QTL MAPPING OF IRON DEFICIENCY CHLOROSIS TOLERANCE IN SOYBEAN USING CONNECTED POPULATIONS

A THESIS
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA
BY

ILENE LOUISE JONES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

MARCH 2014

ACKNOWLEDGEMENTS

I would like to express my appreciation to Dr. Jim Orf for sharing his great depth of knowledge of soybean breeding as well as for his valuable advice navigating through graduate school. I would also like to thank my committee members Dr. Kevin Smith and Dr. Nevin Young for their technical reviews of my thesis work, which have pushed me to further reflect on difficult concepts within my project. Special thanks to Lynne Medgaarden for her kindness and additional support navigating through graduate school. A huge debt of gratitude to Addie Thompson, Liana Nice, and fellow graduate students for their support. Thank you to professors from the Summer Institute of Statistical Genetics, Dr. Ron Mowers, Dr. Elhan Ersoz, Dr. Nicolas Martin, Dr. Suresh Kadaru, Dr. Harish Gandhi, Dr. Joe Curly, Dr. Zhigang Guo, and Dr. Chris Basten for providing invaluable guidance on statistical genetics and data analysis. Lastly, I would like to thank Syngenta for both granting me the opportunity to continue my education during employment and for funding my education and research.

DEDICATION

I would like to dedicate this thesis to my mostest Matthew and to my bestest pals BK and LK with love. Your tough love and support provided the necessary motivation to conquer my greatest fear.

ABSTRACT

Soybean iron deficiency chlorosis or IDC is a yield limiting, abiotic stress condition common to calcareous soil types present in the Upper Midwest. Complex interactions among soil chemical and physical properties within these calcareous soils limit the amount of ferrous iron available to soybean plants. The subsequent nutrient deficiency leads to the classic chlorotic phenotype characterized by interveinal yellowing of new growth trifoliates. IDC is responsible for yield losses up to 0.8 Mg ha^{-1} amounting to an estimated economic loss of $120 million per annum. To mitigate yield losses, growers prefer to plant IDC tolerant cultivars; however, IDC tolerant cultivars have been known to yield less on non-chlorotic soils. In order to improve IDC tolerance without an associated reduction in yield, we evaluated yield and IDC performance using a network of 13 F_4-derived recombinant inbred line (RIL) populations connected by common parents. Chlorosis severity was evaluated using two methods: visual chlorosis ratings and remote sensing via normalized difference vegetative index (NDVI) values collected from the GreenSeeker® RT100 System. NDVI values correlated strongly with visual chlorosis ratings with the largest negative Pearson's correlation coefficient of -0.89 (p-value < 0.0001) captured at the V4 growth stage. NDVI values collected at V4 were moderately correlated to yield with a Pearson's correlation coefficient of -0.61 (p-value < 0.0001), indicating that IDC tolerant lines yield less than IDC susceptible lines on non-chlorotic soils. Co-localization of IDC and yield QTL detected on linkage groups A1/5, J/16, and L/19 confirm that the correlations are in part due to genetically linked loci or pleiotropic effects of a single locus.

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

According to archeological records, soybean (*Glycine max* L. Merr.) was domesticated in China roughly 3,100 years ago (Bray, 1984). Soybeans were first introduced to the United States in 1765 by Samuel Bowen, and since then, U.S. soybean hectarage has steadily increased with much of the increase taking place in the 20th century (Hymowitz and Shurtleff, 2005). Soybean is now the leading oilseed crop in the United States, accounting for approximately 90% of U.S. oilseed production (USDA Economic Research Service - Background). In 2011, 30 million hectares were planted in the U.S., producing an estimated crop value of 35 billion dollars. This amount of hectarage is second only to corn. Globally, soybeans accounted for 56% of the world's oilseed production in 2011.

The versatility of soybean is demonstrated by the variety of products derived from it. Products such as cooking oil, margarine, soy sauce, tofu, chocolate soy milk, soybean meal, biodiesel, soybean plastics, lecithin, etc. continue to drive demand for soybean hectares within the U.S. and Globally thus accentuating the economic importance of this crop.

Iron Deficiency Chlorosis and Associated Yield Reductions

Greater than 80% of U.S. soybean production occurs in the upper Midwest. A portion of the agricultural production areas in the upper Midwest is prone to a yield-limiting, abiotic stress known as iron deficiency chlorosis. IDC is associated with calcareous soils or soils composed of free carbonates. The free carbonates buffer the soil solution so that high soil pH is maintained. The alkalinity of the soil limits the amount of iron biologically available to plants, leading to the notable phenotype of interveinal yellowing of newly developed leaves, stunted growth, and subsequent yield loss. Iron deficiency chlorosis induced on calcareous soils is sometimes referred to as lime-induced chlorosis.

Although lime-induced chlorosis is not exclusive to soybean, soybean is particularly sensitive to iron deficiency stress (Clark, 1982). Hansen et al. (2003)

conducted a survey to assess the extent to which soybean producers are affected by iron deficiency chlorosis. The survey was completed by 79 soybean producers in west-central and southwest Minnesota. In this survey, 99% of the soybean producers indicated that iron deficiency chlorosis was a major production issue. Producers estimated 24% of their soybean crop was affected by iron chlorosis, generating an estimated 0.8 Mg ha^{-1} yield loss per annum. In a later review, Hansen et al. (2004) noted a 160% increase in soybean production area into regions with soil pH of 7.2 or greater from 1979 to 2002. This increase of soybean production area into iron deficiency prone regions has led to yield losses of 340 Mt, worth an estimated $120 million dollars per year. Current production trends are expected to continue, thus limiting or eliminating yield losses due to chlorosis is critical.

Soil Physical and Chemical Factors Leading to Iron Deficiency Chlorosis

Decades of research have been conducted to better understand the soil and environmental properties associated with iron deficiency chlorosis in soybean. Collectively, these studies outline a complex relationship between iron solubility and several soil factors including, but not limited to, the following: soil moisture, soluble salt, calcium carbonate ($CaCO_3$), bicarbonate (HCO_3^-), and nitrate (NO_3^-) content, and soil pH. While all of these soil factors have been implicated with iron deficiency chlorosis, not all have been consistently associated with chlorosis in both field and nutrient solution experiments. Lime-induced chlorosis can be spatially and temporally variable. There can be year to year field variation and even within season field variation. It is also common to see consistent soils types across chlorotic and adjacent nonchlorotic regions within a field. In order to identify the cause of iron deficiency chlorosis, these soil and environmental properties and their complex interactions have been researched extensively. The conclusions from these studies are presented below.

In the Hansen et al. (2003) survey, soybean producers identified soil pH to be one of the most significant factors causing iron deficiency chlorosis. To substantiate this claim,

Hansen et al. (2003) sampled soils from chlorotic, moderately chlorotic, and nonchlorotic regions of fields from 60 sampling sites. Soil pH values ranged from 7.0 to 8.3. Despite a sizeable range in pH values across sampling sites, no significant relationship was found among chlorotic, moderately chlorotic, and nonchlorotic field positions. Prior to the study conducted by Hansen et al. (2003), Inskeep and Bloom (1984) similarly evaluated soil samples from transects across chlorotic and nonchlorotic areas of fields. Their studies showed only slight differences in pH between chlorotic and nonchlorotic locations. Another study conducted by Franzen and Richardson (2000) concluded that soil pH was significantly correlated to iron chlorosis in just 6 of 20 locations.

Conclusions from these studies seemingly lack evidence to decisively support high pH as a causative factor for inducing chlorosis; however, iron deficiency chlorosis occurs mostly in calcareous and alkaline soils with pH values of 7.0-8.5 and >8.5, respectively (Masrchner, 2012). All soil samples collected in the above mentioned experiments were within this pH range. In an oxidized, calcareous nutrient solution with a relatively high pH of 8.3, the concentration of soluble iron is 10^{-10} M, which is far below the critical level of $10^{-7.7}$ M that soybeans require for optimum growth (Lindsay and Schwab, 1982). Within the pH range of 7.4 and 8.5, iron solubility decreases exponentially for each unit increase in pH (Lindsay, 1984). This means that even though iron is quite abundant in these calcareous soils, it is stored in inorganic iron species unavailable to the soybean plants.

The predominant species of inorganic iron in calcareous soils are $Fe(OH)_3$, $Fe(OH)_2^+$, and $Fe(OH)^{4-}$. Ferric hydroxide ($Fe(OH)_3$) is the most soluble form of amorphous iron hydroxides due to its poor crystalline structure. Since dissolution of iron oxide occurs as a surface reaction, crystallinity of iron oxide as well as particle size and reactive surface area all influence the amount of iron available to plants (Loeppert, 1986). Morris et al. (1990) observed that DTPA-extractable iron significantly correlated ($r = 0.82$) with the quantity of amorphous or reactive iron oxide in the soil. In their study, chlorophyll content was also positively correlated with an iron oxide parameter that included amorphous iron content. Likewise, Hansen et al. (2003) observed higher concentrations of DTPA-extractable iron in nonchlorotic areas. Similarly, Franzen and Richardson (2000)

also found that low levels of DTPA-Fe were significantly correlated to iron chlorosis in 10 locations.

Soil pH is affected by several factors including the amount of bicarbonate (HCO_3^- present in the soil. In several published studies, increased soil bicarbonate levels have been associated with incidence of iron deficiency chlorosis. Inskeep and Bloom (1984) measured soil physical and chemical properties from chlorotic, nonchlorotic, and transition areas of the field. In their study, lower concentrations of chlorophyll were associated with higher levels of HCO_3^- at 1 of their 3 sampling sites. They also observed that total calcium bicarbonate ($CaCO_3$) content correlated well with severe chlorotic areas. In another study, Bloom and Inskeep (1986) evaluated soil samples from the field, and also observed higher HCO_3^- concentrations in chlorotic areas of the field. Similarly, Coulombe et al. (1984) observed that increasing concentrations of HCO_3^- increased severity of chlorosis more than any other treatment in their nutrient solution experiments.

Just as soil pH is affected by several factors including concentrations of HCO_3^-, soil HCO_3^- concentrations are also highly dependent on soil chemical and physical properties. In the Bloom and Inskeep (1986) study, they conducted field and growth chamber experiments designed to investigate the relationship between partial pressure of carbon dioxide (pCO_2) in wet soils and HCO_3^- levels and their effects on iron chlorosis. They concluded that increases in soil moisture content decreases the air filled porosity of the soil, which in-turn increases pCO_2 and HCO_3^- concentrations, leading to a greater severity of chlorosis as measured by both visual ratings and total chlorophyll leaf concentrations. With increases in soil moisture, CO_2 dissolves to form carbonic acid (H_2CO_3), which will form HCO_3^- in equilibrium concentrations (Eq. 1) (Lucena, 2000).

$$CO_2 + H_2O \longleftrightarrow H_2CO_3 \text{ (acid)} \longleftrightarrow HCO_3^- \text{ (strong base)} + H^+ \quad \text{[Eq. 1]}$$

$$CaCO_3 \text{ (base)} + H^+ \longleftrightarrow HCO_3^- \text{ (weak acid)} + Ca^{2+} \quad \text{[Eq. 2]}$$

In calcareous soils, the dissolution of $CaCO_3$ into HCO_3^- occurs at pH 8.34 with atmospheric CO_2 levels of 300 ppm (Eq. 2) (Loeppert, 1986). In the first chemical equation, HCO_3^- acts as a strong base, but in the second chemical equation, HCO_3^-acts as a weak acid (Lucena, 2000). This amphoteric behavior of bicarbonate buffers the soil solution to maintain a high pH range, and, therefore, inhibits the ability of the plant to reduce Fe^{3+} to Fe^{2+}. This causes iron deficiency and subsequent chlorosis.

In addition to HCO_3^- sensitivity, nitrate (NO_3^-) can also exacerbate iron deficiency chlorosis. Wiersma (2010) conducted a study to examine the effects of different nitrogen fertilizer rates on two iron efficient, two moderately iron efficient, and two iron inefficient cultivars grown on calcareous soil. Wiersma observed that visual nodulation scores and relative chlorophyll concentrations declined linearly with increased nitrogen rates in all cultivars. Plant height, seed number, and grain yield also decreased greatly with increases nitrogen rate in iron inefficient cultivars. Iron efficient cultivars experienced no significant decrease in plant height, seed number, and grain yield.

