzio and Fehr (1982) also used the inefficient variety Pride B216 as genetic material in a similar study and inferred that polygenes (or multiple genes) controlled the chlorosis response.

Phenotypic evaluations

The four parents and the F_2-derived lines were planted at Humboldt, Iowa, on calcareous soil on June 11, 1993 and May 19, 1994. Each population was evaluated using a randomized complete block design (RCBD) with three replications per year. Twenty-five seeds from each line were planted in a single row plot and no thinning was done. The plot was 76 cm long and 68 cm in distance from adjacent plots. Visual scores and chlorophyll concentrations were evaluated at the V4 stage (Fehr and Caviness, 1977) in 1993 and 1994. Because the chlorosis symptoms were obvious at the V2 stage during 1994, visual scores and chlorophyll concentrations also were evaluated at this stage. The visual scores were assigned on the basis of yellowing of the fully expanded trifoliolate leaves; 1 = no yellowing, 2 = slight yellowing, 3 = moderate yellowing, 4 = intense yellowing, and 5 = severe yellowing with some necrosis (Cianzio et al., 1979). After visual scores were rated, trifoliolate leaves showing chlorosis symptoms were sampled at random from eight plants (one trifoliolate leaf per plant) of each plot to determine chlorophyll concentration. A leaf disc was obtained with a borer (8.8 mm in diam) from the middle leaflet of each

trifoliolate leaf. Chlorophyll from leaf samples was extracted in 80% acetone solution and absorbance was measured at two wavelengths (663 nm and 645 nm) with a spectrophotometer. The chlorophyll concentration ($\mu g/cm^2$) was estimated according to Arnon (1949) as:

Chlorophyll a = 12.7 x A663 - 2.7 x A645

Chlorophyll b = 22.9 x A645 - 4.7 x A663

Chlorophyll a+b = 20.2 x A645 + 8.0 x A663

Phenotypic chlorosis data evaluated at V4 stage in 1993 and 1994 were used in a combined analysis of variance. The effects of replications, lines, and years were considered random factors. Broad sense heritability (Hb^2) was estimated on the basis of the expected mean squares from the combined analysis of variance (Fehr, 1987) as:

$$Hb^2 = \frac{\sigma^2 g}{(\sigma^2 e / rxe + \sigma^2 ge/e + \sigma^2 g)}$$

where $\sigma^2 g$ = genetic variance

$\sigma^2 ge$ = genetic x environment interaction variance

$\sigma^2 e$ = experimental error variance

r = number of replications

e = number of environments

Construction of linkage maps

Leaf samples from 10-15 plants of each $F_{2:4}$ line were harvested at V4 stage and combined for DNA isolation and RFLP and SSR genotyping. Procedures for DNA extraction, restriction enzyme digestion, Southern transfer (Southern, 1975) and autoradiography were as described in Keim et al. (1990b).

To screen for informative RFLP markers, DNA samples from the four parents (Anoka, A7, Pride B216, and A15) were digested with restriction enzymes Dra I, Eco RI, Eco RV, Hind III, and Taq I, separately. The digested samples were used for Southern blottings. Three hundred and eight soybean, 15 mungbean, and 48 common bean marker loci (kindly provided by E. Vallejos at the University of Florida, and N. Young at the University of Minnesota) were screened to identify polymorphisms between each pair of parents. Eighty-two RFLP, 14 SSR, and *I* (hilum color) markers were used to construct a genetic linkage map in the Anoka x A7 population. In the Pride B216 x A15 population, 92 RFLP and ten SSR markers were used to construct a linkage map. Primers and procedures to obtain SSR markers were described by Akkaya et al. (1995). The selected SSR markers were used to fill the gaps of some genetic regions that lacked RFLP markers. The computer program MAPMAKER 3.0b (Lander et al., 1987) was used to develop linkage maps for gene mapping. Markers were assigned to linkage groups using a LOD threshold of 3.0 and a maximum recombination frequency of 0.50. The 'Order' command was used to add new markers to the linkage group. Map distances were estimated using the Haldane mapping function (1919).

QTL mapping

MAPMAKER/QTL1.1 (Lincoln et al., 1992) was used to determine the location of the QTL by using the 'Scan' command and to determine the contribution (R^2) of each detected QTL. A LOD value of 2.0 was used as the threshold for detecting QTL locations. The position of the LOD peak in each significant interval suggested the location of a QTL. One unit of LOD score was

used to establish the confidence interval for detected QTL (Lincoln et al., 1992). The contribution of all QTL also was estimated from multiple loci analysis. One way analysis of variance (ANOVA) using the SAS PROC GLM computer command (SAS Institute, 1990) was applied to detect QTL on linkage groups with only one informative marker and confirm the presence of each QTL.

Transformation from quantitative data into qualitative data

When a single major QTL was detected with high reliability (LOD) and phenotypic contribution (R^2) values, the QTL was hypothesized as a major gene. Because in theory, the means of each QTL genotypic class obtained from QTL mapping should have the same variation for the investigated trait, the averages of two flanking means of QTL genotypic classes could be used as critical points to transform quantitative data into qualitative data. Some progenies having phenotypic values close to the critical points were checked by 'Error detection' and 'Genotype' commands in the MAPMAKER computer program to detect possible misclassification errors. If two single crossovers were found between the possible major gene and two flanking markers, or one double crossover was found between the possible major gene and one of the flanking markers, the phenotypes of these progenies were corrected into the other classes. After the quantitative data were transformed into qualitative data, the segregation data were mapped with 'Group' and ' Map' commands as a general major gene by MAPMAKER.

Results

Genetic variation of iron deficiency

Results of the combined analysis of variances across two years (Table 1) indicated that there were highly significant differences (P = 0.0001) among F2-derived lines for visual scores and chlorophyll concentrations in both populations, Anoka x A7 and Pride B216 x A15. This suggested that it was possible to map gene(s) controlling IDC visual scores and/or chlorophyll concentrations in both populations. Significant differences between years and interactions of the Line x Year for visual scores and chlorophyll concentrations were observed in both the Anoka x A7 and Pride B216 x A15 populations. This indicated that the different $F_{2:4}$ lines of a population responded significantly different response in magnitude of phenotypic expression to the change in field environments during 1993 and 1994.

In both populations, the chlorophyll concentrations were more variable in its response to the environments of different years than visual scores (Figure 1). Chlorophyll concentrations in both populations during 1993 had range values that were lower than in 1994. The range of visual scores was similar over years and populations. According to the normality test for frequency distributions, all distributions for visual scores and chlorophyll concentrations were skewed to the efficient parents in both populations with the exception that the chlorophyll concentrations in the Anoka x A7 population exhibited a normal distribution during 1993. During 1994, frequency distributions for visual scores and chlorophyll concentrations exhibited normal distributions in both populations. Two lines had significantly higher visual scores than the inefficient parent during 1993 in the Pride B216 x A15 population. One line in the Pride B216 x

A15 population, and one line in the Anoka x A7 population had higher chlorophyll concentration than the efficient parents during 1993.

The estimated broad sense heritability for visual scores on an entry mean basis were 82.4% for the Anoka x A7 population and 64.5% for the Pride B216 x A15 population. The heritabilities for chlorophyll concentrations were lower than for visual scores in both populations. For chlorophyll concentrations, the heritabilities were 73.7% in the Anoka x A7 population and 59.9% in the Pride B216 x A15 population.

Construction of linkage maps

In the Pride B216 x A15 population, 89 RFLP and 10 SSR markers were distributed on 21 linkage groups. The short linkage groups D2 (49.8 cM), S (19.1 cM), and W (12.8 cM) of the soybean genetic map contained no markers (Figure 2). Four RFLP (A53, B148, K287, and Bng14) and one morphological (flower color) markers were not linked with any linkage group. The informative markers spanned approximately 1700 cM and the average length of each interval between two adjacent markers was approximately 20.8 cM. Ninety-seven genetic markers consisting of 82 RFLP, 14 SSR, and *I* (hilum color) markers were placed on 24 linkage groups in the Anoka x A7 population (Figure 3). Only one short linkage group W (12.8 cM) of the USDA-ARS/ISU soybean genetic map (Shoemaker and Olson, 1993) was not covered with markers. Three RFLP (A65, A586, and Mng186) markers were not linked with any constructed linkage group. The mapped markers spanned approximately 1650 cM. On average, the intervals between two adjacent markers was approximately 18.9 cM. The order of markers on the maps were similar to the USDA-ARS/ISU

soybean genetic map except that RFLP marker A702 was placed on a different end of linkage group B1 in both the Anoka x A7 and Pride B216 x A15 populations. Due to sampling size and possible scoring error, a few closely linked markers were reversed in order.

Test of polygene mechanism in the Pride B216 x A15 population

V2 growth stage

Two quantitative trait loci (QTL) explaining 28.5 % of the visual score variation at the V2 stage were mapped on linkage groups G, and N. The QTL on linkage group G contributed 15.4% of the visual score variation; and the QTL on linkage group N contributed 23.4 % of the visual score variation (Table 2). Four QTL contributed approximately 42.9 % of the chlorophyll concentration variation at the V2 stage. These QTL, contributing about 7.7 - 25.0 % of the variation in chlorophyll concentration, were mapped on linkage groups B2, G, H, and N (Table 2).

V4 growth stage

When the two year combined data (V4 stage) were used for QTL mapping, four QTL for visual scores were mapped on linkage groups B2 (two), G, and N (Table 2). Each QTL contributed about 8-11% (0.16-0.39 visual score value) of the phenotypic variation for visual scores. Multiple locus analysis indicated that all four QTL contributed about 21.6 % of the phenotypic variation. The QTL on linkage group N was detected in both 1993 and 1994. QTL on linkage groups H, G, and L were detected in 1994 and two QTL on linkage group B2 were detected only from the combined data of years 1993 and 1994.

Three QTL for chlorophyll concentrations at the V4 growth stage were mapped on linkage groups B2, I, and H for the combined data. Each QTL explained 11-22 % (0.98-1.30 $\mu g/cm^2$) of the phenotypic variation of chlorophyll concentrations. All three QTL contributed 34.9 % of phenotypic variation. QTL on linkage group H was detected in both 1993 and 1994. Two QTL on linkage group B2 were detected in 1994 only. QTL on linkage group I was detected only from combined data of 1993 and 1994.

Comparing the mapped QTL for visual scores at V2 and V4 growth stages, we found that QTL on linkage groups G and N were detected at both stages but that the two QTL on linkage group B2 were detected at the V4 stage only using the combined data. Two QTL on linkage groups B2 and H involved in the variation of chlorophyll concentrations were consistent at V2 and V4 stages, but QTL on linkage groups G, N, and I were detected either at V2 or V4 stages suggesting that these QTL were stage specific. Based on QTL mapping, results were not entirely consistent among different environments (growth stages and years) for either visual scores or chlorophyll concentrations. Considering individually detected QTL, all QTL were detected in more than one environment except for the QTL on linkage group L which was detected only at the V4 stage during 1994 (Table 2 and Figure 4). These partially stable QTL, which may not be detected in all environments, were mapped on linkage groups B2, G, H, I, and N.

The QTL detected by the MAPMAKER/QTL program were confirmed by one way analysis of variance using the SAS program. The associations between loosely linked markers and iron deficiency chlorosis also were tested. A RFLP marker, K69, at the end of linkage group G was significantly associated with

visual scores and chlorophyll concentrations at the V2 stage during 1994. Since no QTL for visual scores or chlorophyll concentrations with large phenotypic contribution (R^2 value) and high reliability (LOD value) were found at the V2 or V4 growth stages during 1993 and 1994 in the Pride B216 x A15 population, we confirmed the hypothesis previously proposed by Cianzio and Fehr (1982) that polygenes control iron deficiency chlorosis in this soybean population.

Test of the single major gene with modifying gene mechanism in the Anoka x A7 population

V2 growth stage

Two QTL for visual scores were mapped on linkage groups I and N (Table 3). A QTL on linkage group I contributed 39.2 % of the visual score variation. The iron efficiency allele for this QTL was contributed from the inefficient parent Anoka. High reliability (LOD = 18.2) for the QTL on linkage group N was found with codominance gene action. This QTL contributed 78.7 % of the visual score variation. The total contribution of these two QTL for visual score was 81.2 %. Only one QTL for chlorophyll concentrations was mapped on linkage group N. This QTL explained 52.1% of the variation for chlorophyll concentration (LOD = 10.7) at the V2 growth stage.

V4 growth stage

By using two-year combined data, we mapped two QTL controlling 75.3% of the visual score variation on linkage groups A1 and N. One QTL on linkage group A1 contributed 35.2% of visual score variation. The other QTL on linkage group N had high reliability (LOD = 13.1) and contributed 72.7% (1.24 score unit) of the visual score variation with codominance gene action (Table 3). Two

QTL responsible for chlorophyll concentrations were mapped on linkage group I and N explaining 80.7% of phenotypic variation. A QTL on linkage group N with LOD = 7.3 and R^2 = 68.8% was mapped in the same interval (BLT15-Sat33) as QTL for visual scores as was mapped at the V2 stage (Figure 5). The distance between QTL for visual scores and chlorophyll concentrations was 6 cM. The QTL for chlorophyll concentrations on linkage group N exhibited codominant gene action. QTL on linkage group I contributed a large percentage of the phenotypic variation (R^2 = 80 %) but had relatively low reliability (LOD = 3.5). However, contribution of the efficient alleles from the inefficient parent, Anoka, also was observed at the V4 stage. QTL on linkage groups A1 and I for visual scores or chlorophyll concentration were detected in more than one environment (Table 3 and Figure 5). The QTL on linkage group N was detected in all environments and thus was considered stable. For the QTL on linkage group N, there was much more difference in effect between years than between stages in phenotypic contribution of visual scores and chlorophyll concentration. Due to the large phenotypic contribution (R^2 values), high reliability (LOD scores), and consistent expression in different stages and years, we postulated that the QTL in interval BLT15-Sat33 of linkage N was a major gene.