Bloom et al. (2011) also conducted experiments investigating the effects of NO_3^- on chlorosis. In his first experiment, Bloom investigated factors associated with the phenomenon of decreased chlorosis expression in tractor wheel tracks found in chlorotic fields. The results of the first experiment showed significantly lower NO_3^- concentrations were observed in leaflet samples collected within the green wheel tracks in all 8 field locations that were tested. Significantly lower soil NO_3^- concentrations were also observed within the green wheel tracks of 7 out of the 8 locations. Bloom hypothesizes that the decreased soil NO_3^- concentration observed in compact soils during the early portion of the season is due to increased denitrification. Early season warm weather and increased soil moisture leads to greater denitrification, reducing the overall soil NO_3^- concentrations and thus IDC severity. However, if high moisture levels are maintained at the time of plant emergence, HCO_3^- concentrations will increase via the system of interactions described above and chlorosis will ensue.

Based on the results of the first experiment, Bloom et al. (2011) sought to examine the effects of oat as a companion crop to reduce IDC symptoms by taking up the excess

NO_3^- levels in the soil. Bloom observed higher soybean yields when oats were sowed as a companion crop with the stipulation that soil moisture was not a limiting factor. Oats reduced IDC symptoms by (1) reducing soil moisture content and, therefore, HCO_3^- levels and by (2) taking up the excess NO_3^- levels.

Other soil chemical properties have been associated with incidence of IDC to a lesser extent. High soil soluble salt content as measured by electrical conductivity (EC) has been found to negatively correlate with total chlorophyll content (Franzen and Richardson, 2000; Hansen et al., 2003; Morris et al., 1990). Higher concentrations of mobile ions and insoluble salts are often present around chlorotic rims of depressions in chlorosis prone fields (Inskeep and Bloom, 1984). Increased concentrations of K, P, Ca, Mg, Mn, Cu, Cd, Ni, Zn, and B have all been associated with chlorosis is soybeans, but like other soil chemical properties, correlations have not been consistent (Fleming et al., 1984; Hansen et al., 2003; Inskeep and Bloom, 1984, 1987; Morris et al., 1990).

Physiological Response to Iron Deficiency Chlorosis

To acquire the necessary amounts of iron from the environment, plants have evolved two strategies (Strategy I and Strategy II) that are phylogenetically distinct. Strategy II plants include gramenaceous species. Strategy II involves excretion of phytosiderophores that bind to the insoluble form of ferric iron (Fe^{3+}) creating a soluble complex that Strategy II plants can uptake (Marschner et al., 1986; Marschner and Römheld, 1994). Strategy I plants include dicots like soybeans and non-gramenaceous species. Strategy I plants employ a reduction mechanism that reduces chelated Fe^{3+} to the soluble, biologically available form of iron (Fe^{2+}) (Schmidt, 1999). Specifically, when Strategy I plants are under iron stress, the roots of the plants will respond to the deficiency by (1) acidifying the rhizosphere through excretion of protons from H^+-ATPases, (2) reducing chelated Fe^{3+} to Fe^{2+} mediated by plasma membrane ferric (chelate) reductases, and (3) transferring soluble Fe^{2+} across the plasma membrane and into the cytoplasm via divalent iron transporters.

Strategy I plants are particularly sensitive to additions of HCO_3^-, unlike Strategy II plants. Lucena et al. (2007) conducted several experiments to evaluate expression levels of several genes controlling iron acquisition in Strategy I species in response to additions of HCO_3^-. When HCO_3^- was added to a growth media deficient of iron, expression levels of known iron acquisition genes controlling root responses decreased and chlorosis was induced in Arabidopsis (*Arabidopsis thaliana)*, pea (*Pisum sativum L.*), tomato (*Lycopersicon esculentum*), and cucumber (*Cucumis sativus L*). Conclusions from this study show that HCO_3^- directly induces chlorosis in Strategy I plants by inhibiting expression of genes encoding H^+-ATPases, ferric (chelate) reductases, and iron transporters as well as iron efficiency reaction (FER) (or FER-like) transcription factors that upregulate the previously mentioned iron acquisition genes.

The mechanism for which NO_3^- induces chlorosis is still unclear. Lucena (2000) proposes that the electrons exuded by the ferric (chelate) reductases reduce targets other than Fe^{3+} first. The donated electrons first reduce O_2 to H_2O. When the O_2 is depleted, then NO_3^- is reduced to NH_4^+ (ammonia). When all the nitrogen has been reduced to ammonia, then Fe^{3+} can be reduced to Fe^{2+}. Bloom (2011) also presents a summary of two additional mechanisms proposed by other scientists for which NO_3^- induces chlorosis. The first mechanism proposes that excess NO_3^- uptake results in a release of HCO_3^- from the roots into the soil to maintain a balanced charge. The release of HCO_3^- into the rhizosphere induces chlorosis for explanations formally presented. The second mechanism involves a pH increase in the leaf apoplastic fluid due to an influx of NO_3^-, which interferes with the reduction of Fe^{3+} and iron transport. Further research is needed to determine exactly how NO_3^- intensifies chlorosis.

Genetic Control of Iron Deficiency Chlorosis

Lime-induced chlorosis is a complex trait, [JJ1] whereby multiple loci typically having small effects are responsible for a range of tolerant and susceptible phenotypes (Charlson et al., 2003, 2005; Lin et al., 1997, 2000; O'Rourke et al., 2009; Severin et al.,

2010; Wang et al., 2008). Early experiments studying the genetic control of iron deficiency chlorosis report that IDC is controlled by a single major gene (Cianzio and Fehr, 1980; Weiss, 1943). Weiss (1943) reported a dominance/recessive gene model, where iron efficiency is dominant over iron deficiency. Cianzio and Fehr (1980) likewise confirmed a single major gene model but additionally observed quantitative inheritance patterns, which indicated that IDC is also controlled by modifying genes. Fehr (1982) successively determined that IDC is quantitatively inherited. Subsequent studies conducted by Lin (1997, 2000) confirmed that IDC displayed both single codominant gene and polygenic inheritance patterns in Anoka x A7 and Pride B2/1412 x A1/55 mapping populations, respectively. A summary of QTLs identified in the literature over the past two decades support that iron deficiency chlorosis tolerance and susceptibility is controlled by multiple genes (Table 1). The research conducted to elucidate the physiological response of Strategy I plants to iron deficiency conditions supports a polygenic model as well (Marschner and Römheld, 1994; Marschner et al., 1986).

Quantitative traits like IDC are difficult traits for breeders to improve via traditional phenotypic selection methods due to the inability of a breeder to effectively select and stack numerous favorable alleles that confer IDC tolerance. To improve quantitative trait performance for traits such as IDC, use of marker assisted breeding methods should be employed. One of the challenges of using markers to select for quantitative trait loci, is the ability to first identify the markers that are associated with IDC tolerance. Successful identification of IDC loci having small effect is complicated by fluctuating environmental conditions that lead to temporal and spatial phenotypic variation in the field. Typically, these types of environmental variances are difficult to account for, even in carefully designed and well-replicated experiments. This ultimately leads to larger experimental error variance and lower heritability. Due to heterogeneous soil factors and presence of genotype x environment (GxE) interactions, it is difficult to distinguish genotypic sources of variation from the environmental components responsible for chlorosis (Froechlich and Fehr, 1981; Naeve and Rehm, 2006).

QTL Detection Methods

To effectively detect marker trait associations in complex traits such as IDC, large population sizes (>400) are required in order to provide adequate power for consistent detection of QTL having small effects (Bernardo, 2004). The QTL studies listed in Table 1 all have population sizes of no greater than 150. These smaller population sizes have limited power to detect minor effect QTL typical of a quantitative trait. Large population sizes required to detect small effect QTL are not attractive to breeders as they prefer to breed with not few but many smaller sized breeding populations. Breeding with many smaller populations allows the breeder to sample and recombine the many sources of genetic variation available in their germplasm collections. One drawback of using bi-parental populations for QTL mapping is that only a small portion of the genetic variation available to the breeders is sampled. Frequently, QTLs detected in one population are not detected in other populations, or QTLs detected in one population fail to exhibit the same or similar effect when validated in other genetic backgrounds.

Multi-parental linkage-based QTL mapping methods have been developed to solve the failure to detect QTL due to the QTL being monomorphic in a single mapping population. The failure to detect a QTL that is monomorphic in a mapping population is known as "genetic drift error" (Xu, 1996). By analyzing multiple independent bi-parental mapping populations jointly, one samples more genetic variation and thus increases the likelihood that a QTL will be polymorphic in one or more bi-parental mapping population(s). One caveat of analyzing the disconnected mapping populations jointly is that the QTL effects are nested (in a statistical sense) within populations (Xu, 1996). Because the effects are nested within populations, allelic effects from QTLs discovered in one population cannot not be directly compared to allelic effects of QTLs discovered in another. Consequently, one cannot rank alleles or estimate the number of alleles segregating at a locus.

(Blanc et al., 2006) developed a method that resolves the issue of QTLs nested within populations by analyzing a network of connected populations rather than analyzing

multiple disconnected populations. When a network of connected populations with common parents is analyzed using the methods described in Blanc et al. (2006), the relationships or connections among the parents are considered in the model. By doing so, allelic effects are estimated simultaneously, allowing for direct comparison of parental alleles. Alleles can be ranked, and the number of alleles segregating at a locus can be estimated.

Even more important than the ability to compare alleles across populations, is the additional increase of power that this model gains when parental connections are considered. The connected model reduces the number of parameters in the model compared to the disconnected model proposed by Xu (1996), increasing the power of the model to detect QTL (Jannink and Jansen, 2001; Rebai and Goffinet, 1993). For example, 3 bi-parental recombinant inbred line (RIL) populations genotyped with SNPs and analyzed using a disconnected model will have 6 unique alleles at a locus segregating across the populations (2 unique alleles within each population). In this example, these 3 populations are given a notation of AxB, BxC, and AxC. If those same bi-parental populations were analyzed using a connected model, that model would account for parental relationships, and the number of parameters or alleles considered in that model would be consolidated. For example, the AxB and the BxC populations have a common parent B. Likewise, the AxC and the BxC populations have parent C in common. Considering the parental relationships in the connected model reduces the number of alleles segregating across the populations from 6 down to 3 unique alleles. The reduction of alleles from 6 down to 3, increases the number of progeny classified into each genotype class at a locus, resulting in an increase of power to detect QTL.

Several other advantages of using a connected model exist, including the ability to detect QTL x background interactions (Blanc et al., 2006; Jannink et al., 2008). Additionally, because all markers across populations are included in the linkage map, map resolution increases as well. Combining all genotypic data (i.e. increasing the number of recombination events in the dataset) increases the precision of estimating the QTL position. The single most attractive aspect of this method, however, may be that the connected QTL

mapping population structure more closely represents the diversity of crosses a breeder would make in a conventional breeding program. The connected model allows a breeder to identify QTLs using many smaller sized populations networked together, resembling the structure of a breeding program more closely.

Thesis Objectives

The preferred method to mitigate the negative effects of iron deficiency chlorosis is selecting soybean cultivars with iron deficiency chlorosis tolerance. Previous studies have shown that IDC tolerant cultivars yield better than IDC susceptible cultivars on IDC prone soils; however, when grown on non-IDC prone soils, the IDC susceptible cultivars have higher yields than the IDC tolerant cultivars (Froechlich and Fehr, 1981). The heterogeneity of soil conditions leading to unpredictable iron chlorosis expression make cultivar selection a challenge for growers. In order to obtain optimal yields, growers must select cultivars appropriate for the soil conditions present on their farms, which can be very speculative given the complex nature of IDC expression. Therefore, when improving cultivars for better tolerance to IDC, it is equally important to ensure no negative effects on yield performance coincide with that increase in IDC tolerance.

Since IDC is a polygenic trait, the most effective method to improve soybean cultivars is through marker assisted selection. Markers associated to IDC tolerance can be used to select IDC tolerant cultivars in relevant germplasm. In order to improve IDC tolerance of current Syngenta soybean cultivars, four objectives were addressed in this thesis.

The first two objectives of this thesis aimed to detect quantitative trait loci significantly associated with (1) iron deficiency chlorosis tolerance and (2) yield performance, using both bi-parental and connected composite interval mapping models. The third objective assessed the ability of bi-parental and connected models to effectively detect QTL by comparing the number and location of QTL detected with each model. Lastly, because lower yields have been observed in IDC tolerant cultivars when grown on

non-calcareous soils, co-localization of yield QTL and IDC QTL were evaluated. Breeding strategies to improve Syngenta soybean cultivars for IDC tolerance will be determined based on whether the genetic correlation (as determined by co-localization of QTL) is positive or negative and whether or not the genetic correlation is due to linkage or potential pleiotropy.

MATERIALS AND METHODS

Population Development

Thirteen bi-parental recombinant inbred line (RIL) populations were developed by crossing eight elite inbred lines in various bi-parental combinations. All eight elite inbred lines were developed by Syngenta (Table 2). The 03DL052038 and 04KL108888 lines were selected for their high tolerance to iron deficiency chlorosis. The remaining lines were selected for favorable agronomic traits. RIL populations ranged in size from 39 to 178 individuals.

The F_1 crosses for each population were made in Owatonna, MN during the summer of 2006. The F_1 seeds were harvested in bulk for each population and planted in Oahu, HI in November of 2006. F_2 seeds from this planting were also harvested in bulk for each population and planted again in Oahu, HI in March of 2007. A modified single seed[U2] decent harvest procedure was followed, where a single pod from each F_2 plant was harvested and threshed in bulk. F_3 seeds were planted in Oahu, HI in June of 2007. Again, single pods from F_3 plants were harvested and threshed in bulk. F_4 seeds were planted in Oahu, HI or Salinas, PR in October of 2007. F_5 seeds were harvested from single F_4 plants to create $F_{4:5}$ RILs. $F_{4:5}$ seed was increased for each line in all populations in the summer of 2008 in Owatonna, MN. Another seed increase of $F_{4:6}$ lines was planted 2009 in Salinas, PR.

IDC Evaluation

Three trials were planted in 2008 to evaluate iron deficiency chlorosis severity. One location was planted in Minnesota (Welcome, MN), and two locations were planted in Iowa (Ogden, IA, and Fort Dodge, IA). In 2009, one location was selected to evaluate iron deficiency chlorosis in MN (New Ulm, MN) but was never planted. Two other locations were planted in Iowa (Ogden, IA and Nevada, IA).

The Welcome location in 2008 contained a soil complex of 50% Canisteo or similar soils (Fine-loamy, mixed, superactive, calcareous, mesic Typic Endoaquolls), 35%

Glencoe, depressional, and similar soils (Fine-loamy, mixed, superactive, mesic Cumulic Endoaquolls), and 15% of Harps (Fine-loamy, mixed, superactive, mesic Typic Calciaquolls) and Crippin soil (Fine-loamy, mixed, superactive, mesic Aquic Hapludolls). The Ogden and Fort Dodge locations in 2008 contained a soil type of Canisteo silty clay loam (Fine-loamy, mixed, superactive, calcareous, mesic Typic Endoaquolls). The 2009 New Ulm, MN location contained Delft clay loam (Fine-loamy, mixed, superactive, mesic Cumulic Endoaquolls). The Ogden location in 2009 was classified as Harps loam (Fine-loamy, mixed, superactive, mesic Typic Calciaquolls), and lastly, the Nevada location in 2009 contained a soil type of Webster clay loam (Fine-loamy, mixed, superactive, mesic Typic Endoaquolls). All soils types present at the evaluation sites for the iron deficiency chlorosis consisted of very deep and poorly drained soils typical to areas prone to iron deficiency chlorosis.

Syngenta planted trials in contract fields owned by growers. The fields were chosen by Syngenta based on historical presence of iron deficiency chlorosis. To ensure IDC evaluation trials were planted in locations with adequate iron deficiency chlorosis pressure, the contracted growers would plant their soybean cultivar of choice in their fields using their own production practices. Once the growers had identified a large chlorotic area in their fields, the chlorotic soybean plants would be removed, and the IDC evaluation trials would be planted in the chlorotic area. Evaluation trials were planted in late June through early July each year. Iron deficiency chlorosis was evaluated at V2 and V4 stages, which is approximately 21-28 days after planting and 14 days after V2 evaluation, respectively.

In 2008, the Welcome, MN IDC evaluation trial did not display any chlorotic symptoms despite chlorotic symptoms previously expressed by the soybeans planted by the contracted grower. Both Ogden and Fort Dodge, IA IDC evaluation trials expressed a full range of chlorosis severity[S3].

In 2009, the cultivar planted by the grower in the New Ulm, MN location never produced chlorotic symptoms, so consequently the IDC evaluation trial was never planted at that location. The two Iowa locations planted by the grower did display chlorotic

symptoms in the field; however, the two Syngenta IDC evaluation trials planted within those chlorotic areas previously identified by the growers failed to produce chlorotic symptoms (Figure 1A-1D).

The IDC trials were planted in hill plots. Each hill plot contained 10 seeds. Hill plots were spaced 15 inches from center to center following up the ranges and 10 inches from center to center between rows. $F_{4:5}$ and $F_{4:6}$ RILs were evaluated in 2008 and 2009, respectively.

Severity of iron deficiency chlorosis was evaluated using visual ratings and normalized difference vegetative index (NDVI) values. Visual ratings were taken on a scale from 1 to 9, where a rating of 1 indicated green plants with no chlorosis present, and a rating of 9 indicated severe chlorosis with leaf necrosis and plant death. All ratings were conducted by the same evaluator. NDVI measurements were collected using the GreenSeeker® RT100 System (N-Tech Industries, Ukiah, CA and Oklahoma State Univ., Stillwater). The GreenSeeker® RT100 System is an active lighting optical sensor that uses internal illumination from high intensity light emitting diodes (LED's). The internal light source allows the sensor to be used in all lighting conditions. LED's pulse light at a high frequency, emitting light at 660 nm (red) and 770 nm (NIR) wavelengths. Photodiode detectors measure the amount of reflected light from the leaf canopy. All background illumination is removed by electronic filters. The filtered signal is measured by a multiplexed A/D converter. Twenty readings per second were taken and averaged in order to calculate the average NDVI value per hill plot. NDVI is calculated using the following formula: NDVI = $(\rho_{NIR} - \rho_{Red})/ (\rho_{NIR} + \rho_{Red})$, where ρ_{NIR} is the fraction of emitted near infrared radiation returned from the leaf canopy (reflectance), and ρ_{Red} is the fraction of emitted red radiation returned from the sensed area (reflectance) (Solie et al., 2002).

Sensors were mounted to a boxed frame that reduced the target area exposed to the sensor down to 12 inches, which is approximately the size of a hill plot. This focused the sensor, so that measurements could be taken on a single hill plot without interference from neighboring hill plots.

The IDC evaluation trials were planted using a randomized complete block (RCB) design with two replications and augmented with a repeated check every 10th entry. A moderately susceptible Syngenta cultivar (S21-N6) was used to augment the trials. The performance of the check cultivar was used to evaluate the homogeneity of the field at each testing location. Iron chlorosis most often expresses itself on rims of depressions, so it is important to assess the spatial and temporal variation of fields based on the performance of a common check cultivar. If surface analysis showed significant trends in field variation, the experimental plots were adjusted accordingly.

Surface analysis was conducted using S-Plus by fitting a sequence of linear nested polynomial surface models to the data. The best polynomial surface model is selected based on likelihood ratio tests. The form of the surface is taken as a full third degree polynomial, where $\mu_c(x, y)$ is the surface of the expected values of control plots with x row number and y range number.

$$\mu_c(x,y) = \mu + \beta_1 x + \beta_2 y + \beta_3 x^2 + \beta_4 xy + \beta_5 y^2 + \beta_6 x^3 + \beta_7 x^2 y + \beta_8 xy^2 + \beta_9 y^3$$

The following hypotheses are successively tested. The hypothesis is tested only if the previous hypothesis is significant.

Name	Hypothesis	Parametrically
H_0	No effect of position:	$\beta_i = 0$ for $i = 1,...,9$
H_1	No third order effects:	$\beta_6 = \beta_7 = \beta_8 = \beta_9 = 0$
H_2	No parabolic effects:	$\beta_3 = \beta_5 = 0$
H_3	No first order interaction:	$\beta_4 = 0$

The corrected value of the plot was calculated using the formula below, where $\bar{z}_c$ represents the mean value of all surface control plots and z_i denotes the ith plot result in the field for a given trait.

$$\tilde{z}_i = z_i - \hat{\mu}_c(x_i, y_i) + \bar{z}_c$$

Raw plot values for each trait measured were adjusted based on detectable surface trends according to the methods previously described above. These adjusted plot values were then used to calculate genotypic least squares means (LS-means).

LS-means were calculated using a restricted maximum likelihood (REML) approach with bounded variance components (JMP, Version 10. SAS Institute Inc., Cary, NC, 2012). The following model was used to calculate LS-means for NDVI values and chlorosis ratings at V2 and V4 for genotypes across and within environments. Each RIL population was analyzed separately. Replications, environments, and genotypes were random variables.

$$Y_{jkm} = \mu + R(E)_{k(j)} + E_j + G_m + GE_{mj} + e_{jkm}$$

Y_{jkm} is iron chlorosis severity measured by NDVI or chlorosis rating in the *j*th environment, *k*th replication, and *m*th genotype

μ is the overall mean NDVI value or chlorosis rating

$R(E)_{k(j)}$ is the random effect of the *k*th replication nested in the *j*th environment

E_j is the random effect of the *j*th environment

G_m is the random effect of the *m*th genotype

GE_{mj} is the interaction of *m*th genotype and *j*th environment

e_{jkm} is the random error associated with the *j*th environment, *k*th replication, and *m*th genotype

Least squares means for chlorosis ratings at V2 for the RYS004 population could not be calculated due to iterations not converging properly in the REML model. The REML model did not converge due to unbalanced replication of the RYS004 population. Lack of seed from this population prevented planting balanced replications across all locations[IJ4]. As an alternative approach, LS-means were calculated for all chlorosis traits

using a stepwise method with two models. Firstly, genotypic LS-means were calculated for each environment using the following model below.

$$Y_{km} = \mu + R_k + G_m + e_{km}$$

Y_{km} is iron chlorosis severity measured by NDVI or chlorosis rating in the *k*th replication and *m*th genotype
μ is the overall mean NDVI value or chlorosis rating
R_k is the random effect of the *k*th replication
G_m is the random effect of the *m*th genotype
e_{km} is the random error associated with the *k*th replication and *m*th genotype

The LS-means of the genotypes for each environment were used to calculate the genotypic LS-means within and across environments using the second model below.

$$Y_{jm} = \mu + E_j + G_m + GE_{mj}$$

Y_{jm} is iron chlorosis severity measured by chlorosis rating at V2 in the *j*th environment and *m*th genotype
μ is the overall mean NDVI value or chlorosis rating
E_j is the random effect of the *j*th environment
G_m is the random effect of the *m*th genotype
GE_{mj} is the interaction of *m*th genotype and *j*th environment (also referred to as the error term)

Calculating genotypic LS-means for each environment first and then within and across environments will give more weight to a single replication in the second model if replications are unbalanced. Estimating all parameters simultaneously is preferred;

however, it was not possible given the inability of the iterations to converge in the full model.

The least square means for each genotype were used to conduct composite interval mapping and to calculate Pearson's correlations between yield and IDC trait measures.

Yield Evaluation

Yield was evaluated at 5 locations in 2009 and 2010 (Thorndale, ON; Brookings, SD; Owatonna, MN; Stanton, MN; Nevada, IA). In 2009 and 2010, five of the 13 $F_{4:6}$ and $F_{4:7}$ RIL populations were evaluated for yield, respectively. In 2010, a 6th population was added to create a fully linked network of populations (Figure 2). Due to limited resources, not all populations phenotyped for iron deficiency chlorosis tolerance from the full network could be evaluated for yield. An additional lack of seed supply for some populations prevented them from being planted in every yield evaluation environment.

Yield trials were planted using a replication within sets design (Bernardo, 2002; Hallauer and Miranda, 1981). Individuals from each RIL population were distributed into multiple 36-entry sets based on maturity. The number of sets for each population varied depending on the number of RILs within each population. While entry number assignment within each population remained consistent across environments, each entry was randomly assigned to a plot in the field within each set. Each set was augmented by check cultivars of similar maturity grouping to the RILs within each set. Two replications were planted within each environment, provided enough seed was available.