In order to test if QTL affecting visual scores and/or chlorophyll concentrations on linkage group N was a major gene, the two-year combined data at the V4 growth stage were transformed into qualitative data according to average means of the adjacent QTL genotypic classes. No genotypes for visual scores and two genotypes for chlorophyll concentrations were corrected using ' Genotype' and 'Error detection' commands of the MAPMAKER 3.0b. The numbers in each of the transformed qualitative classes for visual scores and

chlorophyll concentrations are given in Table 4. Results from a χ^2 test revealed that the segregation of F_2-derived lines fit a 1 : 2 : 1 ratio for both visual scores ($p = 0.958$) and chlorophyll concentrations ($p = 0.575$). In addition, the transformed data were analyzed as a major gene by the MAPMAKER program. One putative major gene for visual score and one for chlorophyll concentration were mapped in the same interval (BLT15-Sat33) on linkage group N and located within the QTL confidence interval (Figure 6), and thus confirmed that one major gene with codominant inheritance for iron deficiency chlorosis was involved in the Anoka x A7 population. Consequently, we verified the proposal of Cianzio and Fehr (1980) that a single major gene with modifiers control iron deficiency chlorosis in the Anoka x A7 population.

Comparison between detected QTL in both populations

QTL on linkage groups B2, G, H, and L were detected in the Pride B216 x A15 population and were not detected in the Anoka x A7 population (Figures 4 and 5). Because the LOD value was slightly below the threshold, the QTL on linkage group H was detected in the Pride B216 x A15 population but not detected in the Anoka x A7 population (Figure 7). Two QTL detected on linkage groups I and N in the Anoka X A7 population also were detected in the Pride B216 x A15 population. However, the QTL contributed different percentages of phenotypic variations in the two populations. Comparison of detected QTL in both populations suggested that the different phenotypic variations or inheritance mechanisms between populations may depend on the combination of allelic polymorphisms of QTL and the magnitude of gene effect.

Discussion

Linkage maps

Soybean is a diploid species with twenty pairs of chromosomes (2n = 40) (Newell and Hymowitz, 1983). Some researchers reported that the current soybean species are tetraploids that evolved through diploidization (2n = 4x = 40) (Lackey, 1980). The current soybean molecular genetic map encompasses approximately 2900 cM (Shoemaker and Olson, 1993). One difficulty for soybean genome study is the lack of polymorphisms available for linkage map construction (Keim et al., 1990b). Although genetic studies from the populations developed by crossing elite lines were thought to be more practical for breeding programs, the frequency of polymorphism between two *G. max* cultivars was only about 20 % (Apuya et al., 1988). In this study, 25.1% of the tested probes detected polymorphisms in the Pride B216 x A15 population and 22.9% detected RFLP polymorphisms in the Anoka x A7 population. The frequency of polymorphisms detected by SSR markers was 45.8 % in the Pride B216 x A15 population and 58.3 % in the Anoka x A7 population.

The use of SSR and some RFLP markers, detected using mungbean and common bean probes, in this study allowed us to fill gaps within many linkage maps. Thus the distributions of markers covered most regions of the USDA-ARS/ISU soybean genetic map (Shoemaker and Olson, 1993). However, a few linkage groups still had large intervals between markers so that QTL with minor effect in these intervals might not be detected.

Genetic variation of iron deficiency

The highly susceptible cultivars (Pride B216 and Anoka) and the resistant lines (A15 and A7) were selected as parents to develop populations segregating for IDC. No $F_{2:3}$ lines in either population had significantly lower visual scores than the efficient parents in 1993 and 1994. However, one line in the Anoka x A7 population, and one line in the Pride B216 x A15 population had significantly higher chlorophyll concentrations than the efficient parents during 1993. These observations suggest that the inefficient parents (Anoka and Pride B216) also contributed favorable alleles at some QTL and that more resistant cultivars could be developed through genetic combination of selected alleles. The phenotypic distribution of F_2-derived progenies was skewed to the efficient parents indicating that dominant gene action or gene interaction is involved in response to iron deficiency, or that some iron inefficient genes with lower penetrance or expressivity did not express in the field tests. The difference of heritability values between the Pride B216 x A15 and the Anoka x A7 populations may also imply that diverse genetic components existed in the two populations.

Polygene mechanism in the Pride B216 x A15 population

Falconer (1989) discussed two reasons to explain the continuous variation of metric characters. One was the simultaneous segregation of many genes affecting the character, and the other was the superimposition of truly continuous variation arising from non-genetic causes. In our experiment, seven QTL were mapped in the Pride B216 x A15 population, but no completely consistent results were observed between growth stages and years (Table 2),

although most QTL were detected in more than one environment including stages and years. This finding is in agreement with the results of analysis of variances showing highly significant ($p = 0.0001$) effect of years, and interaction of the line x year in the evaluated traits. The significant effect of years, and of the interaction of genotype x year on the expression of iron deficiency chlorosis in the analysis of variance of our populations also reported by others (Diers et al., 1992c; Dragonuk et al., 1989), indicated the need to conduct QTL mapping in multiple environments. The minor phenotypic contribution and unstable expression of each detected QTL has supported the 'buffering mechanism' of gene expression proposed by Mather (1943) for a typical polygene mechanism controlling iron deficiency chlorosis in the Pride B216 x A15 population.

Major gene and modifying gene mechanism in the Anoka x A7 population

One QTL controlling visual score and chlorophyll concentration on linkage group N was verified to be a major gene with codominant inheritance. Weiss (1943), however, concluded that iron efficiency is dominant over iron efficiency.

Typically when measuring recombination between a major gene and two flanking markers, the sum of the two interval recombinations is greater than the recombinant frequency between two outside loci (Griffiths et al., 1993). Because QTL (or interval) mapping identifies the location of QTL within an interval between flanking markers, this procedure shouldn't change the distance between two flanking markers. Although the distance between the major gene for visual score and major gene for chlorophyll concentration was 6.0 cM in QTL

mapping, and 23.4 cM in major gene mapping, these distances were within the confidence intervals of QTL mapping. Based on statistical inference and the phenotypic evaluations used as indicators for chlorosis, we postulated one major gene on linkage group N with pleiotropic effects on visual score and chlorophyll concentration.

One QTL on linkage group A1 contributed 35.2 % of visual score variation combined over years. However, most of the contribution of this locus was caused by overdominance gene action. This QTL might act as a modifier to disturb discontinuous phenotypic distribution into a continuous distribution and to increase the difficulty of selection in the segregating population. Because the difference between two homozygous QTL genotypic classes was very small (0.2 score unit), this QTL wouldn't affect the phenotypic variation too much in later generations, especially for an inbreeding species for which homozygous lines are released as commercial cultivars.

QTL on linkage group I contributed 80% of phenotypic variation of chlorophyll concentrations. However, this QTL was only detected in the combined data from 1993 and 1994. It was likely that this QTL had poor penetrance or expressivity for chlorophyll and that this QTL was very sensitive to field environments. This QTL could be detected only when the number of replications or environments was increased. The allele encoding high chlorophyll concentrations was, however, contributed by the inefficient parent, Anoka. This result is contradictory to the assumption that Anoka is a highly inefficient parent or that A7 is a highly efficient parent, and might be one reason used to explain that the sum of individual QTL contribution for visual scores or chlorophyll contribution was more than 100%. Accordingly, results of

multiple locus analysis revealed that the QTL on linkage groups N and A1 contributed 75.3 % of phenotypic variation in visual scores, and QTL on linkage groups N and I contributed 80.7 % of phenotypic variation in chlorophyll contribution. Other possible explanations are that this QTL is affected by the major gene on linkage group N (Jansen, 1994), duplicated regions of chromosome, an error from colinear marker data (Lincoln et al., 1992), or other unknown conditions (Lincoln et al., 1992).

Relationship between visual scores and chlorophyll concentrations

Visual score is a fast and convenient method to evaluate iron deficiency chlorosis. Since the number of genes, gene action, and the magnitude of gene effect controlling chlorosis are unknown, a five score scale may not be appropriate to evaluate the phenotypic segregation of the trait. In addition, visual score is a subjective evaluation method so that phenotypic evaluation may vary from person to person, or date of evaluation. On the other hand, chlorophyll concentration evaluation is an objective method. In our study, we can not be certain that the genetic variation of chlorophyll concentration in non-stressed environments is related to that in stressed environments, or that genetic variation of chlorophyll concentration in non-stressed environments will affect the evaluation in stressed environments, or that other pigment systems, for example, carotenoid and xanthophyll (Terry and Abadia, 1986), might be involved in chlorosis phenotype. The physiological mechanisms of IDC in soybean are still not clearly understood. We can not conclude which indicator is more representative of IDC in soybean. However, most genomic regions associated with the variation of visual scores also were involved in the variation

of chlorophyll concentrations (Figures 4 and 5). In the region where QTL for one indicator was detected, there was often a corresponding peak of LOD values for the other indicator (Figure 7). Therefore, visual score and chlorophyll concentration evaluations apparently detected similar genetic mechanisms affecting iron deficiency in soybean.

Comparison between detected QTL in both populations

QTL on linkage groups I and N were detected in both populations. In the Anoka x A7 population, QTL on linkage group N were detected with a high reliability and contributed to the largest proportion of the phenotypic variation, and thus provided evidence of a single major gene involved in iron efficiency. In the Pride B216 X A15 population, neither the QTL on linkage group N, nor other detected QTL contributed large proportions of phenotypic variation, and hence evidence from this population support a polygene mechanism controlling IDC in soybean. Different phenotypic contributions of a QTL in two populations might be caused from gene dosage compensation (Mather, 1943), different genetic backgrounds (Doebley et al., 1995; Tanksley and Hewitt, 1988), multiple alleles at a locus, or be due to different mutations in the locus (Robertson, 1989).

QTL on linkage groups B2, G, H, and L were detected in the Pride B216 x A15 population only, although, the QTL on linkage group H deviated near the acceptance threshold in the Anoka x A7 population. This might indicate that for QTL on linkage group B2, G, and L, no allelic variation existed in these loci in the Anoka x A7 population (Dudley, 1993), epistatic effect was present (Lark et al., 1994; Lark et al., 1995), or that the major gene on linkage group N contributed the largest proportion of phenotypic variation. Thus other QTL

could not be detected because the genetic variation was less than the experimental error (Cox, 1995). Therefore, the conclusion that the inheritance of iron deficiency chlorosis in soybean can vary, depending on the parents used in developing the populations (Cianzio and Fehr, 1982), implies not only different QTL, but involvement of different magnitudes of gene effects.

Relationship between major genes and polygenes

Thompson and Thoday (1979), and Allard (1970) described the nature of major genes and quantitative traits very well. Thompson and Thoday (1979) pointed out that the history of quantitative genetics was separated from traditional Mendelian genetics by methodology, which was used to classify phenotypes of continuous traits into discrete groups and make counts of the number of individuals in each group. Allard (1970) described that if the non-genetic variation was large enough, even for a trait controlled by a single major gene, the F_2 population could exhibit a continuous distribution. Weiss (1943) noted some variation among iron inefficient cultivars and among efficient cultivars for degrees of iron efficiency. He stated that the magnitude of expression of modifying genes was negligible in comparison with that of the major gene. Clearly, he ignored the minor genes and treated iron efficiency in soybean as a qualitative trait.

Robertson (1989) proposed that loci with qualitative mutants are the same loci that affect the expression of quantitative traits. Tanksley (1993) proposed that if a gene contributing to quantitative variation was allelic to a gene controlling qualitative variation, then these genes should be mapped to the same locus along the chromosome. Beavis et al. (1991) compared the

positions of QTL affecting plant height of maize with previously known positions of qualitative variation for the same trait. Their results showed that most QTL were close to mapped qualitative loci. Eck et al. (1994) studied tuber shape of potato as qualitative and quantitative traits and found that the same RFLP markers were associated with both quantitative and qualitative traits. They transformed quantitative data into qualitative data with flanking RFLP markers. High recombination frequency, however, was observed disturbing the effect for the tagging markers. Our approach has shown that transforming quantitative data into qualitative data resulted in mapping of a major gene to the same region in which a QTL was mapped in the Anoka x A7 population. This result suggested that if a trait is controlled by a major gene that is difficult to be qualitatively evaluated due to environmental effects, or to disturbances by modifying genes, the trait can be treated as a quantitative trait and mapped to the same region as a major gene. Our results also provided evidence at the molecular level confirming the hypothesis that two genetic systems, ie., a major gene with modifying genes and/or polygene systems, control iron deficiency chlorosis of soybean.

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Table 1. Combined analysis of variance of visual scores and chlorophyll concentrations evaluated at V4 stage in the $F_{2:4}$ lines developed from Pride B216 x A15 and Anoka x A7 populations.

Source of Variation	Degrees of Freedom	Visual Score Mean Square	Visual Score F value	Visual Score Pr > F	Chlorophyll Concentration Mean Square	Chlorophyll Concentration F Value	Chlorophyll Concentration Pr > F
			Pride B216 x A15				
Rep/Year	4	6.29	32.78	0.0001	7.05	5.42	0.0003
Year	1	15.17	42.14	0.0001	1217.40	498.93	0.0001
Line	119	1.01	2.81	0.0001	6.04	2.48	0.0001
Line x Year	119	0.36	1.87	0.0001	2.44	1.87	0.0001
Error	476	0.19			1.30		
			Anoka x A7				
Rep/Year	4	3.41	16.50	0.0001	3.46	1.62	0.1688
Year	1	0.84	1.50	0.0001	1435.76	286.00	0.0001
Line	91	1.46	2.61	0.0001	8.03	1.60	0.0130
Line x Year	91	0.56	2.67	0.0001	5.02	2.35	0.0001
Error	364	0.21			2.14		

Table 2. Intervals significantly associated with variations for visual scores and chlorophyll concentrations at V2 and V4 growth stages in the Pride B216 x A15 population, during 1993, 1994 and combined over years.

Interval	Linkage Group	R^2 Value (%)	LOD Value	Means of QTL Genotypic Classes		
				A 1A1	A1A2	A2A2
			Visual scores at V2 stage of 1994			
Satt12-T36	G	15.4	4.0	2.61	2.10	2.28
Mng456-K418	N	23.4	2.4	2.51	2.13	1.93
Multiple loci		28.5				
			Visual scores at V4 stage of 1993			
A404-B69	H	29.9	2.4	2.96	2.37	2.35
Satt9-A71	N	7.8	2.1	2.64	2.52	2.28
Multiple loci		36.9				
			Visual scores at V4 stage of 1994			
T36-K227	G	12.2	2.6	2.44	2.04	2.27
A132-A461	L	15.3	2.8	2.41	2.29	1.92
Satt9-A71	N	17.2	2.6	2.42	2.03	1.96
Multiple loci		34.1				
			Visual scores at V4 stage of 1993 & 1994			
A593-Satt70	B2	10.8	2.5	2.50	2.37	2.11
A519-Satt63	B2	7.7	2.4	2.41	2.40	2.13
T36-K227	G	9.2	2.2	2.53	2.21	2.37
Satt9-K418	N	8.0	2.1	2.43	2.38	2.15
Multiple loci		21.6				

(continued)

		Chlorophyll concentrations at V2 stage of 1994				
A593-Satt20	B2	7.7	2.1	7.88	8.15	9.11
Satt12-T36	G	16.0	4.2	7.22	8.92	8.29
A404-B69	H	25.0	2.4	7.65	7.93	9.35
Mng456-K418	N	11.5	2.1	7.78	8.93	9.56
Multiple loci		42.9				
		Chlorophyll concentration at V4 stage of 1993				
A404-B69	H	16.3	2.6	4.29	5.02	5.29
		Chlorophyll concentration at V4 stage of 1994				
Satt70-Satt20	B2	11.9	3.1	6.79	7.50	8.24
A183-A519	B2	16.3	2.9	7.04	6.95	7.49
A404-B69	H	13.7	2.2	6.93	7.20	8.12
Multiple loci		31.5				
		Chlorophyll concentration at V4 stage of 1993 & 1994				
Satt70-Satt20	B2	11.3	2.4	5.82	6.16	6.80
A515-K644	I	19.3	2.6	5.35	6.31	6.58
A404-B69	H	21.7	2.4	5.52	6.29	6.82
Multiple loci		34.9				

Table 3. Intervals significantly associated with variations for visual scores and chlorophyll concentrations at V2 and V4 growth stages in the Anoka x A7 population, during 1993, 1994 and combined over years.

Interval	Linkage Group	R^2 value (%)	LOD Value	Means of QTL Genotypic Classes A 1A1	A1A2	A2A2
		Visual scores at V2 stage of 1994				
A515-K644	I	39.2	2.2	1.99	2.52	2.83
BLT15-Sat33	N	78.7	18.2	3.04	2.47	1.69
Multiple loci		81.2				
		Visual scores at V4 stage of 1993				
K644-B39	I	57.0	3.4	2.10	2.72	2.90
Sat33-A280	N	26.8	4.9	2.73	2.34	2.10
Multiple loci		75.5				
		Visual scores at V4 stage of 1994				
K258-A256	A1	38.6	3.1	2.65	1.91	2.85
K644-B39	I	77.4	3.1	1.30	2.87	3.04
BLT15-Sat33	N	77.6	14.6	3.13	2.36	1.32
Multiple loci		91.1				
		Visual scores at V4 stage of 1993 & 1994				
K258-A256	A1	35.2	2.8	2.54	2.07	2.75
BLT15-Sat33	N	72.7	13.1	2.92	2.35	1.68
Multiple loci		75.3				
		Chlorophyll concentrations at V2 stage of 1994				
BLT15-Sat33	N	52.1	10.7	7.07	7.66	10.59
		Chlorophyll concentration at V4 stage of 1993				
Sat33-A280	N	30.5	4.7	4.57	5.19	5.42
		Chlorophyll concentration at V4 stage of 1994				
BLT15-Sat33	N	52.3	6.6	6.15	8.64	10.15
		Chlorophyll concentration at V4 stage of 1993 & 1994				
A515-K644	I	80.0	3.5	8.12	6.86	5.03
BLT15-Sat33	N	68.8	7.3	5.20	6.96	8.04
Multiple loci		80.7				

Table 4. χ^2 test for goodness of fit to a 1 homozygous efficient : 2 heterozygote : 1 homozygous inefficient ratio for qualitatively classified data in the Anoka x A7 population.

	Number of Lines				
	A1A1	A1A2	A2A2	χ^2 (1:2:1)	p value
	Visual scores (V4 stage)				
Observed	24	46	22	0.086	0.96
Expected	23	46	23		
	Chlorophyll concentrations (V4 stage)				
Observed	20	51	21	1.107	0.58
Expected	23	46	23		

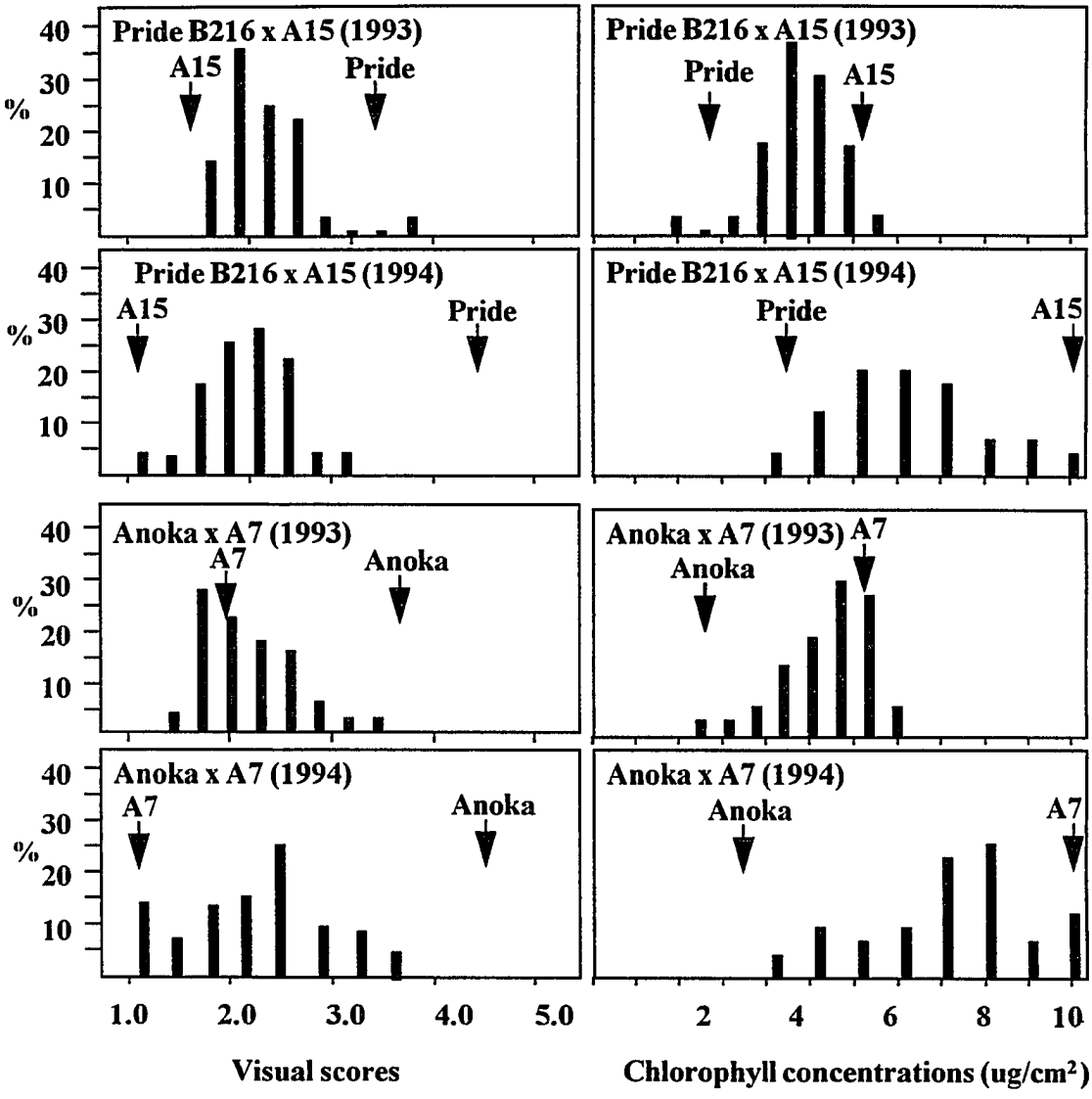


Figure 1. Frequency distributions of visual scores and chlorophyll concentrations at the V4 stage during 1993 and 1994 in the Pride B216 x A15 and Anoka x A7 populations.

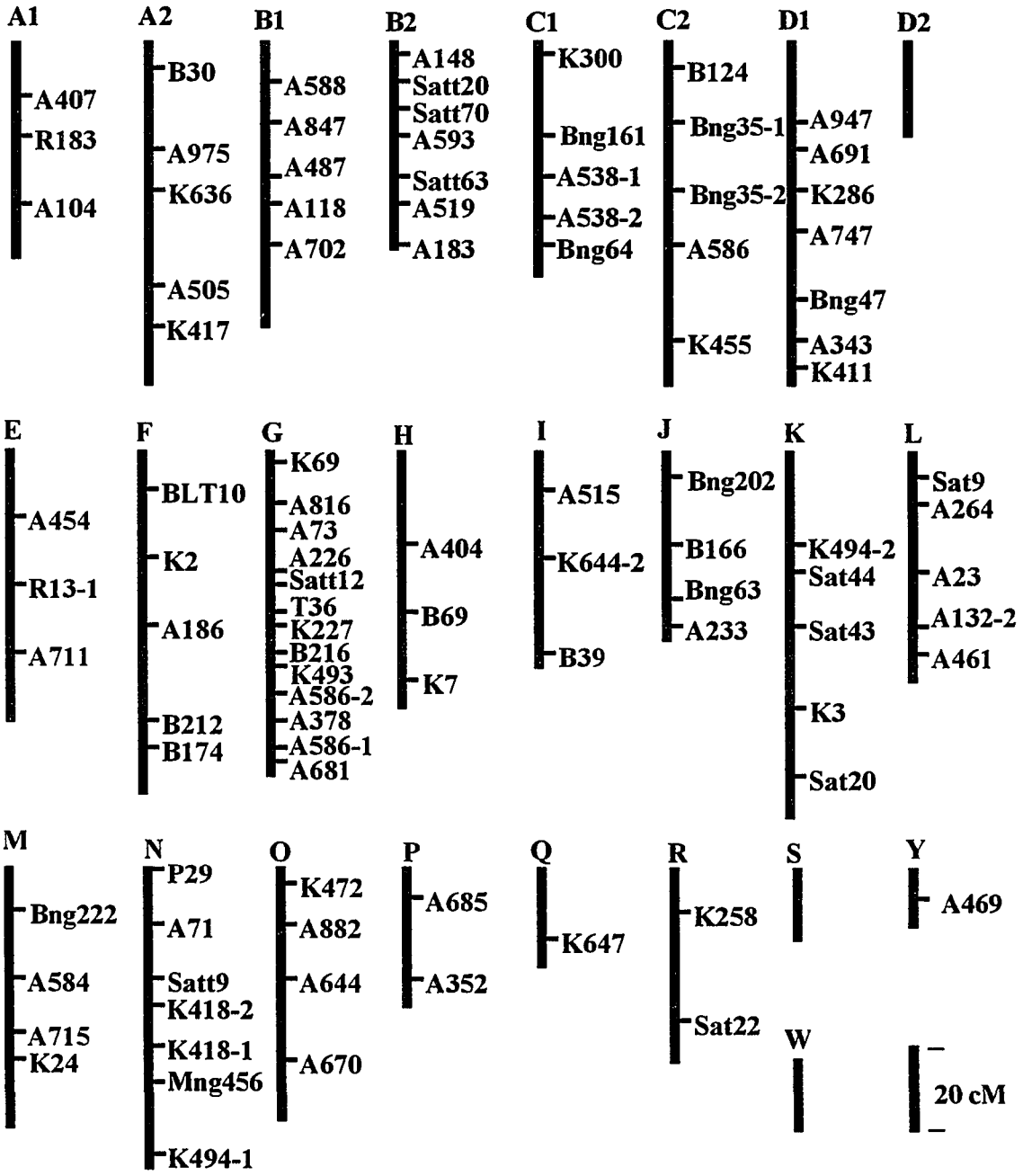


Figure 2. Distribution of molecular markers used to map QTL for iron deficiency chlorosis in the Pride B216 x A15 population.