Two row plots 3.7 m in length with 76.2 cm row spacing were mechanically planted at all locations in 2009 and 2010. Three hundred seeds were planted within each plot. Maturity notes were taken at the Brookings and Owatonna locations in 2009 and 2010, and at the Stanton location in 2009. Harvest maturity was calculated by counting the number of days after planting to the date when 95% of the pods had reached their mature color. Plants were mechanically harvested, and yield was adjusted to 13% moisture.

Least squares means were calculated using a restricted maximum likelihood (REML) approach with bounded variance components. The following model was used to calculate LS-means of yield for genotypes across and within environments. Each RIL population was analyzed separately. All model components were considered random effects with the exception of the overall mean which is always considered a fixed effect.

$$Y_{ijkl} = \mu + E_i + S_j + SE_{ij} + R(S/E)_{k(ji)} + G(S)_{l(j)} + EG(S)_{il(j)} + e_{ijkl}$$

Y_{ijkl} is yield measured in bushels per acre in the *i*th environment, *j*th set, *k*th replication, and *l*th genotype
μ is the overall mean yield measured in bushels per acre
E_i is the random effect of the *i*th environment
S_j is the random effect of the *j*th set
SE_{ij} is the interaction of the *i*th environment and *j*th set
$R(S/E)_{k(ji)}$ is the random effect of the *k*th replication nested in the *j*th set and *i*th environment
$G(S)_{l(j)}$ is the random effect of the *l*th genotype nested in the *j*th set
$EG(S)_{il(j)}$ is the interaction of *i*th environment and the *l*th genotype nested in the *j*th set
e_{ijkl} is the pooled error of the *i*th environment, *j*th set, *k*th replication, and *l*th genotype

The least squares means for each genotype were used to conduct composite interval mapping and to calculate Pearson's correlations between yield and iron chlorosis tolerance trait measures.

Genotypic Evaluation

Leaf tissue was collected from $F_{4:5}$ plants from each RIL population. Tissue was lyophilized and submitted to the Syngenta genotyping laboratory in Stanton, MN. All genotyping laboratory work was conducted following proprietary protocols specified by Syngenta. In general, DNA was extracted using a variation of a CTAB DNA extraction

method similar to methods described in (Kidwell and Osborn, 1992). PCR was conducted using ABI 9700 thermocyclers, and data was visualized using an ABI 7900HT Fast Real-Time PCR System. A total of 867 SNP markers were run on the network of 13 populations. The number of markers run on each population ranged from 161 to 376 markers. Heterozygous genotypes were treated as missing data.

Consensus Map and QTL Mapping

Consensus genetic map positions were used to map QTL via composite interval mapping (CIM). Syngenta colleagues created the consensus map by combining genotypic data from 9 bi-parental F_5-derived populations. Population sizes ranged from 241 to 309 RILs. The original marker order was determined by physical map positions. Intermarker distances were estimated using internal mapping software. The software used a maximum likelihood mapping algorithm. The first version of the consensus map included 7,135 SNP markers. The total length of the map was estimated to be 4,478 cM with an average intermarker distance across chromosomes of < 1 cM and a maximum intermarker distance of 32.5 cM. An additional 9,594 SNP markers were added to the consensus map, finalizing the total marker number to 16,729. The additional 9,594 SNP markers were not screened on the original 9 bi-parental F_5-derived RIL populations. To obtain genetic map positions for these additional markers, a regression method was conducted to interpolate the genetic positions of the markers. The final version of the consensus map reduced the average intermarker distance to < 1 cM and the maximum intermarker distance to 22 cM. Both public and private SNPs are included in the Syngenta soybean consensus map. Linkage group and corresponding chromosome number are noted together throughout this thesis.

The current public soybean Consensus Map 4.0 consists of 5,500 genetic markers, including an additional 2,500 new SNPs added from the previous 1,141 mapped SNPs from version 3.0 (Choi et al., 2007; Hyten et al., 2010). The total length of the public Consensus Map 4.0 is 2,296.4 cM. The Syngenta consensus map is considerably expanded compared to the public consensus map. This is likely due to different algorithms used to create the

consensus map. Genotyping errors can also expand genetic map length, but this is not a likely cause for an expanded map as several iterations of data review and culling was performed during the creation of the consensus map.

Syngenta consensus map positions were used in the QTL analysis conducted to identify QTL significantly associated with iron deficiency chlorosis tolerance/susceptibility and yield. R/QTL was used to evaluate segregation ratios of the genotypes within each bi-parental mapping population (Broman et al., 2003). Distorted, problematic markers were removed using an ($\alpha = 0.05$) significance threshold after a Bonferroni correction for multiple tests was applied. Markers with moderate departures from the expected segregation ratio were either kept or removed using the investigators discretion as some segregation distortion is indicative of a partially lethal genotype and not due to marker quality.

Composite interval mapping (CIM) was conducted on individual bi-parental populations using algorithms from QTL Cartographer that were adapted for Syngenta mapping software. Quantitative trait loci were also detected using CIM on the entire network of populations using the 'network population mapping' methods developed and patented by Syngenta (Guo et al., 2010). Network population mapping has increased power to detect QTL over current joint connected population mapping by grouping common alleles at a locus thereby reducing the number parameters in the model compared to that of the connected model. Composite interval mapping was performed using a default 10 cM window and a walking speed of 2 cM. Cofactors were selected using forward-reverse stepwise regression. Five hundred and 1,000 permutation tests with a genome-wide significance of ($\alpha = 0.05$) were conducted on bi-parental and connected models, respectively, to obtain QTL significance thresholds for each trait across and within environments (Doerge and Churchill, 1996).

RESULTS AND DISCUSSION

Surface Analysis of Iron Deficiency Chlorosis Trials

Heat map images displaying the trends in field heterogeneity for each environment were created using the raw chlorosis trait data from the repeated check cultivar S21-N6. The maps indicated that no surface trends were detected in the NDVI and chlorosis rating data collected at the V2 stage in the Ogden environment (figures not shown). Similarly, no trends were detected in the NDVI data collected at the V4 stage in the Fort Dodge environment (figures not shown). Lack of detectable surface trends may be due to either no significant variation in the performance of the repeated check cultivar or due to no patterns of variable performance of the repeated check cultivar.

Surface analysis did detect surface trends in the NDVI and chlorosis rating data collected at the V4 stage in the Ogden environment (Figures 3-4). At this location, chlorosis expression increased in severity around the perimeter of the field compared to chlorosis expression at the center of the field with V4 ratings ranging from 7.9 to 3.6, respectively. NDVI values showed similar trends with values ranging from 0.4 at the center of the field and decreasing towards the perimeter with a value of 0.2. This surface pattern could be indicative of a slight depression present in the center of the field. Chlorosis has been known to express more severely around the rims of these types of depressions, which coincides with the surface patterns observed here (Inskeep and Bloom, 1984).

The lack of field variation detected at the V2 stage in the Ogden environment may be due to early wet field conditions uniformly distributed across the trial. Early wet conditions exacerbate chlorosis expression by increasing soil HCO_3^- concentrations via (Eq. 1) (Lucena, 2000). As field conditions begin to dry out, soybeans recover from their chlorotic symptoms; however, rims of depressions still remain prone to chlorosis. Higher concentrations of mobile ions and insoluble salts, known to correlate with chlorosis expression, tend to collect around these rims (Inskeep and Bloom, 1984). Within the Ogden location, chlorosis more severely expressed at the V2 stage than the V4 stage with chlorosis remaining severe around the perimeter of the experiment (Table 3). This pattern

in chlorosis expression is consistent with the hypothesis of a depression present within the testing location. [IJ5]

Unlike the absence of trends in variation at the V2 stage in the Ogden location, the Fort Dodge location did display trends in field variation at the V2 stage. Both NDVI and chlorosis ratings indicated chlorosis expressed more severely in the 'upper left' portion of the heat map and decreased diagonally across the field (Figures 5-6). Chlorosis ratings ranged from 7.0 in the 'upper left' to 3.1 in the 'lower right' quadrant of the heat map. Consequently, NDVI values displayed similar field trends with a more severe NDVI value of 0.3 in the 'upper left' and a less severe value of 0.4 in the 'lower right' quadrant of the heat map.

Similar spatial patterns of field variation observed in Ogden at the V2 growth stage were not sustained at V4. The diagonal spatial patterns observed at the V2 stage changed to a more complex pattern of variation at the V4 stage as detected by the chlorosis rating data only (Figure 7). No trends were detected with NDVI values at V4. This change in temporal and spatial variation highlights the complexity of chlorosis expression due to the frequently dynamic soil chemical and physical properties in the field.

It is unknown why the NDVI values collected at V4 did not display the same trends in variation as observed with the chlorosis ratings collected at V4 in Fort Dodge. One likely explanation is that the limited scanning area implemented by the N-Tech GreenSeeker® RT100 System does not adequately scan the plant surface area affected by chlorosis. The instrument only scans a 1-inch band width in a fixed position, so it is possible that the N-Tech GreenSeeker® RT100 System is unable to scan a representative sample of the larger area of vegetative growth present at the V4 stage. Soybean plants often recover from chlorosis at the V4 stage, so less vegetative growth may be affected by chlorosis. Because the N-Tech GreenSeeker® RT100 System only scans a small portion of the plant, it is possible to miss the few trifoliates that may express chlorosis. Visual ratings in contrast are based on whole plant evaluations and would account for all chlorosis symptoms displayed by the plant.

Objective phenotyping methods are always preferred over subjective rating data collection methods; however, experimenters must understand the limitations of technology. The N-Tech GreenSeeker® RT100 System is a medium throughput phenotyping technology that provided objective data; however, the narrow bandwidth of the scanning area limited the amount of surface area considered for evaluation. This could have introduced a sampling bias. Conversely, visual ratings are a subjective method of evaluation, which could have also introduced the bias of the evaluator and misclassification error. It is important to consider both data types, keeping in mind the limitations of both.

Raw plot values for each trait measured were adjusted based on detectable surface trends, according to the methods previously described above. These adjusted plot values were then used to calculate genotypic LS-means.

Variance Components and Least Squares Means Values

Iron deficiency chlorosis and yield experiments were analyzed using mixed models. Variance components of the mixed models were estimated using a restricted maximum likelihood (REML) method (JMP, Version 10. SAS Institute Inc., Cary, NC, 2012). Least squares means were calculated for each trait across and within environments. The LS-mean values were used for subsequent calculation of Pearson's correlations and QTL analysis.

Variance components are represented as percentages of the total variation (Table 3). Percent of total variation for each parameter in the REML models varied widely from population to population for each trait. In the IDC evaluation experiment, the percentages of variation attributable to genotypes ranged from 7.86% to 46.02%. The percent of variation explained by genotypes was far less than the percentage of variation due to error for all populations. Percentages of error variances ranged from 40.16% to 78.43%. This result, while concerning, was not unexpected. Iron deficiency chlorosis expression is very complex and is affected by several interacting soil chemical and physical factors. Of the three chlorosis locations planted for IDC evaluation in 2008, one of the locations failed to

produce chlorotic symptoms. In 2009, none of the three locations produced chlorotic symptoms. The two replications of data collected within the two 2008 environments is not nearly sufficient to minimize error variances given the variability of IDC expression within environments. Unfortunately, no additional resources were available for conducting supplementary IDC evaluations in the years following. If additional resources were available for phenotyping, additional replications, environments, and use of a different experimental design such as an alpha-lattice or a row-column design should be considered to better control error variances. Despite larger than desired error variances, genotypic LS-means calculated via REML methods were used to evaluate Pearson's correlations between yield and chlorosis traits as well as to conduct QTL mapping.

No other gross trends in the variance components were observed in the IDC models with the exception of a slight tendency for chlorosis ratings at V2 to have higher percentages of variation due to genotype compared to the other chlorosis measures.

Environments explained the majority of the variation in yield performance. The percent of total variance explained by environments ranged from 42.95% to 60.28% across the six populations evaluated for yield (Table 3). The ten yield environments represent trials grown in five cities over two years. The trialing locations were geographically dispersed over maturity groups I and II. Yield environments were selected to represent the diverse set of field conditions present in grower's fields, thus, the large percentage of variation due to environment was not unexpected.

The percentages of variation explained by genotypes nested within sets ranged from 5.40% to 9.25% (Table 3). These percentages were less than the percentages of variation due to error, which ranged from 11.56% to 19.94%. Yield is a complex trait with low heritability, so it was not surprising to observe error variances attributable to a larger portion of the total phenotypic variance. Additional environments should have been tested to reduce error variances, but due to limited resources, the six populations were submitted to preliminary yield trialing locations only as opposed to additional trialing sites reserved for more advanced stage materials.