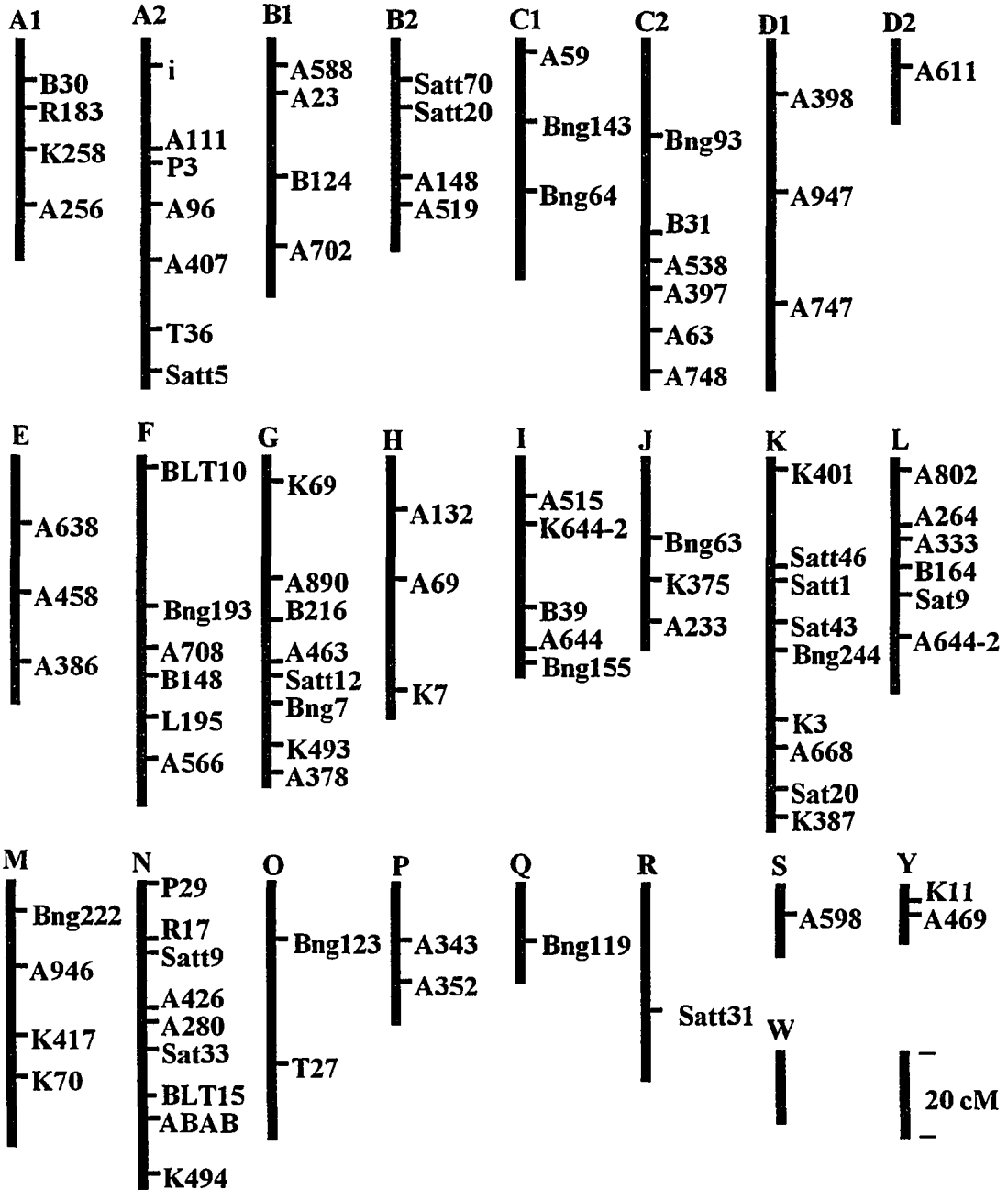
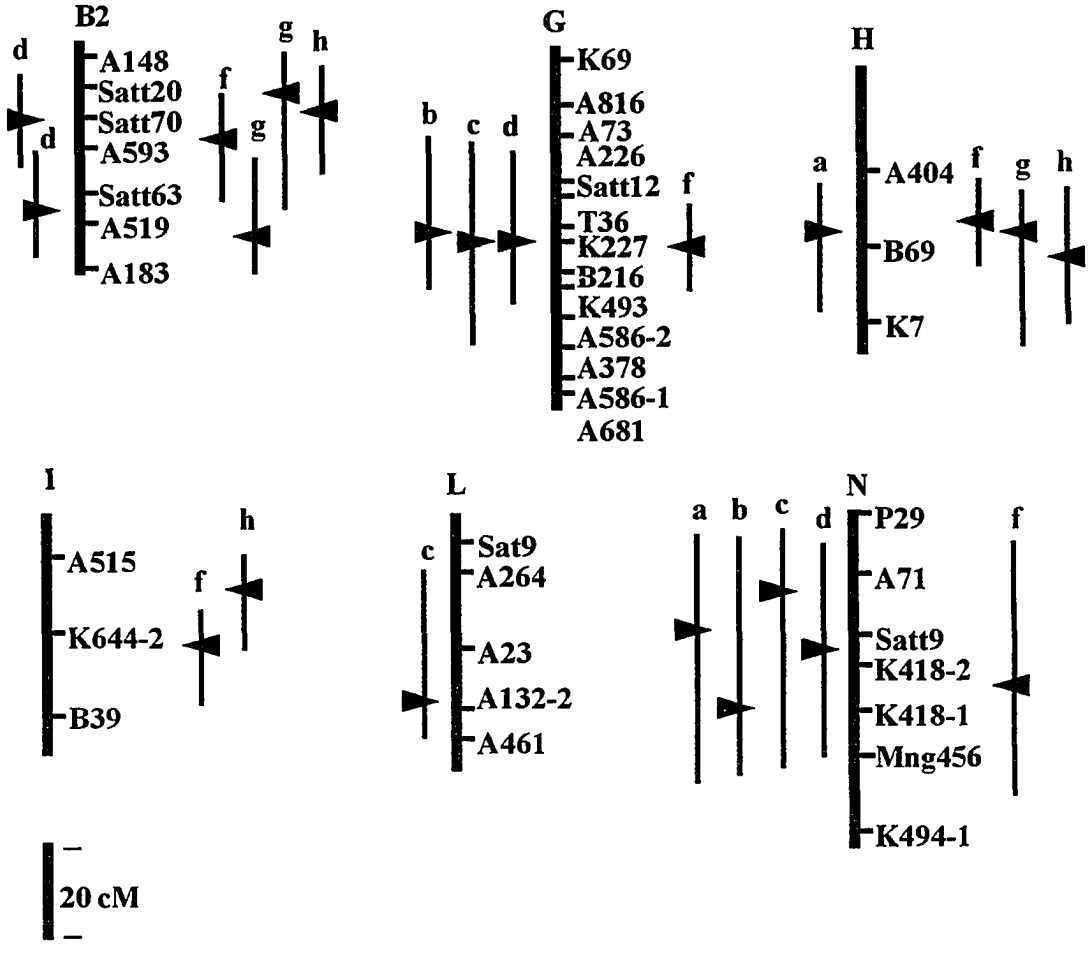
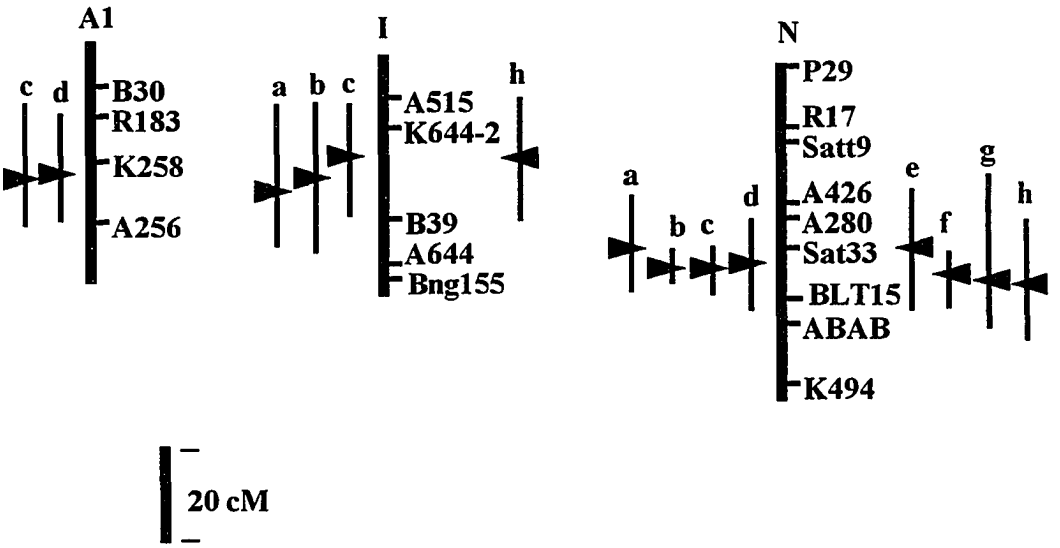


Figure 3. Distribution of molecular markers used to map QTL for iron deficiency chlorosis in the Anoka x A7 population.



a = visual scores at V4 stage in 1993
b = visual scores at V2 stage in 1994
c = visual scores at V4 stage in 1994
d = combined visual scores at V4 stages in 1993 & 1994
e = chlorophyll concentrations at V4 stage in 1993
f = chlorophyll concentrations at V2 stage in 1994
g = chlorophyll concentrations at V4 stage in 1994
h = combined chlorophyll concentratins at V4 stages in 1993 & 1994

Figure 4. Locations of detected QTL for iron deficiency chlorosis in the Pride B216 x A15 population. The straight lines and arrows beside the linkage groups show the confidence intervals and locations of the detected QTL, respectively.



a = visual scores at V4 stage in 1993
b = visual scores at V2 stage in 1994
c = visual scores at V4 stage in 1994
d = combined visual scores at V4 stages in 1993 & 1994
e = chlorophyll concentrations at V4 stage in 1993
f = chlorophyll concentrations at V2 stage in 1994
g = chlorophyll concentrations at V4 stage in 1994
h = combined chlorophyll concentrations at V4 stages in 1993 & 1994

Figure 5. Locations of detected QTL for iron deficiency chlorosis in the Anoka x A7 population. The straight lines and arrows on the left side of linkage groups show the confidence intervals and locations of detected QTL, respectively.

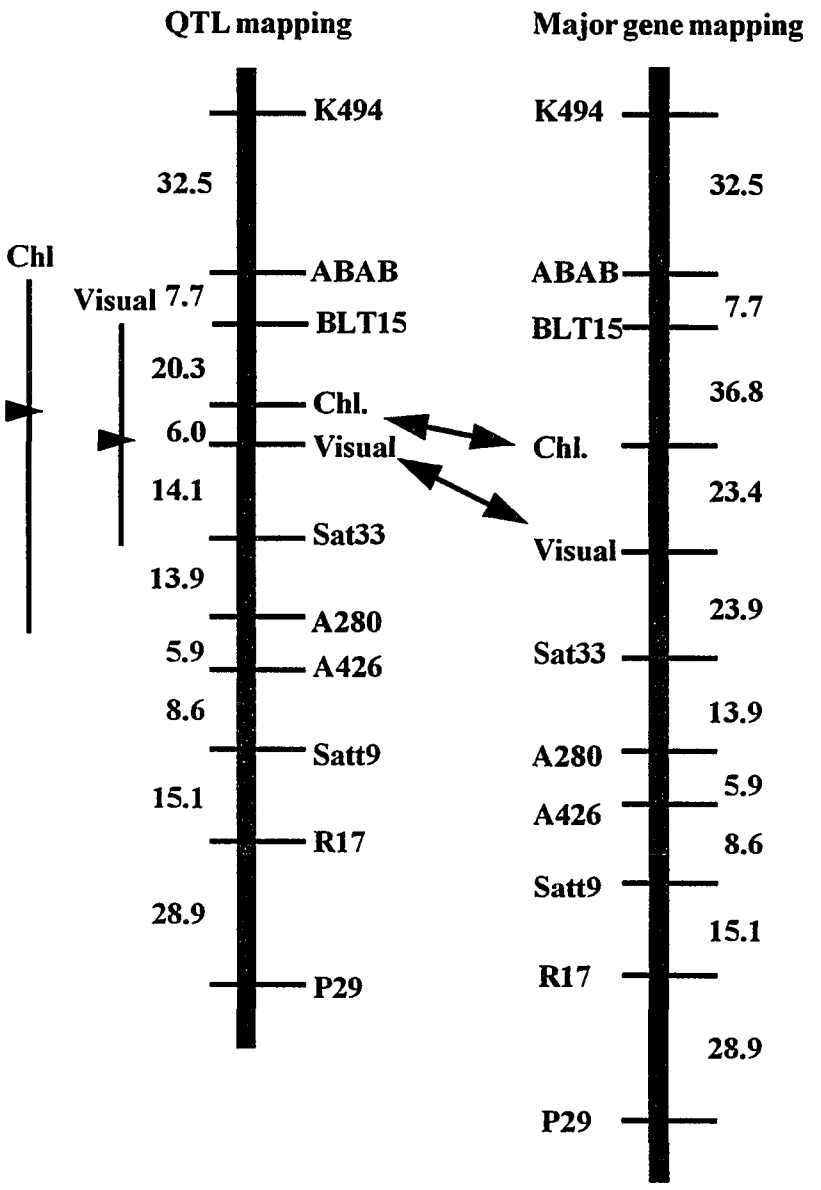


Figure 6. Comparison between linkage maps (linkage group N) from QTL mapping and major gene mapping for visual scores and chlorophyll concentrations in the Anoka x A7 population. The straight lines and arrows on the left side of linkage group N showing the confidence intervals and locations of detected QTL, respectively. The symbol 'Visual' stands for visual scores and 'Chl.' stands for chlorophyll concentrations.

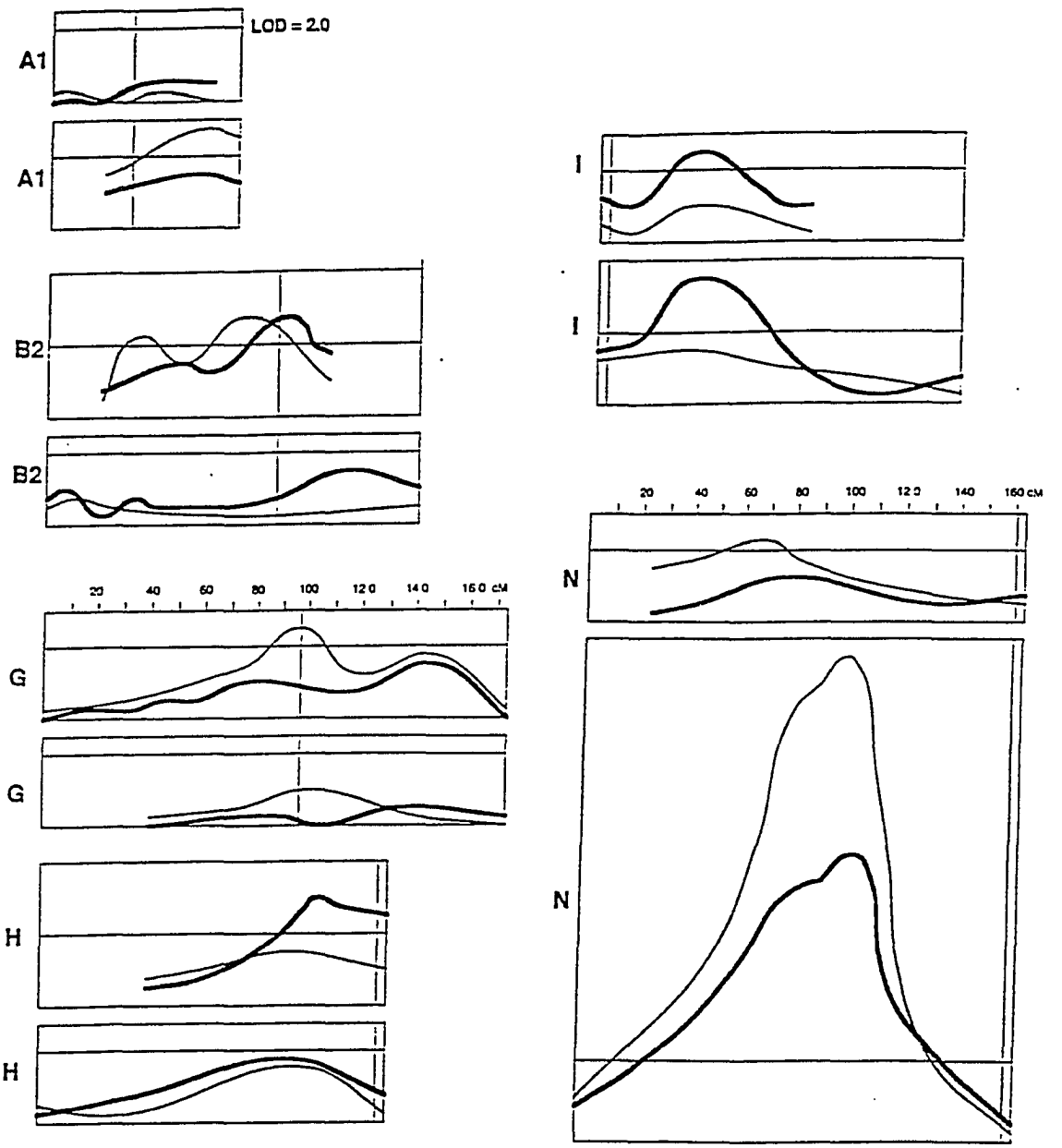


Figure 7. Likelihood distributions of visual scores (_____) and chlorophyll concentrations (_____) averaged across years at V4 growth stage on linkage groups A1, B2, G, H, I, and N in Pride B216 x A15 (upper) and Anoka x A7 (lower) populations. The vertical lines on each figure show the location of common marker loci (anchors) and horizontal lines show the threshold of LOD = 2.0 in both populations.

II. MOLECULAR MARKER EVALUATION OF THE EFFICIENCY OF NUTRIENT SOLUTION TEST FOR IRON DEFICIENCY CHLOROSIS IN SOYBEAN

A paper to be submitted to Crop Science

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Silvia Rodriguez de Cianzio, and Randy C. Shoemaker

Abstract

Nutrient solution systems have been considered an alternative method to field evaluations for studies of iron deficiency chlorosis (IDC) and for breeding soybeans with improved iron efficiency. To determine the efficiency of nutrient solution evaluation for soybean IDC, 120 $F_{2:4}$ lines in a Pride B216 x A15 population, and 92 $F_{2:4}$ lines in an Anoka x A7 population were grown in nutrient solution in greenhouse plantings and evaluated for IDC by visual scores and determinations of chlorophyll concentrations. Eighty-nine RFLP and ten SSR markers in the Pride B216 x A15 population, and 82 RFLP, 14 SSR and *I* (hilum color) markers in the Anoka x A7 population were used to construct linkage maps and to locate quantitative trait loci (QTL) controlling iron deficiency chlorosis. In the Anoka x A7 population, one major gene on linkage group N, and modifying QTL on linkage groups A1 and I previously mapped during field tests also were identified in the nutrient solution test. One newly identified QTL was mapped on linkage group B2. In the Pride B216 x A15

population, one QTL previously mapped on linkage group I during field tests was not identified in the nutrient solution test, and two newly identified QTL were mapped on linkage groups A2 and B1. QTL on linkage groups B2, G, H, L and N were identified in both field and nutrient solution tests. Due to significant interaction between genotype and environment (G x E) in both field and nutrient solution tests, QTL identifications from multiple environments were used to compare the similarity between field and nutrient solution tests. We conclude that similar QTL are identified in nutrient solution test and field test and that nutrient solution tests demonstrated high effectiveness in evaluating IDC of soybean.

Introduction

Iron deficiency chlorosis (IDC) is a common problem in soybean grown on calcareous soil in the midwestern United States (Fehr, 1982). Some soybean varieties are genetically more efficient in iron utilization than others (Cianzio et al., 1979). A single major gene with modifiers (Cianzio and Fehr, 1980) and a polygene mechanism (Cianzio and Fehr, 1982) governing iron deficiency chlorosis in soybean have been reported.

Field evaluations for iron efficiency in soybean have been limited by the severity of chlorosis due to environmental variability (Jessen et al., 1986), and alternative evaluation methods have been studied. The decreased sensitivity of IDC determination for soybean grown in pots resulted in the lack of identification of some moderately inefficient genotypes (Fairbanks et al., 1987). Chlorosis symptoms also were much less severe in the growth chamber than in

the field (Byron and Lambert, 1983). Detopping of seedlings was suggested as a means to increase the severity of chlorosis (Jessen et al., 1986; Piper et al., 1986). Tissue culture techniques also were reported to be useful to evaluate IDC in soybeans (Stephens et al., 1990).

Nutrient solution has been used to evaluate IDC in soybean (Coulombe et al., 1984). The advantage to this system is that it; a) can be done during winter; b) each cycle of evaluation can be completed in only about one month; c) a higher severity of chlorosis can be detected; and d) the problems of the heterogeneity of calcareous soils and varying environmental conditions can be avoided. High rank correlation between field and nutrient solution evaluations was obtained using eight soybean cultivars with a wide range of chlorosis ratings (Jessen, 1988a). Diers and Fehr (1989) concluded that single plant selection for IDC in either field or nutrient solution evaluation was less effective than selection among lines in replicated tests or selection based on progeny tests. Dragonuk et al. (1989b) compared the genetic improvement in terms of genetic gain for IDC between nutrient solution and field tests in a soybean recurrent selection program and suggested that nutrient solution and field evaluations may partially select for different mechanisms of response to iron stress.

Cianzio and Fehr (1980) reported that a single major gene and modifying genes controlled soybean iron efficiency in a segregating population developed from the inefficient parent, Anoka, and an efficient line. Cianzio and Fehr (1982) also studied a segregating population developed from the inefficient variety, Pride B216, crossed to an efficient line. They inferred a different

genetic mechanism implying that polygenes (or multiple genes) controlled the IDC in soybean.

Molecular markers have proven to be useful for selection of single plants and lines (Paterson et al., 1991). If nutrient solution and field evaluations test the same or similar genetic mechanisms controlling IDC, the combined use of molecular markers and nutrient solution should increase the efficiency of breeding for this trait. RFLP markers associated with iron efficiency evaluated in field tests on calcareous soil have been identified (Diers et al., 1992; Lin et al., submitted). One major gene and several QTL controlling IDC evaluated in field tests were positioned on soybean linkage maps and two separate genetic mechanisms controlling the expression of iron deficiency chlorosis in soybeans were confirmed (Lin et al., submitted).

The objectives of our study were to map genes for iron deficiency chlorosis evaluated in nutrient solution, and to determine the degree of similarity between the iron efficiency QTL mapped using nutrient solution and field tests.

Materials and Methods

Plant materials

One hundred and twenty $F_{2:4}$ lines from Pride B216 x A15, and 92 $F_{2:4}$ lines from Anoka x A7 were used in this study. The lines had been tested in the field on calcareous soil in 1993 and 1994 (Lin et al., submitted). Anoka and Pride B216 were inefficient parents, both used to develop populations to study the inheritance of the trait (Cianzio and Fehr, 1980 and 1982). The efficient

parents A7 and A15 were selected from a recurrent selection program (Fehr et al., 1984; Jessen et al., 1988b).

Nutrient solution evaluations

Four periods of nutrient solution evaluations were conducted in the greenhouse facilities at Land O'Lakes Inc., Webster City, Iowa, during 1994. Each population was evaluated in two different greenhouse environments or periods. In each period, one population was evaluated in a randomized complete block design (RCBD) with two replications. Eight seeds of each $F_{2:4}$ lines and 72 seeds of each parent were germinated in plastic trays containing moistened peat moss. After 7-8 days, four uniform seedlings of each line and 32 of each parent were transplanted to plastic buckets containing 10 liters of nutrient solution. Each bucket was covered with a black plexiglass plate containing 12 holes, and three plants were grown in each hole.

From each line and parent, two plants were evaluated per replication; therefore each plant was identified with a plastic tag. In each bucket, there were 36 tagged plants including two plants from each of 15 lines assigned at random to each bucket, the two parents, and one check cultivar. Preparation of nutrient solution medium, greenhouse growth conditions, and plant management were done following the methodology described by Dragonuk et al. (1989a).

Visual scores and chlorophyll concentrations were evaluated at the V2 growth stage (Fehr and Caviness, 1977). The visual scores were evaluated on the basis of yellowing of the young leaves; 1 = no yellowing, 2 = slight yellowing, 3 = moderate yellowing, 4 = intense yellowing, and 5 = severe yellowing with

some necrosis (Cianzio et al., 1979). After visual scores were rated, one trifoliolate leave showing chlorosis symptoms was sampled at random from the two plants of a line or parent to determine chlorophyll concentration. A leaf disc was obtained with a borer (8.8 mm in diam) from the middle leaflet of each trifoliolate leaf. Chlorophyll from leaf samples was extracted in 80% acetone solution and absorbance values were measured at two wavelengths (663 nm and 645 nm) with a spectrophotometer. The chlorophyll concentration ($\mu g/cm^2$) was estimated according to Arnon (1949) as:

Chlorophyll a = 12.7 x A663 - 2.7 x A645

Chlorophyll b = 22.9 x A645 - 4.7 x A663

Chlorophyll a+b = 20.2 x A645 + 8.0 x A663

Phenotypic data evaluated at the V2 stage in the two periods were combined for the analysis of variance. The effects of replication, lines, and periods were considered random factors. Broad sense heritability (Hb^2) was estimated on the basis of the expected mean squares from the combined analysis of variance (Fehr, 1987) as:

$$Hb^2 = \frac{\sigma^2 g}{(\sigma^2 e / rxe + \sigma^2 ge/e + \sigma^2 g)}$$

where $\sigma^2 g$ = genetic variance

$\sigma^2 ge$ = genetic x environment interaction variance

$\sigma^2 e$ = experimental error variance

r = number of replications

e = number of environments

The missing data were estimated as described in Gomez and Gomez (1984).

Test of the relationship between the chlorosis symptoms and Fe

Three plants of each parent (Pride B216, Anoka, A7, and A15) were grown in two replications at each of two nutrient solutions. One nutrient solution contained a limiting amount of iron (2 μM $Fe(NO_3)_2$) (Dragonuk et al., 1989a). The other nutrient solution contained 20 μM of $Fe(NO_3)_2$ and was considered a high Fe availability solution. The response of plants in these two nutrient solutions was evaluated as described at the V2 growth stage and was used to test the relationship between the chlorosis of plants in nutrient solution and the availability of Fe.

Construction of linkage maps

Leaf samples from 10-15 plants of each line were harvested from field planting and bulked for DNA isolation and RFLP and SSR genotyping. Procedures for soybean DNA extraction, restriction enzyme digestion, Southern transfer (Southern, 1975), and autoradiography were as described in Keim et al. (1990). In order to screen informative RFLP markers, DNA samples from each of the four parents (Anoka, A7, Pride B216, and A15) were separately digested with restriction enzymes Dra I, Eco RI, Eco RV, Hind III, and Taq I. The digested samples were electrophoresed on agarose gels and were used for Southern blottings. Three hundred and eight soybean, 15 mungbean, and 48 common bean probes (kindly provided by E. Vallejos at the University of Florida, and N. Young at the University of Minnesota) were screened by identifying polymorphisms between each pair of parents. Eighty-two RFLP, 14 SSR, and *I* (hilum color) markers were used to construct a genetic linkage map in the Anoka x A7 population. In the Pride B216 x A15 population, 92 RFLP and ten SSR

markers were used to construct a linkage map. Primers and procedures to obtain SSR markers were described by Akkaya et al. (1995). Selected SSR markers were used to fill gaps of some genomic regions that lacked RFLP markers. The computer program MAPMAKER 3.0b (Lander et al., 1987) was used to develop linkage maps for gene mapping. Markers were assigned to linkage groups using a LOD threshold of 3.0 and a maximum recombination frequency of 0.50. The 'Order' command was used to add a new marker to the linkage group. Map distances were estimated using the Haldane (1919) mapping function.

QTL mapping

The MAPMAKER/QTL1.1 computer program (Lincoln et al., 1992) was used to determine the location of QTL and to estimate the phenotypic contribution (R^2) of each detected QTL. In order to detect QTL with minor effects, a LOD value of 2.0 was used as the threshold for detecting QTL locations. The position of the LOD peak in each significant interval suggested the location of QTL. The contribution of all QTL also was estimated from multiple loci analysis. One-way analysis of variance (ANOVA) using the SAS PROC GLM computer command was applied to detect QTL on linkage groups with only one informative marker and to confirm the presence of each QTL (SAS Institute, 1990).

Results

Test of the relationship between Fe availability and chlorosis in nutrient solution

Both efficient and inefficient parents had significantly different visual scores and chlorophyll concentrations in the high Fe availability [20 μM $Fe(NO_3)_2$] nutrient solution than in the low Fe availability [2 μM $Fe(NO_3)_2$] nutrient solution. However, no chlorosis symptoms were observed in the two efficient parents in the high Fe nutrient solution. Chlorophyll concentrations of both efficient parents and inefficient parents in the high Fe availability nutrient solution were at least twice as much as in the low Fe availability nutrient solution (Table 1). These observations indicated that the chlorosis evaluated in the nutrient solution system was in fact due to iron deficiency.