The percentage of variance explained by replications nested within sets and environments was predictably small for all populations. The remaining variance components of the yield model showed no other major trends across the six populations evaluated for yield performance.

Genotypic LS-means were used to calculate Pearson's correlations between yield and IDC trait measures as well as to conduct composite interval mapping.

Pearson's Correlations between Yield, NDVI, and IDC Ratings

Pearson's correlations among yield, NDVI measurements at V2 and V4, and iron deficiency chlorosis ratings at V2 and V4 were calculated using genotypic LS-mean estimates within and across populations and environments. Correlations were computed using the 'Multivariate and Correlation Procedure' in SAS JMP (JMP, Version 10. SAS Institute Inc., Cary, NC, 2012). A pairwise estimation method was used.

The Pearson's correlations indicate a linear association between traits caused by both genetic and non-genetic factors. If the correlation is due to genetic factors, selection for one trait will lead to a correlated response to selection in the other trait (Bernardo, 2010). For example, if lower yields are associated with increased IDC tolerance due to a genetic correlation, selection for IDC tolerance will indirectly lead to lower yields. If the genetic correlation is due to linkage, the correlation will eventually diminish with generations of recombination, thus any undesired association between traits such as low yield and high IDC tolerance may be broken. If the genetic correlation is due to pleiotropy, it will be impossible to select simultaneously for high yield and increased IDC tolerance at that locus (Bernardo, 2010).

Correlations between yield and iron chlorosis trait measures were calculated using a subset of the data determined by common entries across yield and IDC evaluation trials. Five of the six populations evaluated for yield were also evaluated for iron deficiency chlorosis. In total, 217 entries were used to calculate correlations between yield and IDC severity. Limited resources prevented all entries from being evaluated for both IDC and

yield. Despite having to use a reduced dataset, yield significantly correlated to IDC severity across populations at a significance levels of ($p < 0.001$) (Table 4). Moderate positive correlations between yield and chlorosis ratings were observed at V2 and V4 stages with Pearson's correlation coefficients of 0.56 and 0.54, respectively (Table 4). NDVI values showed slightly stronger correlations with negative correlation coefficients of -0.55 and -0.61 at V2 and V4 stages, respectively. Keeping in mind larger positive NDVI values indicate healthy plants, the negative correlations between yield and NDVI values and the positive correlations between yield and chlorosis ratings provide evidence that increased IDC tolerance may lead to reduced yields. Evaluation of correlations between yield and IDC severity within populations showed lower yields significantly associated with increased IDC tolerance in RYS002 and RYS008 populations only (Appendix A). Although not significant, there is a low correlation of higher yields and increased IDC tolerance, indicating that the undesired association of low yields with increased IDC tolerance is dependent on the population.

Correlations between NDVI and chlorosis ratings using LS-mean estimates across and within populations and environments were also calculated in order to evaluate the utility of the data collected by the N-Tech GreenSeeker® RT100 System. Again, Pearson's correlations were computed using the pairwise estimation method available in SAS JMP. NDVI values strongly correlated to visual ratings across the twelve populations and across all environments with significance levels of ($p < 0.0001$) (Table 4). At V2, NDVI values negatively correlated with visual ratings with a correlation coefficient of -0.79. The negative relationship between NDVI and visual ratings increased strength to -0.89 at V4. Moderate to high correlations between NDVI and visual ratings within populations and environments were also all consistently significant with p-values of ($p < 0.001$) (Appendixes B-D). Bearing in mind the NDVI value is an objective measure of IDC severity, evaluators of IDC should strongly consider using an objective tool such as the N-Tech GreenSeeker® RT100 System to evaluate IDC severity given the strong correlations to visual ratings. The GreenSeeker® is not a perfect technology as it has a limited scanning width that may introduce a sampling bias; however, other imaging technologies that do not

suffer from this limitation and can provide high-throughput phenotyping capabilities should be researched further as a means to reduce error variances in IDC severity measurements.

Lastly, NDVI measurements taken at V2 strongly correlated to NDVI measurements taken at V4 with a correlation of 0.86. Similarly visual ratings taken at V2 strongly correlated to visual ratings taken at V4 with a correlation coefficients of 0.84. Both correlations were significant at a level of ($p < 0.0001$) (Table 4). The strong correlations between chlorosis severity measured at V2 and V4 indicated that chlorosis severity observations taken during the initial flash of chlorotic symptoms are tightly associated with chlorotic symptoms expressed 14 days after V2. Breeders sometimes observe differentiation of genotypes' abilities to recover from chlorosis at V4, but the strong correlations observed within this experiment suggest that the majority of the genotypes recovered similarly. Chlorosis severity means measured within populations indicate a general reduction of chlorotic symptoms from V2 to V4 across all populations; the strong correlations of chlorosis severity suggest that genotypes recovered from chlorosis similarly (Table 3).

Composite Interval Mapping of Iron Deficiency Chlorosis Traits

In total, 50 QTLs detected via CIM using the disconnected bi-parental model were significantly associated to IDC traits (Table 5). In contrast, a total of 22 QTLs detected via CIM using the connected model described in Guo et al. (2010) were significantly associated to IDC traits (Table 5). Of those 72 QTL detected by both models, only a subset of the most interesting regions are considered for discussion here. Due to the large error variances calculated from both IDC and yield REML models, only regions with multiple QTL identified within the same confidence interval for similar traits were considered for discussion. Large error variances lead to lower heritabilities and can increase chances of falsely detecting a QTL. Considering regions with multiple QTL of the same trait type should provide evidence that the QTLs are true.

Quantitative trait loci associated with chlorosis ratings at V2 were detected on linkage group (LG) A1/5 across and within environments for populations RYS004 and RYS008 (Table 5). The 1-LOD support interval for the QTL identified in RYS004 ranged from 17.0 to 22.5 cM, whereas the 1-LOD support interval identified in the RYS008 population ranged from 17.2 to 23.1 cM. Both populations reported consistent QTL LOD score, R^2, and additive effect values within the Ogden and Fort Dodge environments as well as across both environments, indicating the stability of this QTL across environments. The RYS008 population reported slightly larger LOD score, R^2, and additive effect value compared with the RYS004 population.

Joint connected analysis similarly detected a significant QTL on LG A1/5 associated with visual ratings taken at V2 across populations. Unlike the bi-parental model, the connected model detected no significant regions within environments. The position of the QTL detected across environments was upstream of the QTLs identified by the disconnected bi-parental model with a 1-LOD support interval of 7.1 to 17.2 cM (Table 5). The connected model was unable to narrow the QTL confidence interval to locate more precisely the position of QTL as expected. A small monomorphic region at the top of LG A1/5 existed in the RYS004 and RYS008 populations. When analyzing the entire network of populations, the polymorphic marker coverage expanded into this region, shifting the location of the confidence interval upstream. The QTL LOD score, R^2, and additive effect values detected by the connected model were similar to those detected by the bi-parental model although the LOD score and additive allelic effect were somewhat smaller in the connected model. A region on LG A1/5 had previously been associated with chlorosis (Lin et al., 1997, 2000); however, the region was located downstream on the consensus map at 110 cM, suggesting that the QTLs are different.

Several significant QTL associated with both chlorosis rating and NDVI values measured at V4 were identified on LG A2/8 in populations RYS002 and RYS003. Given the strong correlations between NDVI values and visual ratings, one expects to observe co-localization of NDVI and rating QTL, supporting the notion that NDVI values measure the same genetic mechanism responsible for chlorosis as visual ratings (Table 4). For

population RYS003, overlapping 1-LOD supports extended across two large regions ranging from 43.0 to 163.3 cM and 184.9 to 216.7 cM (Table 5). These QTL were identified only in the Ogden environment. Significant QTLs associated with chlorosis rating measured in the RYS002 population at V4 indicated the QTL was stable across and within each environment; however, a very large 1-LOD support interval ranging from 94.1 to 215.6 cM was observed (Table 5). These large 1-LOD supports coincided with large gaps in marker coverage across this region. When all population data was analyzed simultaneously using the connected model, the large region associated with chlorosis was narrowed to a single region at 185.1 cM as defined by a QTL associated with the NDVI trait measured at V2 (Table 5). Again, on average, the connected model detected a smaller LOD score and R^2 value compared to the bi-parental model. The additive effects of the NDVI values detected by the connected model were also smaller than the effects detected in the RYS003 population, where an NDVI QTL was detected. Diers et al. (1992) identified a QTL on the top of LG A2/8, explaining 17% of the phenotypic variance. This QTL is located 140 cM upstream of the QTL identified across environments using the connected model, suggesting that the QTLs are not the same. It is worth noting that the 1-LOD support interval associated with visual ratings measured in the RYS003 population at V4 nearly overlaps with the QTL identified by Diers et al. (1992).

Regions associated with chlorosis ratings and NDVI values measured at V2 and V4 were identified on LG D1b/2 using the bi-parental CIM model for populations RYS003 and RYS007. The following 1-LOD support intervals were detected in population RYS003: 25.7 to 41.1 cM, 43.3 to 87.7 cM, and 103.4 to 108.7 cM (Table 5). The QTLs located at the top of the chromosome, significantly associated with visual ratings taken at V4, explained 13.1% to 18.9% of the variation in the trait, while the QTLs associated with NDVI measured at V2 and V4 explained a higher percentage of variation with R^2 values ranging from 35.3% to 47.3% (Table 5). The QTLs detected in the RYS003 population were stable across locations. Similar regions associated with NDVI measured at V4 were also identified in the RYS007 population. The following 1-LOD support intervals corresponded with the three QTLs discovered in RYS007: 99.1 to 99.5 cM and 90.0 to

109.4 cM and 108.4 to 138.3 cM (Table 5). The R^2 values had a similar range to the NDVI QTL discovered in RYS003; values ranged from 29.9% to 45.6%. Again, the QTLs exhibited stability across environments.

The connected model detected significant chlorosis QTL on LG D1b/2 at 108.4 cM within the Ogden and Fort Dodge locations. The connected model more precisely located the QTL within 106.9 to 108.4 cM and 107.9 to 108.4 cM respective to the Ogden and Fort Dodge environments (Table 5). Reduced LOD scores and R^2 values were observed when compared to the traditional bi-parental model. The additive effects of the visual ratings collected at V4 detected by the connected model were also smaller than the additive effects detected by the bi-parental model.

A region on LG H/12 previously identified to be associated with chlorosis by Lin et al. (1997, 2000) was similarly identified in population RYS008 (Table 5). The QTL was identified in both Ogden and Fort Dodge locations as well as across locations, indicating QTL stability. The IDC tolerant parent 03DL052038 carried the favorable allele conferring an increase in NDVI value of 0.018 at V2. The across locations QTL explained 28.3% of the variation in NDVI measured at V2.

Another region on LG I/20 was also significantly associated with NDVI values collected from RYS003 at V4 in the Ogden environment (Table 5). The 1-LOD support interval ranged from 67.4 to 98.6 cM. Using connected CIM, a smaller region located just upstream from 118.8 to 120.0 cM was found to be significantly associated with NDVI measured at the V2 stage in Ogden and across locations (Table 5). The direction and magnitude of the additive effects as well as the differences in R^2 suggest that the bi-parental and the connected QTL are distinct. Interestingly, the QTLs identified via connected CIM explain a large percentage of variation ranging from 42.1% to 46.4% within the Ogden location and across locations, respectively. Wang et al. (2008) identified a region upstream of the QTLs identified here. One cannot be certain that the region identified on LG I/20 within this study confers IDC tolerance due to the same genetic mechanism as the region associated in Wang (2008) study.

Linkage group L/19 contained several significant QTL identified by both bi-parental and connected CIM models. Populations RYS002 and RYS004 identified two large overlapping 1-LOD support intervals significantly associated with chlorosis rating at V2. The RYS002 1-LOD support interval ranged from 54.4 to 72.9 cM, and the RYS004 1-LOD support interval ranged from 34.1 to 99.9 cM (Table 5). The LOD scores, R^2, and additive effects detected by both populations were consistent within and across environments, indicating the QTL is stable across locations. The connected CIM model detected two significant QTL associated with chlorosis rating at V2 with 1-LOD support intervals of 90.4 to 102.3 cM and 97.9 to 102.3 cM for Ogden and across locations, respectively. Again, the connected model was able to significantly reduce the large 1-LOD support intervals from the bi-parental model and more precisely locate the QTL to a region between 90.4 and 102.3 cM (Table 5). LOD scores and additive values were comparable between the traditional bi-parental and the connected models. The QTLs identified using the connected model, however, explained a larger portion of the variation in the trait with R^2 values ranging from 19.5% to 27.6%. Charlson et al. (2003, 2005) similarly identified a QTL significantly associated with chlorosis rating on LG L/19. The QTL identified by Charlson et al. (2003, 2005) has an estimated position on the Syngenta consensus map of 106.5 cM, which is just downstream of the 1-LOD support interval identified here.

Another QTL significantly associated with NDVI at V2 was identified within and across locations using the connected CIM model. The NDVI QTL was located within a 1-LOD support interval ranging from 157.2 to 164.3 cM (Table 5). This QTL had a similar range of LOD scores and R^2 values within and across locations as the RYS004 bi-parental QTL on LG L/19.

The major QTL underlying the single major gene model with modifiers detected on LG N/3 by Lin et al. (1997, 2000) was not detected in this study. Lin et al. (1997, 2000) identified a single QTL accounting for 72% of visual chlorosis variation in an F_2 population created from crossing Anoka by A7. This region physically correlated with a Fe-Deficiency-Induced Transcription Factor (FIT)/bHLH heterodimer known to induce expression of other iron acquisition genes (O'Rourke et al., 2009; Peiffer et al., 2012;

Severin et al., 2010). The connected CIM model did identify an unlinked region downstream of FIT on LG N/3 that accounted for 17.0% to 27.3% of variation in visual chlorosis at V2. The region identified by the connected model is not expected to be associated with the transcription factor located on LG N/3.

Composite Interval Mapping of Yield

Yield is positively associated with maturity as later maturing cultivars tend to have higher yield. Many QTL identified for yield may actually instead identify QTL due to variation in maturity, especially if models calculating mean values for yield do not control for variation in yield due to maturity. Experimental designs grouping cultivars with similar maturities can help control for some of the variation in yield due to maturity; however, one should consider adding maturity date as a covariate in models that calculate mean values for yield. Unfortunately, an incomplete dataset was collected on maturity for this experiment with maturity notes taken only on 5 of the 10 yield environments. For this reason, a maturity covariate was not considered in the model when calculating LS-mean values for yield. Alternatively, yield QTLs identified were compared to an internal list of genomic regions known to be associated with maturity (maturity data not shown).

Both bi-parental and connected CIM models detected QTL significantly associated with yield within and across environments (Tables 5). Regions identified on linkage groups C1/4, C2/6, J/16, and O coincide with regions previously identified as being significantly associated with maturity (maturity data not shown). The bi-parental CIM model detected several QTL on LG C2/6 within all 5 environments for 2009 and 2010 as well as across all environments. Overlapping 1-LOD support intervals ranged from 182.1 to 218.0 cM (Table 5). Across environments, the connected model was able to resolve this large region to a 1-LOD support interval of 183.4 to 199.6 cM (Table 5). This QTL explained 38.1% of the variation in yield performance across environments. Several within environment yield QTLs were also identified via the connected model. Two regions identified by overlapping 1-LOD supports ranged from 165.9 to 173.8 cM and 183.4 to

209.7 cM. The region ranging from 183.4 to 209.7 cM explained similar percentages of variation as the across environment QTL. The connected model also detected two regions on LG O/10 significantly associated with yield across locations. These two regions have corresponding 1-LOD support intervals of 141.4 to 147.8 cM and 166.8 to 170.5 cM and explain 28.7% and 24.5% of variation in yield, respectively. Similar to the yield region on C2/6, several within environment loci were found to significantly associate with yield as well using the connected model. Consistent detection of QTL within and across locations indicate stability of the QTL.

The yield QTL corresponding to regions associated with maturity on LGs C2/6 and O/10 did not co-localize with any IDC QTL. All other yield QTL identified are discussed in relation to co-localizing with chlorosis QTL in the next section.

Co-localization of Iron Deficiency Chlorosis and Yield Trait QTL

Iron efficient cultivars have been known to perform well on chlorotic soils; however, when grown on non-chlorotic soils, these iron efficient cultivars yield less than the iron deficient cultivars (Froechlich and Fehr, 1981). When developing new cultivars for improved IDC tolerance, a breeder must be careful not to inadvertently co-select genomic regions with negative effects on yield. To prevent selecting IDC tolerant cultivars with associated negative effects on yield, genetically correlated regions or regions where yield and IDC QTL co-localize should be inspected. There are two mechanisms for which a genetic correlation exists. Pleiotropy defines a situation where a single locus may effect seemingly unrelated traits due to that locus effecting multiple pathways. Linkage can also simulate pleiotropy when multiple loci conferring different phenotypic effects are inherited together due to linkage. Positive genetically correlated traits are linked in coupling and negatively correlated traits are linked in repulsion. Generations of random mating will eventually lead to a recombination event between linked loci, absolving the genetic correlation due to linkage (Hallauer and Miranda, 1981).

Regardless of the whether pleiotropy or linkage is the cause of the genetic correlation, both mechanisms should be investigated and should be considered when designing a breeding approach to improve cultivars. If two linked loci (one loci with a positive effect on IDC and another loci with negative effects on yield) are detected within 50 cM of each other but do not have overlapping confidence intervals, it is likely that the genetic correlation is due to linkage and not pleiotropy. In this scenario, large breeding populations may be necessary in order to increase the probability of observing a recombinant that confers IDC tolerance with no negative effects on yield. If on the other hand, two QTL are observed to have overlapping confidence intervals, it is possible that either pleiotropy or linkage is the cause of the genetic correlation. Additional crosses and investigation of progeny segregation ratios would be necessary to determine if the cause was due to linkage. If the two QTL identified are pleiotropic, it would be impossible to make a selection in this region conferring IDC tolerance with no reduction in yield, thus, other modifying loci will need to be considered for selection in cultivar improvement. If of course the genetic correlation is positive, the breeder could made use of correlated selection responses (i.e. indirect selection) by improving both traits simultaneously. The remaining yield QTL identified in this study will be discussed in reference to co-localization with IDC QTL below.

The QTL significantly associated with visual chlorosis at V2 on LG A1/5 (7.1 to 17.2 cM) is near two yield QTL identified in the Brookings and Owatonna environments in 2010 with respective 1-LOD support intervals of 28.3 to 36.2 cM and 23.9 to 46.1 cM (Table 5). If a breeder selects for the 04KL10888 allele that reduces visual IDC severity by 0.18 units in the RYS002 population, a subsequent decrease in yield of 2.46 bu ac^{-1} would also be expected (Table 5). A breeder should not consider making this selection because the small gain in IDC tolerance coincides with a larger decrease in yield.

Several other co-localized regions significantly associated with yield and IDC were detected on linkage groups A2/8, B1/11, C1/4, C2/6, F/13, J/16, I/20, L/19, M/7, and N/3 (Table 5). Many of these QTL were only identified in single populations and environments

and will not be discussed further. Two of those 10 linkage groups, however, did contain multiple IDC and yield QTLs, co-localizing together.

Two yield/maturity QTL on LG J/16 overlapped with two chlorosis QTL. The two chlorosis QTL 1-LOD support intervals overlapped and ranged from 39.9 to 174.8 cM; the two yield QTL 1-LOD support intervals ranged from 64.1 to 141.7 cM and 147.5 to 164.9 cM (Table 5). Interestingly, a QTL explaining 93.8% of the visual chlorosis severity at V2 in Ogden detected by the connected model is linked to a yield QTL also detected by the connected model, which explained 31.1% of the variation within the Owatonna 2010 environment (Table 5). The locations for phenotyping IDC and yield differed, but if these QTL were found to be stable across environments, the 03DL052038 genotype would be undesirable as it contains a negative allelic effect of -2.31 bu ac^{-1} linked with an allele conferring a positive reduction in visual IDC severity by -2.66 units. This type of linkage is undesirable and would need to be considered when developing breeding populations with this line.

Lastly, two yield QTL on the end of LG L/19 co-localized with three stable NDVI QTL measured at V2. Respective overlapping 1-LOD support intervals ranged from 159.5 to 164.9 cM and 157.2 to 164.3 cM (Table 5). The yield QTL explained a smaller portion of the variance with R^2 values of 13.4% and 15.4% of the variation observed in the Owatonna 2009 and the Brookings 2010 environments, respectively. Consequently, the 04KL111519 genotype confers correlated positive effects on both yield and NDVI values favorable for breeding.

In general, when selecting IDC tolerant genotypes, one should consider the source of favorable alleles and magnitude of their additive effects to ensure that selecting for IDC tolerance does not come with a large penalty on yield. Consideration of parental genotypes and associated allelic effects should direct selection of parents and choice of breeding strategy to improve cultivar performance.

Assessment of Bi-parental and Connected CIM Models

In most cases, joint analysis of connected populations via CIM reduced the size of QTL confidence intervals previously detected using the individual bi-parental populations. Several examples were observed where the connected model was able to resolve several larger confidence intervals detected by the bi-parental model on LGs A1/5, A2/8, C1/4, C2/6, D1b/2, L/19, M/7, and N/3 (Table 5). Large 1-LOD support intervals identified in individual bi-parental populations often coincided with large monomorphic regions. Analyzing multiple connected populations using the connected model increased the number of recombination events analyzed simultaneously in the dataset, which resulted in better resolution of QTL location.

In addition to having the ability to more precisely locate the QTL, the connected model also allowed for direct comparison of allelic effects. For example several chlorosis QTL were detected on LG D1b/2 in populations RYS003 and RYS007. Because the bi-parental model only considers single populations, one cannot rank the allelic effects of those QTL, so there is no way to determine which of the favorable alleles identified in the two populations will confer the greatest reduction in chlorosis expression (Table 5). Joint analysis using the connected model allows for simultaneous comparison of alleles, so ranking among parental alleles across all parents is possible.

A comparison of the number of QTLs identified by each model yielded mixed conclusions. Many of the bi-parental populations, when analyzed individually, failed to detect significant QTL due to small population sizes. Despite no significant QTLs detected in populations RYS005, RYS006, RYS010, RYS011, RYS012, and RYS013, their genotypic and phenotypic data was still informative in the connected CIM model. Combining data using the connected CIM model, allowed information to be extracted from these datasets that would otherwise be uninformative.

In theory, connected models should benefit from an increase in power to detect QTL as the number of individuals in a marker class increases when combining populations. Despite this theoretical advantage, there were a few instances when QTLs were identified within and across environments using the bi-parental CIM model with no corresponding identification of significant QTL by the connected CIM model. The increase in power to

detect QTL should result in more QTLs detected by the connected model compared that of the bi-parental model; however, this was not observed. The connected model detected 60 significant QTL compared to the 96 QTLs detected by the bi-parental model. Figure 8 (A-B) depict the number of chromosomal regions associated with IDC severity and yield detected by the connected and the bi-parental models as well as by both models. This diagram illustrates the trend, where the connected model failed to detect more regions associated with IDC tolerance/susceptibility and yield. This discrepancy in the connected CIM model could be due to several factors. Firstly, it is likely that spurious QTLs were identified in the bi-parental CIM model. Exceedingly large LOD scores may be indicative of a spurious QTL. This occurs in regions with low genotypic information available such as when large gaps in marker coverage exist (Broman et al., 2003). QTLs were detected within regions of large monomorphic gaps for many of the bi-parental populations within this study, and it is quite possible that singleton QTL within those regions are spurious.

The network of populations analyzed within this study suffered from an unbalanced crossing design. It is recommended to develop a network of connected populations using a half diallel crossing scheme to ensure the probability of balanced genotypic classes. Unbalanced designs may be more prone to the prevalence of minor allele frequencies that can reduce the connected CIM model's power to detect QTL. The network of populations analyzed within this thesis not only suffered from an unbalanced crossing design, but it also suffered from small population sizes. Combing population data from this unbalanced network could have decreased the power to detect QTL in some instances. Lastly, because the connected model assumes identity by state (IBS) and not identity by decent (IBD), the connected model may group alleles inappropriately, creating noise in the dataset. The model would be more effective if haplotypes defined by a similarity threshold were used to group genotypes into genotypic classes. Higher density of markers would be necessary to conduct this appropriately, however.