Genetic variation

In the two populations, there were significant differences between the two periods of nutrient solution tests, and among $F_{2:4}$ lines of each population for visual scores and chlorophyll concentrations (Table 2). The interaction between lines and periods for visual scores and chlorophyll concentrations at the V2 growth stage of both populations also was significant. Severe chlorosis was observed at the V2 stage in the Period 1 tests in both populations (Figure 1). Due to low temperature during the testing periods, the chlorosis symptoms were less severe in the Period 2 tests of both populations. In the previous field tests, the estimated broad sense heritability of the visual scores was 82.4% and the heritability of the chlorophyll concentration was 64.5% for the Pride B216 x A15 population. The heritabilities of visual scores was 73.7% and for the chlorophyll

concentrations was 59.9% for the Anoka x A7 population (Lin et al., submitted). Less phenotypic variation was observed in the nutrient solution tests (except Period 1 test of the Anoka x A7 population) than in the field tests (Table 3), therefore the estimated broad sense heritabilities from nutrient solution tests were less than the field test. In nutrient solution tests, the estimated heritabilities on an entry mean basis were 25.5 % for visual scores and 37.4% for chlorophyll concentrations in the Pride B216 x A15 population. The heritabilities were 34.5 % and 49.2 % for visual scores and chlorophyll concentrations in the Anoka x A7 population, respectively.

Linkage maps

Because SSR and RFLP markers were specifically selected to fill gaps between RFLP markers, the markers covered most regions of the USDA-ARS/ISU soybean genetic map (Shoemaker and Olson, 1993). In the Pride B216 x A15 population (Figure 2), 89 RFLP and ten SSR markers were distributed on all linkage groups except the short linkage groups D2 (49.8 cM), S (19.1 cM), and W (12.8 cM). The informative markers spanned approximately 1700 cM and the average length of each interval between two adjacent markers was about 20.8 cM. Ninety-seven genetic markers consisting of 82 RFLP, 14 SSR, and *I* (hilum color) markers were placed on 24 linkage groups in the Anoka x A7 population (Figure 3). Only one short linkage group W (12.8 cM) of the USDA-ARS/ISU soybean genetic map was not covered with markers. These markers spanned about 1650 cM. On average, the intervals between two adjacent markers were about 17.6 cM. The order of markers on the maps were similar to those reported for the USDA-ARS/ISU soybean genetic map except that RFLP

marker A702 was placed on a different end of linkage group B1 in both the Anoka x A7 and Pride B216 x A15 populations. A few closely linked markers were reversed in order due to sampling size and/or possible scoring errors.

Mapping of QTL in the Pride B216 x A15 population

In previous field tests, QTL controlling chlorosis, based on visual scores or chlorophyll concentrations were detected on linkage groups B2, G, H, I, L, and N of the Pride B216 X A15 population (Lin et al., submitted). In the nutrient solution the measures of chlorophyll concentration detected more QTL than visual score evaluation (Table 4). Chlorophyll concentration seems to be more effective in detecting QTL for chlorosis than visual score evaluation in nutrient solution. Due to the significant environmental effects and significant interaction between QTL and genotype, no single periods of nutrient test detected the same QTL as the field test did. When both periods of nutrient solution tests were evaluated, QTL on linkage groups A2, B1, B2, G, H, L and N were detected for chlorophyll concentration evaluation at the V2 stage (Figure 4). Only a subset of common QTL for visual scores on linkage groups B1, B2, G, L, and N were detected at different stages in both period tests. Therefore, most QTL identified in the field test also were identified in the nutrient solution test. Two newly identified QTL were mapped on linkage groups A2 and B1. One RFLP marker, K69, was associated with visual scores and chlorophyll concentration in one test (Period 2) by using one way analysis of variance. This QTL previously had been identified to be correlated with chlorosis in the field. The iron efficient alleles of all detected QTL were contributed from efficient parent A15. This result was in agreement with field test.

Mapping of QTL and a major gene in the Anoka x A7 population

One major gene affecting visual scores and chlorophyll concentrations was mapped on linkage group N in the Anoka x A7 population in the previous field test (Lin et al., submitted). Results from the nutrient solution test revealed that this gene also was detected by visual score evaluation at the V2 stage in both periods of test, but it contributed less to the phenotypic variation than was observed in the field tests (Table 5). In the Period 2 test, due to less severe chlorosis symptoms, the effect of the major gene was not detected by chlorophyll concentrations (Tables 3 and 5). However, when visual scores were determined at the V4 growth stage, the major gene on linkage group N contributed 82.9 % (LOD = 12.0) and 40.4 % (LOD = 5.4) to visual score variation in the Period 1 and Period 2 tests, respectively.

Two QTL for visual scores and/or chlorophyll concentration on linkage groups A1 and I detected in the field test also were detected in the nutrient solution test for visual scores at stage V4 period 1, and the two periods combined (Table 5). On QTL on linkage group I was also detected for chlorophyll concentration measured at period 1. One newly identified QTL for visual score was mapped on linkage group B2. The iron efficient alleles of the QTL on linkage groups A1, I, and B2 were contributed by the inefficient parent Anoka.

Due to significant environmental (period or year) effects and interaction between lines and environments in both field and nutrient solution tests, some QTL were detected only in one environment (Figures 4 and 5). When we took into account all test environments simultaneously, however most QTL detected in the field tests also were detected in at least one period of nutrient solution tests for both populations. One QTL associated with chlorophyll concentration

on linkage group I of the Pride B216 x A15 population was detected only in the field tests (Figure 4).

Discussion

The nutrient solution developed to select iron efficient genotypes was based on the field conditions of calcareous soil; i.e. high bicarbonate (HCO_3^-) concentration, and low Fe availability (Coulombe et al., 1984). Because the severity of chlorosis was mainly induced by increasing the concentration of bicarbonate (Jesssen et al., 1986; Dragonuk et al., 1989a), and because many nutritional deficiencies, such as nitrogen, manganese, phosphorus, boron, calcium, and iron, produce chlorosis symptoms on leaves (Taiz and Zeiger, 1991), we were concerned as to whether the chlorosis variation in nutrient solution systems was truly related to iron deficiency. The demonstration of an essential element deficiency involved growing plants in nutrient solutions containing all essential elements except the element in question (Arnon and Stout, 1939). Our experimental results showed that increasing the concentration of available Fe could reduce or relieve the symptoms of chlorosis, and thus demonstrated that the chlorosis of plants grown in nutrient solution system was caused by Fe deficiency.

On the whole, the variation of chlorosis in the nutrient solution system was less than in the field test. This result was in accordance with a previous study (Dragonuk et al., 1989b). Additionally, Diers and Fehr (1989) reported that single plant evaluations had lower heritabilities than those of line evaluations in field tests and nutrient solution tests. Because of limitations in

cost and space for growing plants in the greenhouse, only two plants were grown to represent an $F_{2:4}$ line. This number might be insufficient to estimate the performance of each $F_{2:4}$ line. As expected, the estimated broad sense heritabilities on an entry mean basis for visual scores and for chlorophyll concentrations were less than in the field test. The low heritability of an evaluated trait could reduce the power and reliability in QTL mapping (Hyne et al., 1995).

Chlorophyll concentrations may be better at representing the true phenotypes of iron deficiency chlorosis than visual scores. Still, it was found that these two indicators often shared common QTL in field and nutrient solution tests. Since chlorophyll concentration evaluation detected more QTL than visual score evaluation, it seemed that the former was a better indicator than the latter in the nutrient solution test.

Diers and Fehr (1989) reported that there was no detectable difference between field and nutrient solution tests due to the lack of significant difference between the tests for heritabilities. Previous work (Dragonuk et al., 1989b) has indicated that nutrient solution and field tests might be partially selecting for different genetic mechanisms of responses to Fe stress. In our work, although the detected QTL for Fe deficiency chlorosis were unstably expressed in both field and nutrient solution tests, most QTL detected in the field tests were also detected in the nutrient solution tests for both populations. We concluded therefore that nutrient solution and field tests evaluated very similar mechanisms controlling iron deficiency chlorosis in soybean.

Since the nutrient solution system identifies mechanisms of iron deficiency chlorosis of soybean similar to that detected in the field test and the

nutrient solution technique can be used during winter and be completed in a short period of time (about one month), use of field and nutrient solution tests sequentially, may increase the efficiency of breeding for iron efficiency. Caution however, needs to be exercised due to the unstable expression of detected QTL and major genes in different environments, which can reduce the effectiveness of selection. Unstable detection of QTL is likely caused by degree of stress, phenotypic variation, and environmental effects (Tanksley, 1993). Improvements in methodology, such as modifications in the nutrient solution, and determining the best growth stage for visual evaluation and chlorophyll concentration determination will increase the possibility of using nutrient solution tests for the selection of iron efficient genotypes.

In practical plant breeding programs, breeders are careful about selecting a few of the most iron efficient plants. Diers and Fehr (1989) reported that the actual genetic gains for plants identified in nutrient solution was significantly higher than the gains from selection in the field when the upper 20 % of progenies were selected. Because severe chlorosis can be adjusted in the nutrient solution test, a gridding procedure that is selecting for one or a few of the best genotypes in each bucket (Gardner, 1961; Fehr, 1987) might be suitable to control environmental effects and increase efficiency in the selection of the most iron efficient individuals. In order to obtain high indirect selection efficiency and to minimize the cost of marker tests, applying marker-assisted selection in single plant selection of early generation populations, in which the linkage disequilibrum between markers and QTL can be maintained for the trait with environmentally unstable expression is necessary. Recently, a very effective method has been suggested to increase the frequencies of both major

and minor genes simultaneously in a recurrent selection program using linked markers to selected for favorable alleles, or discarding all individuals with more than one major favorable allele and then selecting the most efficient of the remaining individuals (Cox, 1995). This study has demonstrated the high effectiveness of nutrient solution test and has provided the possibility for combined use of nutrient solution test, field test, and marker-assisted selection in improving soybean iron deficiency chlorosis.

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Table 1. Means and standard errors of visual scores and chlorophyll concentrations at V2 stage of four parents in 2μM $Fe(NO_3)_2$ and 20 μM $Fe(NO_3)_2$ nutrient solutions.

	Visual Score		Chlorophyll Concentration ($\mu g/cm^2$)	
Cultivars/Lines	2 μM Fe	20 μM Fe	2 μM Fe	20 μM Fe
Pride B216	3.75 ± 0.25	2.50 ± 0.50	0.95 ± 0.59	3.25 ± 1.18
Anoka	3.25 ± 0.20	1.80 ± 0.18	1.19 ± 0.05	4.96 ± 1.31
A15	1.25 ± 0.20	1.00 ± 0.00	4.27 ± 0.35	11.46 ± 1.15
A7	2.25 ± 0.20	1.00 ± 0.00	3.43 ± 0.89	8.59 ± 0.64

Table 2. Analysis of variance of visual scores and cholrophyll concentrations in two $F_{2:4}$ populations developed from Pride B216 x A15 and Anoka x A7, evaluated in nutrient solution.

Source of Variation	Degree of Freedom	Visual Score Mean Square	Visual Score F Value	Visual Score Pr > F	Chlorophyll Concentration Mean Square	Chlorophyll Concentration F Value	Chlorophyll Concentration Pr > F
			Pride B216 x A15				
Rep/Period	2	0.80	5.04	0.0072	11.93	18.88	0.0001
Period	1	57.75	240.60	0.0001	98.43	90.30	0.0001
Line	119	0.33	1.38	0.0401	1.74	1.60	0.0054
Line x Period	119	0.24	1.50	0.0045	1.09	1.73	0.0002
Error	235*	0.16			0.63		
			Anoka x A7				
Rep/Period	2	0.07	0.48	0.6205	1.17	1.01	0.3679
Period	1	333.50	980.88	0.0001	396.09	338.54	0.0001
Line	91	0.52	1.53	0.0219	4.39	3.75	0.0001
Line x Period	91	0.34	2.16	0.0001	2.25	1.93	0.0001
Error	182	0.16			1.17		

* Three degrees of freedom from missing data were eliminated.

Table 3. Means and ranges of visual chlorosis score and chlorophyll concentration ($\mu g/cm^2$) distributions for the Pride B216 x A15 and Anoka x A7 populations in the field and nutrient solution tests.

	Visual Score		Chlorophyll Concentration	
	Mean	Range	Mean	Range
Pride B216 x A15 population				
Field				
1993	2.49	1.83-4.50	4.92	1.24- 7.17
1994	2.20	1.00-3.50	7.52	4.41-11.25
Nutrient solution				
Period 1	3.43	2.25-4.25	2.36	1.03- 4.65
Period 2	2.65	1.50-3.25	3.26	1.64- 5.27
Anoka x A7 population				
Field				
1993	2.38	1.50-3.67	5.18	2.55- 7.15
1994	2.30	1.00-3.83	8.41	3.56-11.90
Nutrient solution				
Period 1	3.51	2.25-4.75	3.69	1.42- 6.67
Period 2	1.61	1.00-2.50	5.77	2.61- 9.78

Table 4. Intervals significantly associated with variations for visual scores and chlorophyll concentrations at V2 growth stage in the Pride B216 x A15 population.

Visual Score					Chlorophyll Concentration				
Interval	Linkage Group	R^2 (%)	LOD Value	Parental Contrib.	Interval	Linkage Group	R^2 (%)	LOD Value	Parental Contrib.
Period 1									
A118-A702	B1	14.6	2.3	A15	A118-A702	B1	15.8	2.9	A15
					A519-Satt63	B2	8.8	2.0	A15
					Satt12-T36	G	9.5	2.6	A15
					A404-B69	H	20.6	2.2	A15
					Mng456-K418	N	21.8	2.9	A15
					Multiple loci		52.7		
Period 2									
A404-B69	H	18.4	3.1	A15	K636-A505	A2	20.1	3.0	A15
Mng456-K418	N	14.9	2.1	A15	A118-A702	B1	20.6	2.1	A15
Multiple loci		27.4			A404-B69	H	23.0	3.5	A15
					Mng456-K418	N	21.3	3.9	A15
					Multiple loci		55.5		
Periods 1 & 2 combined									
Satt12-T36	G	11.3	2.7	A15	A118-A702	B1	19.6	2.3	A15
Mng456-K418	N	21.6	3.7	A15	A519-Satt63	B2	12.1	3.0	A15
Multiple loci		28.0			Satt12-T36	G	9.6	2.6	A15
					A132-A461	L	16.3	2.7	A15
					Mng456-K418	N	21.7	3.8	A15
					Multiple loci		50.9		

Table 5. Intervals significantly associated with variations for visual scores and chlorophyll concentrations at V2 and V4 growth stages in the Anoka x A7 population.