CONCLUSIONS

Similar to the results published by Froechlich and Fehr (1981), IDC tolerant lines yielded less than IDC susceptible lines on non-IDC soils (Table 4). Co-localization of yield and IDC QTLs on A1/5, J/16, and L/19 would suggest that the correlation is due in part to a genetic correlation between yield and IDC (Table 5). Overlapping yield and IDC QTL confidence intervals indicated that the genetic correlation is due to either linkage or pleiotropy. In order to determine the cause of the correlation, a large amount of additional crossing would be needed to observe a recombinant that broke the genetic correlation in the case of a tight linkage. This type of experiment may be unrealistic in a breeding program. Rather it may be more practical to select co-localized QTLs that have favorable additive effects in coupling. For example, selecting for the 04KL111519 parental genotype on LG L/19 between 157.2 and 164.9 cM would increase both IDC tolerance and yield performance (Table 5).

Other yield and IDC QTLs that did not co-localize were also detected. Bi-parental and connected CIM models detected QTLs significantly associated with IDC across and/or within environments on linkage groups A1/5, A2/8, D1b/2, H/12, I/20, L/19, and N/3 (Table 5). No single major QTL explaining the majority of chlorosis variation was detected in any of the populations, individually or combined, except for the Ogden V2 visual chlorosis QTL detected on LG J/16 via the connected model (Table 5). The number of IDC QTL detected in this study was congruent with a complex trait, where multiple loci with smaller effects conferred the trait phenotype. As expected, multiple loci with smaller effects were also significantly associated with yield with the exception of a few larger effect QTL detected on linkage groups C1/4, C2/6, J/16, and O. These larger effect QTL coincide with previously identified regions associated with maturity.

It is highly recommended to screen this network of populations in additional chlorotic environments in order to reduce the percentage of IDC phenotypic variation due to error. High percentages of error can reduce your heritabilities and increase your chance of identifying a false QTL. This thesis focused on reporting genomic regions associated with multiple QTLs detected within multiple populations and/or environments. Knowing that IDC was evaluated in two environments, the error variances were quite large, and false

QTL may have been detected. Permutation tests should minimize the number of false QTL detected; however, further screening is necessary before these QTL should be used for selection in a breeding program.

In general, the connected CIM model was able to resolve large confidence intervals previously detected by the bi-parental CIM model. In addition to more precisely locating the QTL position, the connected model was able to identify QTLs using genotypic and phenotypic data of uninformative bi-parental populations that failed to detect QTL. The connected model also allowed all parental allelic effects to be compared and ranked simultaneously. Unfortunately, however, it is likely that the connected model was unable to detect more QTLs than the bi-parental model due to the unbalanced design of the connected network populations as well as spurious QTL detected in the smaller bi-parental populations. A balanced network of populations like a half diallel should be considered for future QTL studies.

Lastly, objective high throughput phenotyping systems such as the GreenSeeker® RT100 System show promise to help reduce measurement error. Subjective visual ratings often result in misclassification of genotypes into phenotypic classes thus increasing error variances and decreasing power to detect QTL. In this study, visual ratings were highly correlated to NDVI values, supporting the validity of the tool. Each phenotyping method used to evaluate chlorosis within this study was not without fault, however. Other objective high throughput phenotyping technologies are available and should be investigated further.

REFERENCES

Bernardo, R. (2002). Breeding for quantitative traits in plants (Woodbury, Minn: Stemma Press).

Bernardo, R. (2004). What proportion of declared QTL in plants are false? Theoretical and Applied Genetics *109*, 419–424.

Bernardo, R.N. (2010). Breeding for quantitative traits in plants (Woodbury, Minn.: Stemma Press).

Blanc, G., Charcosset, A., Mangin, B., Gallais, A., and Moreau, L. (2006). Connected populations for detecting quantitative trait loci and testing for epistasis: an application in maize. Theoretical and Applied Genetics *113*, 206–224.

Bloom, P.R., and Inskeep, W.P. (1986). Factors affecting bicarbonate chemistry and iron chlorosis in soils. Journal of Plant Nutrition *9*, 215–228.

Bloom, P.R., Rehm, G.W., Lamb, J.A., and Scobbie, A.J. (2011). Soil nitrate is a causative factor in iron deficiency chlorosis in soybeans. Soil Science Society of America Journal *75*, 2233.

Bray, F. (1984). Science and civilization in China, Volume 6. Part II: agriculture (Cambridge University Press, Cambridge).

Broman, K.W., Wu, H., Sen, S., and Churchill, G.A. (2003). R/qtl: QTL mapping in experimental crosses. Bioinformatics *19*, 889–890.

Charlson, D., Cianzio, S., and Shoemaker, R. (2003). Associating SSR markers with soybean resistance to iron deficiency chlorosis. Journal of Plant Nutrition *26*, 2267–2276.

Charlson, D.V., Bailey, T.B., Cianzio, S.R., and Shoemaker, R.C. (2005). Molecular marker Satt481 is associated with iron-deficiency chlorosis resistance in a soybean breeding population. Crop Science *45*, 2394.

Choi, I.-Y., Hyten, D.L., Matukumalli, L.K., Song, Q., Chaky, J.M., Quigley, C.V., Chase, K., Lark, K.G., Reiter, R.S., Yoon, M.-S., et al. (2007). A soybean transcript map: gene distribution, haplotype and single-nucleotide polymorphism analysis. Genetics *176*, 685–696.

Cianzio, S.R. de, and Fehr, W.R. (1980). Genetic control of iron deficiency chlorosis in soybeans. Iowa State Journal of Research *54*, 367–375.

Clark, R.B. (1982). Iron deficiency in plants grown in the great plains of the US. Journal of Plant Nutrition *5*, 251–268.

Coulombe, B.A., Chaney, R.L., and Wiebold, W.J. (1984). Bicarbonate directly induces iron chlorosis in susceptible soybean cultivars. Soil Science Society of America Journal *48*, 1297.

Doerge, R.W., and Churchill, G.A. (1996). Permutation tests for multiple loci affecting a quantitative character. Genetics *142*, 285–294.

Fehr, W.R. (1982). Control of iron-deficiency chlorosis in soybeans by plant breeding. Journal of Plant Nutrition *5*, 611–621.

Fleming, A.L., Chaney, R.L., and Coulombe, B.A. (1984). Bicarbonate inhibits Fe-stress response and Fe uptake-translocation of chlorosis-susceptible soybean cultivars. Journal of Plant Nutrition *7*, 699–714.

Franzen, D.W., and Richardson, J.L. (2000). Soil factors affecting iron chlorosis of soybean in the Red River Valley of North Dakota and Minnesota. Journal of Plant Nutrition *23*, 67–78.

Froechlich, D.M., and Fehr, W.R. (1981). Agronomic performance of soybeans with differing levels of iron deficiency chlorosis on calcareous soil. Crop Science *21*, 438.

Guo, Z., Kishore, V., Kadaru, S., Li, M., Warner, T., and Caton, H. (2010). Network population mapping. PCT/US2010/030983. http://patentscope.wipo.int/search/en/WO2010120844.

Hallauer, A.R., and Miranda, J.B. (1981). Quantitative genetics in maize breeding (Springer).

Hansen, N.C., Schmitt, M.A., Anderson, J.E., and Strock, J.S. (2003). Iron Deficiency of Soybean in the Upper Midwest and Associated Soil Properties. Agronomy Journal *95*, 1595.

Hansen, N.C., Jolley, V.D., Naeve, S.L., and Goos, R.J. (2004). Iron deficiency of soybean in the North Central U.S. and associated soil properties. Soil Science and Plant Nutrition *50*, 983–987.

Hymowitz, T., and Shurtleff, W.R. (2005). Debunking soybean myths and legends in the historical and popular literature. Crop Science *45*, 473–476.

Hyten, D.L., Choi, I.-Y., Song, Q., Specht, J.E., Carter, T.E., Shoemaker, R.C., Hwang, E.-Y., Matukumalli, L.K., and Cregan, P.B. (2010). A High Density Integrated Genetic Linkage Map of Soybean and the Development of a 1536 Universal Soy Linkage Panel for Quantitative Trait Locus Mapping. Crop Science *50*, 960.

Inskeep, W.P., and Bloom, P.R. (1984). A comparative study of soil solution chemistry associated with chlorotic and nonchlorotic soybeans in western Minnesota. Journal of Plant Nutrition *7*, 513–531.

Inskeep, W.P., and Bloom, P.R. (1987). Soil chemical factors associated with soybean chlorosis in calciaquolls of western minnesota. Agronomy Journal *79*, 779–786.

Jannink, J.-L., and Jansen, R. (2001). Mapping epistatic quantitative trait loci with one-dimensional genome searches. Genetics *157*, 445–454.

Jannink, J.-L., Moreau, L., Charmet, G., and Charcosset, A. (2008). Overview of QTL detection in plants and tests for synergistic epistatic interactions. Genetica *136*, 225–236.

Kidwell, K.K., and Osborn, T.C. (1992). Simple plant DNA isolation procedures. In Plant Genomes: Methods for Genetic and Physical Mapping, J.S. Beckmann, and T.C. Osborn, eds. (Springer Netherlands), pp. 1–13.

Lin, S., Cianzio, S.R., and Shoemaker, R.C. (1997). Lin - 1997 - Mapping genetic loci for iron deficiency chlorosis in soybean. Molecular Breeding *3*, 219–229.

Lin, S.F., Grant, D., Cianzio, S., and Shoemaker, R. (2000). Molecular characterization of iron deficiency chlorosis in soybean. J. of Plant Nutrition *23*, 1929–1939.

Lindsay, W.L. (1984). Soil and plant relationships associated with iron deficiency with emphasis on nutrient interactions. Journal of Plant Nutrition *7*, 489–500.

Lindsay, W.L., and Schwab, A.P. (1982). The chemistry of iron in soils and its availability to plants. Journal of Plant Nutrition *5*, 821–840.

Loeppert, R.H. (1986). Reactions of iron and carbonates in calcareous soils. Journal of Plant Nutrition *9*, 195–214.

Lucena, J.J. (2000). Effects of bicarbonate, nitrate and other environmental factors on iron deficiency chlorosis. A review. Journal of Plant Nutrition *23*, 1591–1606.

Lucena, C., Romera, F.J., Rojas, C.L., García, M.J., Alcántara, E., and Pérez-Vicente, R. (2007). Bicarbonate blocks the expression of several genes involved in the physiological responses to Fe deficiency of Strategy I plants. Functional Plant Bioliology *34*, 1002–1009.

Marschner, H., and R\ömheld, V. (1994). Strategies of plants for acquisition of iron. Plant and Soil *165*, 261–274.

Marschner, H., Römheld, V., and Kissel, M. (1986). Different strategies in higher plants in mobilization and uptake of iron. Journal of Plant Nutrition *9*, 695–713.

Masrchner, P. (2012). Marschner's mineral nutrition of higher plants (Elsevier Ltd.).

Morris, D.R., Loeppert, R.H., and Moore, T.J. (1990). Indigenous soil factors influencing iron chlorosis of soybean in calcareous soils. Soil Science Society of America Journal *54*, 1329–1336.

Naeve, S.L., and Rehm, G.W. (2006). Genotype × environment interactions within iron deficiency chlorosis-tolerant soybean genotypes. Agronomy Journal *98*, 808.

O'Rourke, J., Nelson, R., Grant, D., Schmutz, J., Grimwood, J., Cannon, S., Vance, C., Graham, M., and Shoemaker, R. (2009). Integrating microarray analysis and the soybean genome to understand the soybeans iron deficiency response. BMC Genomics *10*, 376.

Peiffer, G.A., King, K.E., Severin, A.J., May, G.D., Cianzio, S.R., Lin, S.F., Lauter, N.C., and Shoemaker, R.C. (2012). Identification of candidate genes underlying an iron efficiency quantitative trait locus in soybean. Plant Physiol. *158*, 1745–1754.

Rebai, A., and Goffinet, B. (1993). Power of tests for QTL detection using replicated progenies derived from a diallel cross. TAG Theoretical and Applied Genetics *86*, 1014–1022.

Schmidt, W. (1999). Review: Mechanisms and regulation of reduction-based iron uptake in plants. New Phytologist *141*, 1–26.

Severin, A.J., Peiffer, G.A., Xu, W.W., Hyten, D.L., Bucciarelli, B., O'Rourke, J.A., Bolon, Y.-T., Grant, D., Farmer, A.D., May, G.D., et al. (2010). An integrative approach to genomic introgression mapping. Plant Physiology *154*, 3–12.