Visual Score					Chlorophyll Concentration				
Interval	Linkage Group	R^2 (%)	LOD Value	Parental Contrib.	Interval	Linkage Group	R^2 (%)	LOD Value	Parental Contrib.
V2 stage of period 1									
BLT15-Sat33	N	28.7	5.9	A7	A515-K644	I	29.4	2.9	Anoka
					BLT15-Sat33	N	23.5	5.0	A7
					Multiple loci		35.3		
V2 stage of period 2									
BLT15-Sat33	N	14.8	2.2	A7	A519-A148	B2	11.7	2.3	Anoka
V2 stage of periods 1 & 2 combined									
BLT15-Sat33	N	34.7	6.2	A7	BLT15-Sat33	N	19.2	2.8	A7
V4 stage of period 1 (visual score only)									
K258-A256	A1	54.2	3.8	Anoka					
A515-K644	I	68.8	4.6	Anoka					
BLT15-Sat33	N	82.9	12.0	A7					
Multiple loci		92.9							
V4 stage of period 2									
BLT15-Sat33	N	40.4	5.4	A7	BLT15-Sat33	N	39.5	5.2	A7
V4 stage of periods 1 & 2 combined									
K258-A256	A1	28.4	4.0	Anoka					
A515-K644	I	66.3	3.9	Anoka					
BLT15-Sat33	N	82.0	13.2	A7					
Multiple loci		85.3							

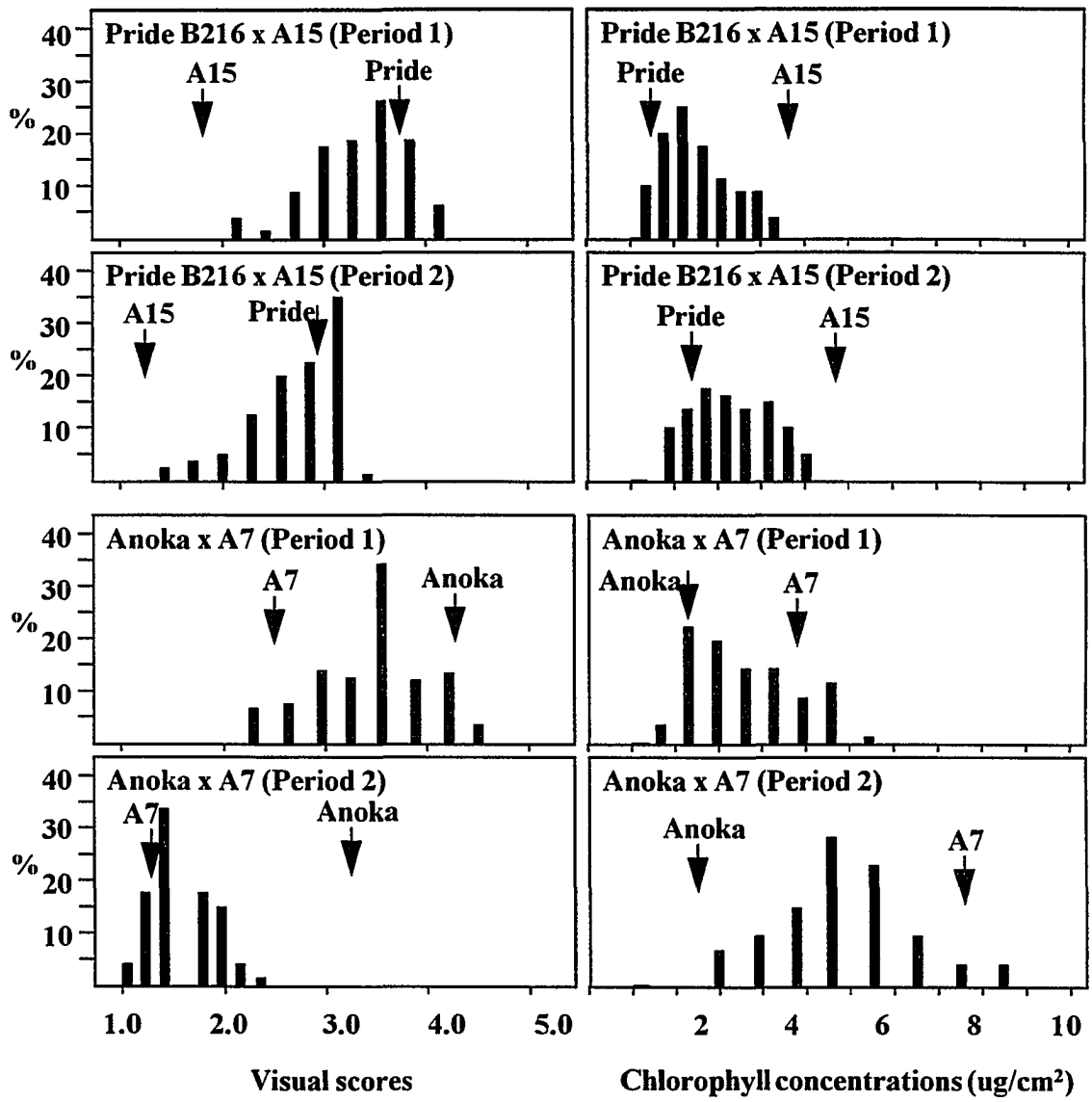


Figure 1. Frequency distributions of visual scores and chlorophyll concentrations at V2 stage averaged over replications in the Pride B216 x A15 and Anoka x A7 populations.

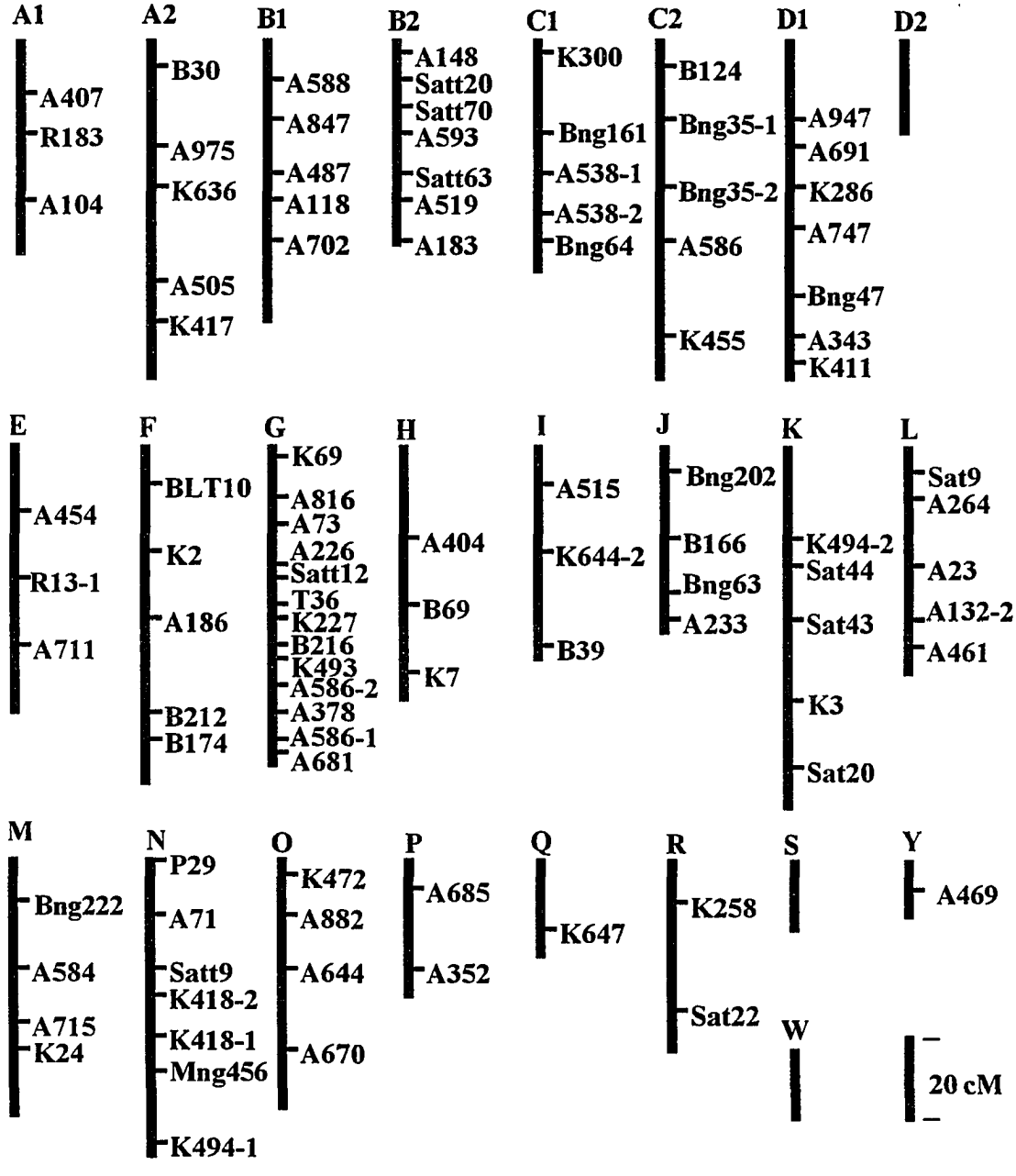


Figure 2. Distribution of molecular markers used to map QTL for iron deficiency chlorosis in the Pride B216 x A15 population.

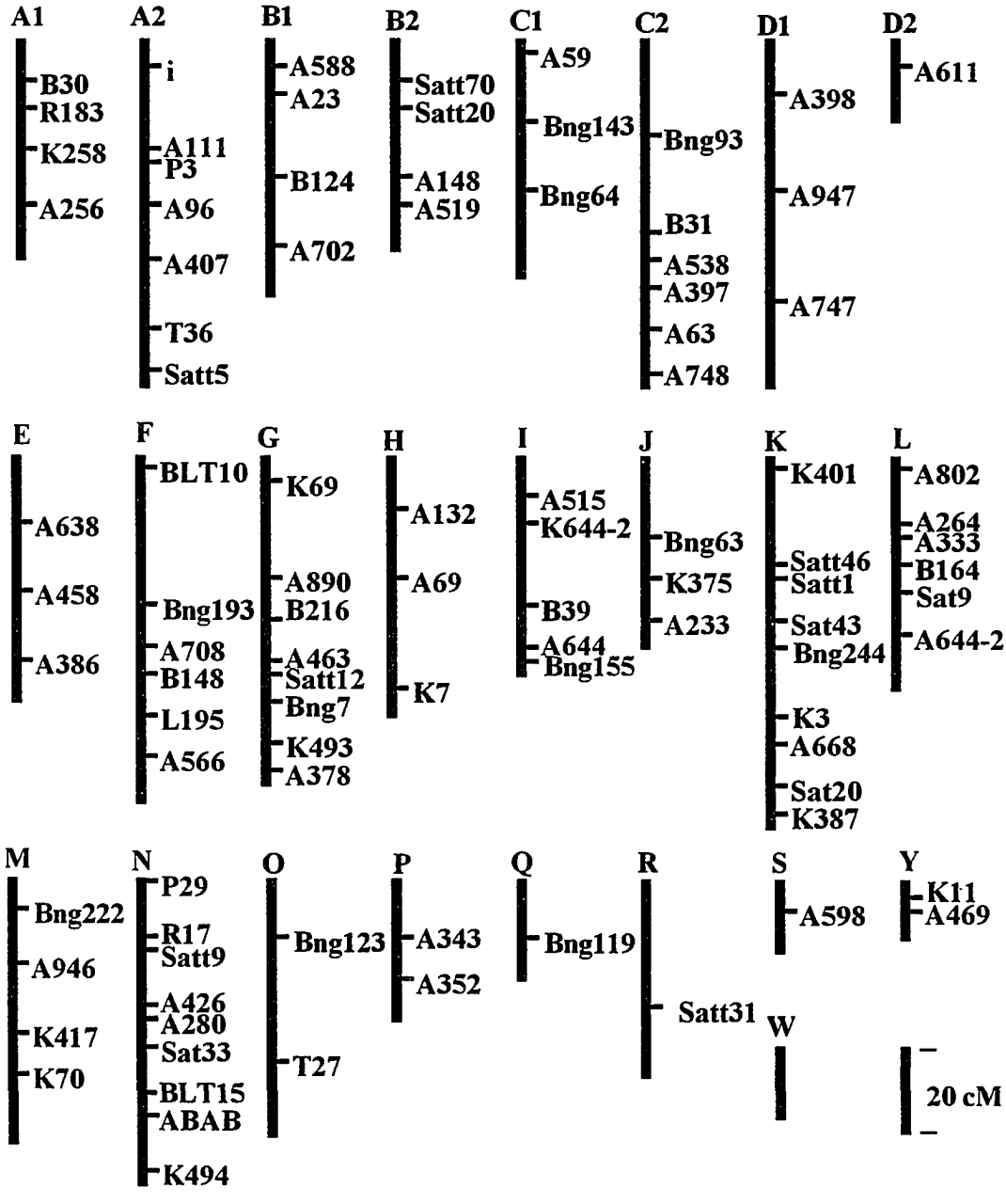


Figure 3. Distribution of molecular markers used to map QTL for iron deficiency chlorosis in the Anoka x A7 population.

Figure 4. Locations of detected QTL for iron deficiency chlorosis from field (left side of the linkage group) (Lin et al., submitted) and nutrient solution (right side of the linkage group) tests in the Pride B216 x A15 population. The straight lines and arrows beside the linkage groups show the confidence intervals and locations of detected QTL, respectively.

Field tests:
a = visual scores at V4 stage in 1993
b = visual scores at V2 stage in 1994
c = visual scores at V4 stage in 1994
d = combined data of visual scores at V4 stages in 1993 & 1994
e = chlorophyll concentrations at V4 stage in 1993
f = chlorophyll concentrations at V2 stage in 1994
g = chlorophyll concentrations at V4 stage in 1994
h = combined data of chlorophyll concentrations at V4 stages in 1993 & 1994

Nutrient solution tests:
j = visual scores at V2 stage in Period 1
k = visual scores at V2 stage in Period 2
l = combined data of visual scores at V2 stages in Periods 1 & 2
m= chlorophyll concentrations at V2 stage in Period 1
n = chlorophyll concentration at V2 stage in Period 2
o = combined data of chlorophyll concentrations at V2 stages in Periods 1 & 2

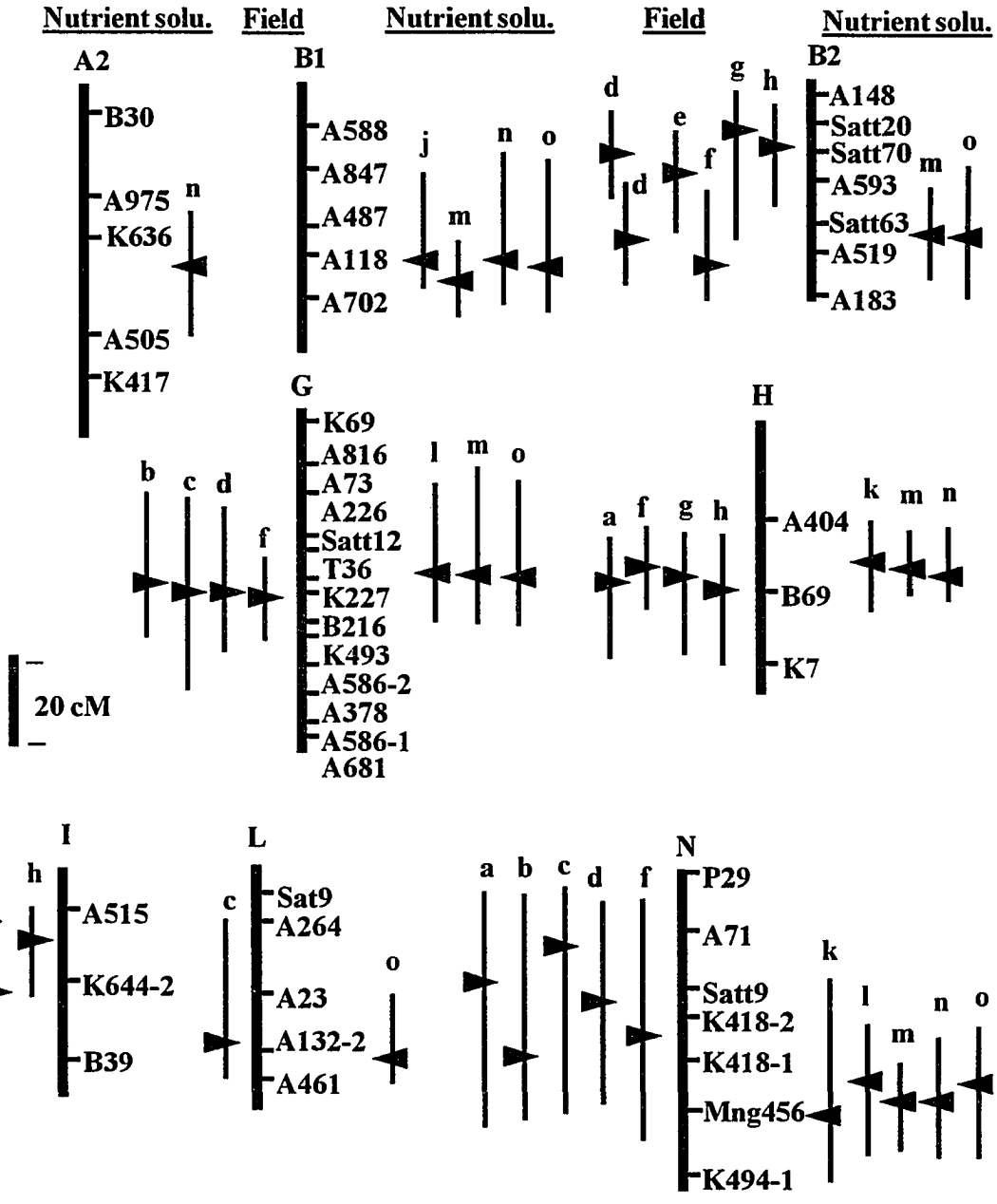
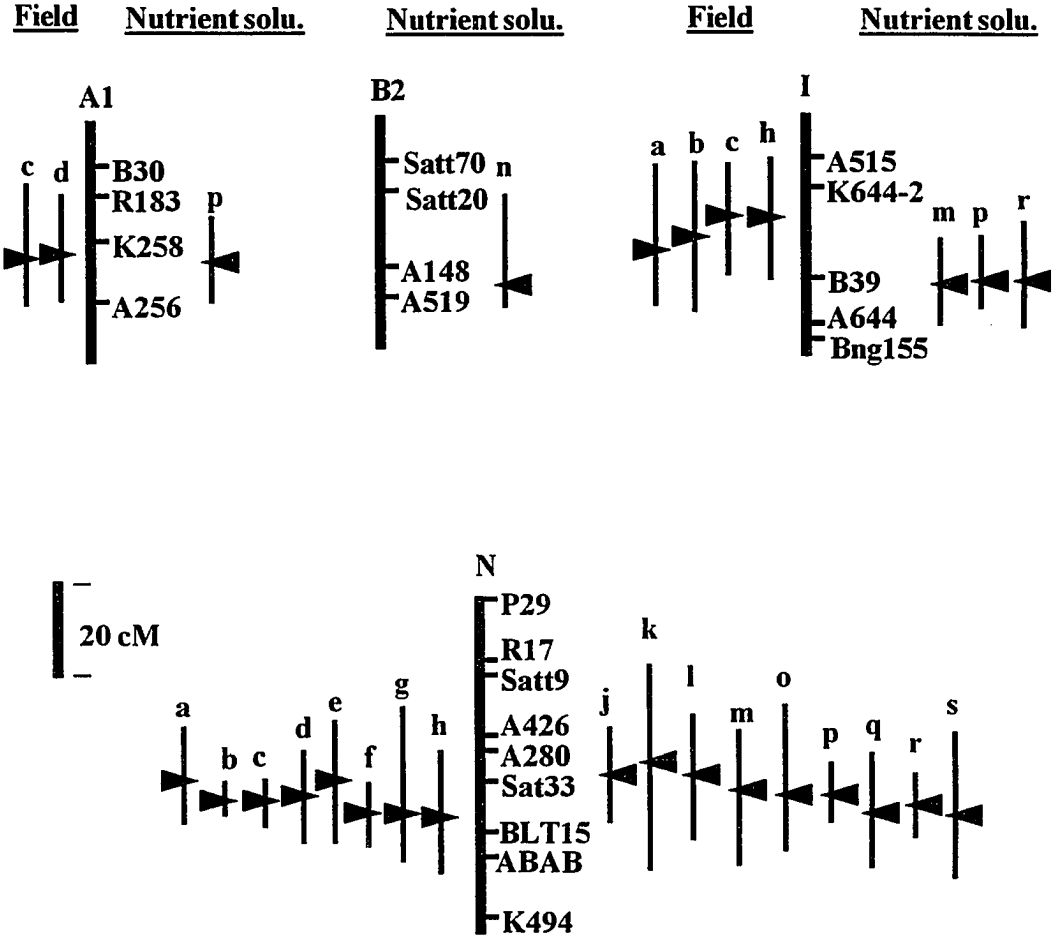

Nutrient solu.
Field
Nutrient solu.
Field
Nutrient solu.
A2
B30
A975
K636
A505
K417
n
B1
A588
A847
A487
A118
A702
j
m
n
o
d
d
e
f
g
h
B2
A148
Satt20
Satt70
A593
Satt63
A519
A183
m
o
G
K69
A816
A73
A226
Satt12
T36
K227
B216
K493
A586-2
A378
A586-1
A681
b
c
d
f
l
m
o
H
A404
B69
K7
a
f
g
h
k
m
n
20 cM
I
A515
K644-2
B39
f
h
L
Sat9
A264
A23
A132-2
A461
c
o
N
P29
A71
Satt9
K418-2
K418-1
Mng456
K494-1
a
b
c
d
f
k
l
m
n
o

Figure 5. Locations of detected QTL for iron deficiency chlorosis from field (left side of the linkage group) (Lin et al., submitted) and nutrient solution (right side of the linkage group) tests in the Anoka x A7 population. The straight lines and arrows beside the linkage groups show the confidence intervals and locations of detected QTL, respectively.

Field tests:
a = visual scores at V4 stage in 1993
b = visual scores at V2 stage in 1994
c = visual scores at V4 stage in 1994
d = combined data of visual scores at V4 stages in 1993 & 1994
e = chlorophyll concentrations at V4 stage in 1993
f = chlorophyll concentrations at V2 stage in 1994
g = chlorophyll concentrations at V4 stage in 1994
h = combined data of chlorophyll concentratins at V4 stages in 1993 & 1994

Nutrient solution tests:
j = visual scores at V2 stage in Period 1
k = visual scores at V2 stage in Period 2
l = combined data of visual scores at V2 stages in Periods 1 & 2
m= chlorophyll concentrations at V2 stage in Period 1
n = chlorophyll concentration at V2 stage in Period 2
o = combined data of chlorophyll concentrations at V2 stages in Periods 1 & 2
p = visual scores at V4 stage in Period 1
q = visual scores at V4 stage in Period 2
r = combined data of visual scores at V4 stages in Periods 1 & 2
s = chlorophyll concentrations at V4 stage in Period 2


Field
Nutrient solu.
Nutrient solu.
Field
Nutrient solu.
A1
c
d
B30
R183
p
K258
A256
B2
Satt70
n
Satt20
A148
A519
I
a
b
c
h
A515
K644-2
m
p
r
B39
A644
Bng155
20 cM
N
P29
R17
Satt9
A426
A280
Sat33
BLT15
ABAB
K494
a
b
c
d
e
f
g
h
j
k
l
m
o
p
q
r
s

GENERAL CONCLUSION

The objectives of this study were to map genes affecting iron deficiency chlorosis in soybean, to test the hypothesis that two genetic mechanisms control iron deficiency chlorosis in soybean, and to determine the degree of similarity in genetic mechanisms controlling soybean iron deficiency chlorosis between field and nutrient solution tests.

One hundred and twenty $F_{2:4}$ lines in Pride B216 x A15, and 92 $F_{2:4}$ lines in Anoka x A7 populations were evaluated by visual scores and spectrometric chlorophyll determinations in a field of calcareous soil in 1993 and 1994. Each of the Anoka x A7 and the Pride B216 x A15 populations also was separately evaluated with two periods (runs) of nutrient solution tests conducted in greenhouse conditions. Eighty-nine RFLP and ten SSR markers in the Pride B216 x A15 population, and 82 RFLP, 14 SSR and *I* (hilum color) markers in the Anoka x A7 populations were used to construct linkage maps and to locate quantitative trait loci (QTL).

In the first paper of this dissertation, one QTL contributed an average of 72.2% of the visual score variation and 68.8 % of the chlorophyll concentration variation and was mapped on linkage group N of the Anoka x A7 population. Due to the large phenotypic contribution (R^2) and reliability (LOD value) for the QTL on linkage Group N, quantitative data were reclassified according to the means of the QTL genotypic classes and transformed into qualitative data fitting

a major gene model. The putative major gene was mapped in the same interval of linkage group N for both visual scores and chlorophyll concentrations, thus verifying that one major gene is involved in the Anoka x A7 population. One other QTL for visual score variation, and another for chlorophyll concentration variation were mapped on linkage groups A1 and I, respectively. Accordingly, the segregation for iron deficiency chlorosis in the Anoka x A7 population supported a single major gene with modifying gene mechanism, which previously was proposed by Cianzio and Fehr (1980).

QTL affecting visual scores and/or chlorophyll concentrations were detected on linkage groups B2, G, H, I, L, and N of the Pride B216 x A15 population at different growth stages or different years. No QTL were detected with large gene effects (R^2) or high reliability (LOD value) in the Pride B216 x A15 population, supporting the hypothesis that a typical polygene mechanism, previously proposed by Cianzio and Fehr (1982), controls IDC in this population.

This study has verified that two genetic mechanisms control iron deficiency chlorosis in soybean. These data suggest that in the development of breeding programs to select for iron efficient cultivars of soybean, strategies to exploit the genetic elements involved in two genetic mechanisms should be considered. The discovery of a major gene may contribute information for the genetic improvement of this trait and provide starting material for map-based cloning of a gene controlling nutrient efficiency in a higher plant.

In the second paper of this dissertation, due to significant interaction between genotype and environment (G x E) in both field and nutrient solution tests, QTL identified from multiple environments were used to determine the degree of similarity between nutrient solution and field tests. In the Anoka x A7 population, one major gene on linkage group N, and QTL on linkage groups A1 and I previously mapped during field tests also were identified in the nutrient solution test. One newly identified QTL was mapped on linkage group B2. In the Pride B216 x A15 population, one QTL previously mapped on linkage group I during the field tests was not identified in the nutrient solution tests, and two newly identified QTL were separately mapped on linkage groups A2 and B1. QTL on linkage groups B2, G, H, L, and N were identified in both field and nutrient solution tests. The nutrient solution and field tests demonstrated high similarity in the genetic mechanisms for iron deficiency chlorosis of soybean. Therefore, this study has provided evidence at the molecular level indicating that the efficiency of breeding iron efficient cultivars of soybean can be increased by using nutrient solution evaluation and makes possible the application of marker facilitated selection of iron efficient genotypes.

This dissertation has presented the basic information to dissect and manipulate the genetic factors controlling iron efficiency in soybean. The

possibility of increasing breeding efficiency for the trait through combined use of field and nutrient solution evaluations, and marker-assisted selection was discussed.

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