Solie, J.B., Stone, M.L., Raun, R.W., and Johnson, G.V. (2002). Real-time sensing and N fertilization with a field scale greenseekerTM applicator. In In P.C. Robert (ed.) Proc. Int. Conf. Precision Agriculture and Other Precision Resources Management, 6th, Minneapolis, MN.

Wang, J., McClean, P.E., Lee, R., Goos, R.J., and Helms, T. (2008). Association mapping of iron deficiency chlorosis loci in soybean (Glycine max L. Merr.) advanced breeding lines. Theoretical and Applied Genetics *116*, 777–787.

Weiss, M.G. (1943). Inheritance and physiology of efficiency in iron utilization in soybeans. Genetics *28*, 253–268.

Wiersma, J.V. (2010). Nitrate-induced iron deficiency in soybean varieties with varying iron-stress responses. Agronomy Journal *102*, 1738.

Xu, S. (1996). Mapping quantitative trait loci using four-way crosses. Genetics Research *68*, 175–181.

USDA Economic Research Service - Background.
http://www.ers.usda.gov/topics/crops/soybeans-oil=crops/background.aspx#.UYazYrWG2IU

TABLES

Table 1: Summary of quantitative trait loci identified in the literature.

Population	LG	Flanking Loci	R2 (%)	LOD	Software/Analysis	Reference
A81-356022 x PI 468916 60 F2:3	A2	I Locus	17	-	Anova	Diers et al., 1992c
	D1a	C063_1	31	-		
	G	K069_1	11	-		
Pride B216 x A15 120 F2:4	B2	Satt 70-Satt 20 *	11	2.4	Mapmaker QTL	Lin et al., 2000
	I	A515-K644 *	19	2.6		
	H	A404-B69 *	22	2.4		
	B2	A583 - Satt 70 +	11	2.5		
	B2	A519 - Satt 63 +	8	2.4		
	G	T36 - K227 +	9	2.2		
	N	Satt 9 - K418 +	8	2.1		
Anoka x A7 92 F2	A1	K258 - A256 +	35	2.8		
	N	BLT15 - Sat 33 +	72	13		
	I	A515-K644 *	80	3.5		
	N	BLT15 - Sat 33 *	69	7.3		
Pioneer 9254 x A79-770012 150 F2:4	L	Satt448 +	4.3	-	Anova	Charlson et al., 2003
	L	Satt481 +	4.1	-		
Pioneer 9254 x A79-770012 145 F2:4	L	Satt481 +	12	-	Anova	Charlson et al., 2005
Panel A 139 lines; Panel B 115 lines	F	Satt114 +			AM	Wang et al., 2007
	I	Satt239 +				

* Chlorophyll concentration measured.

\+ Visual score measured.

Table 2: Mapping populations. Populations labeled with population identification code, female and male parent names, and number of recombinant inbred lines per mapping population.

Population ID	Female Parent	Male Parent	Number of RILS Per Population
RYS004	04KL109378	03DL052038*	134
RYS005	03KL015763	03DL052038*	81
RYS006	04KL111519	03DL052038*	53
RYS007	04KL109428	03DL052038*	52
RYS008	04KL111531	03DL052038*	178
RYS002	04KL108888*	04KL109428	118
RYS003	04KL108888*	WW221162	158
RYS009	WW221162	04KL111519	170
RYS010	04KL109378	04KL109428	45
RYS011	04KL111531	04KL109378	84
RYS012	04KL109378	03KL015763	98
RYS013	04KL111531	WW221162	39
RYS014	04KL109378	04KL111519	86

* Cultivar is iron deficiency chlorosis tolerant.

Table 3: Summary of REML models. Mean, minimum, and maximum least squares means values calculated using a restricted maximum likelihood method. Variance components are captured as percentages of total variance for each term in the iron deficiency chlorosis and yield generalized linear model for all traits. NDVI (normalized difference vegetative index measured at V2 and V4 stages), chlorosis ratings measured using a 1 to 9 scale at V2 and V4 stages, and yield measured in bushels per acre.

						Iron Deficiency Chlorosis Evaluation					Yield Evaluation						
Population	Trait	Scale or Units	Mean	Min	Max	$\sigma^2_{r(e)}$	σ^2_{e}	σ^2_{g}	σ^2_{eg}	σ^2_{error}	σ^2_{e}	σ^2_{s}	σ^2_{se}	$\sigma^2_{r(se)}$	$\sigma^2_{g(s)}$	$\sigma^2_{eg(s)}$	σ^2_{error}
RYS002	NDVI (V2)	-1 to 1	0.33	0.25	0.37	5.57	2.91	16.45	0.00	75.07	-	-	-	-	-	-	-
	NDVI (V4)	-1 to 1	0.39	0.32	0.45	8.69	0.00	17.46	0.00	73.84	-	-	-	-	-	-	-
	Chlorosis Rating (V2)	1 to 9	5.48	4.16	6.90	9.35	0.00	30.70	0.00	59.95	-	-	-	-	-	-	-
	Chlorosis Rating (V4)	1 to 9	3.61	1.64	6.76	14.60	0.00	35.36	0.00	50.03	-	-	-	-	-	-	-
	Yield	bu ac^{-1}	47.44	39.04	55.90	-	-	-	-	-	45.33	9.59	10.16	1.39	9.25	6.25	18.03
RYS003	NDVI (V2)	-1 to 1	0.33	0.25	0.39	0.00	0.00	34.30	16.48	49.22	-	-	-	-	-	-	-
	NDVI (V4)	-1 to 1	0.37	0.32	0.39	11.86	0.00	8.93	39.05	40.16	-	-	-	-	-	-	-
	Chlorosis Rating (V2)	1 to 9	5.58	4.35	6.65	8.08	0.00	25.83	25.45	40.64	-	-	-	-	-	-	-
	Chlorosis Rating (V4)	1 to 9	3.51	1.81	4.87	20.71	0.00	21.60	7.39	50.30	-	-	-	-	-	-	-
	Yield	bu ac^{-1}	51.45	44.71	59.07	-	-	-	-	-	57.98	2.91	7.37	3.05	7.16	6.31	15.22
RYS004†	NDVI (V2)	-1 to 1	0.35	0.30	0.40	-	28.91	16.05	55.04	-	-	-	-	-	-	-	-
	NDVI (V4)	-1 to 1	0.43	0.40	0.47	-	64.30	7.86	27.84	-	-	-	-	-	-	-	-
	Chlorosis Rating (V2)	1 to 9	4.75	3.55	5.87	-	7.20	46.02	46.77	-	-	-	-	-	-	-	-
	Chlorosis Rating (V4)	1 to 9	2.36	1.08	4.40	-	9.87	43.97	46.16	-	-	-	-	-	-	-	-
	Yield	bu ac^{-1}	44.10	32.54	51.80	-	-	-	-	-	53.65	9.87	10.56	1.99	5.53	6.85	11.56

† Unbalance replication caused convergence errors when estimating variance components and least squares means using a single model; therefore, least squares means were first calculated using a model with replication and genotypes for each environment. Genotypic least squares means were then used in the second model to calculate environment, genotype, and genotype by environment variance components (the error component is confounded within the genotype by environment variance component). For simplification, only the second model without replication is shown.

Table 3 Continued

Population	Trait	Scale or Units	Mean	Min	Max	Iron Deficiency Chlorosis Evaluation					Yield Evaluation						
						$\sigma^2_{r(e)}$	σ^2_{e}	σ^2_{q}	σ^2_{eq}	σ^2_{error}	σ^2_{e}	σ^2_{s}	σ^2_{se}	$\sigma^2_{r(se)}$	$\sigma^2_{q(s)}$	$\sigma^2_{eq(s)}$	σ^2_{error}
	NDVI (V2)	-1 to 1	0.34	0.28	0.39	1.60	13.82	14.34	0.00	70.24	-	-	-	-	-	-	-
	NDVI (V4)	-1 to 1	0.39	0.33	0.46	0.00	7.19	13.34	4.73	74.74	-	-	-	-	-	-	-
RYS005	Chlorosis Rating (V2)	1 to 9	5.15	3.87	6.20	3.21	0.58	22.26	0.00	73.94	-	-	-	-	-	-	-
	Chlorosis Rating (V4)	1 to 9	3.30	1.57	5.43	0.00	8.79	31.27	3.13	56.80	-	-	-	-	-	-	-
	Yield	bu ac^{-1}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NDVI (V2)	-1 to 1	0.31	0.27	0.33	0.00	12.95	9.55	0.00	77.50	-	-	-	-	-	-	-
	NDVI (V4)	-1 to 1	0.38	0.33	0.42	11.18	0.00	11.37	0.00	77.45	-	-	-	-	-	-	-
RYS006	Chlorosis Rating (V2)	1 to 9	4.90	3.99	6.12	0.14	5.94	21.69	0.43	71.80	-	-	-	-	-	-	-
	Chlorosis Rating (V4)	1 to 9	3.65	2.98	5.26	12.06	0.00	12.72	3.78	71.44	-	-	-	-	-	-	-
	Yield	bu ac^{-1}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NDVI (V2)	-1 to 1	0.33	0.30	0.36	11.40	0.00	8.17	7.08	73.35	-	-	-	-	-	-	-
	NDVI (V4)	-1 to 1	0.38	0.29	0.45	1.34	0.00	20.88	3.68	74.10	-	-	-	-	-	-	-
RYS007	Chlorosis Rating (V2)	1 to 9	4.76	3.64	6.12	0.03	8.54	26.47	10.66	54.29	-	-	-	-	-	-	-
	Chlorosis Rating (V4)	1 to 9	3.47	2.56	5.16	6.84	7.14	21.35	6.57	58.09	-	-	-	-	-	-	-
	Yield	bu ac^{-1}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NDVI (V2)	-1 to 1	0.35	0.27	0.41	2.56	0.09	29.62	0.36	67.37	-	-	-	-	-	-	-
	NDVI (V4)	-1 to 1	0.38	0.30	0.49	3.91	0.00	29.22	0.00	66.86	-	-	-	-	-	-	-
RYS008	Chlorosis Rating (V2)	1 to 9	5.05	3.21	6.64	4.03	6.31	34.95	0.00	54.71	-	-	-	-	-	-	-
	Chlorosis Rating (V4)	1 to 9	3.99	2.42	5.81	14.41	0.00	25.57	0.00	60.02	-	-	-	-	-	-	-
	Yield	bu ac^{-1}	44.70	36.70	49.81	-	-	-	-	-	60.28	2.73	8.96	0.85	5.40	8.24	13.55
	NDVI (V2)	-1 to 1	0.26	0.21	0.30	0.00	6.15	17.32	0.00	76.53	-	-	-	-	-	-	-
	NDVI (V4)	-1 to 1	0.26	0.19	0.31	3.37	0.00	15.51	14.87	66.25	-	-	-	-	-	-	-
RYS009	Chlorosis Rating (V2)	1 to 9	6.11	5.43	6.72	4.07	4.97	10.90	2.59	77.47	-	-	-	-	-	-	-
	Chlorosis Rating (V4)	1 to 9	5.59	4.68	6.64	13.61	0.00	8.66	5.52	72.21	-	-	-	-	-	-	-
	Yield	bu ac^{-1}	51.05	46.88	55.04	-	-	-	-	-	46.53	0.38	16.43	2.09	6.01	11.03	17.52

Table 3 Continued

						Iron Deficiency Chlorosis Evaluation					Yield Evaluation						
Population	Trait	Scale or Units	Mean	Min	Max	$\sigma^2_{r(e)}$	σ^2_{e}	σ^2_{q}	σ^2_{eq}	σ^2_{error}	σ^2_{e}	σ^2_{s}	σ^2_{se}	$\sigma^2_{r(se)}$	$\sigma^2_{q(s)}$	$\sigma^2_{eq(s)}$	σ^2_{error}
	NDVI (V2)	-1 to 1	0.27	0.24	0.32	0.91	0.00	15.68	4.98	78.43	-	-	-	-	-	-	-
	NDVI (V4)	-1 to 1	0.34	0.27	0.41	0.00	2.89	25.82	4.16	67.13	-	-	-	-	-	-	-
RYS010	Chlorosis Rating (V2)	1 to 9	6.65	5.15	7.73	8.23	0.00	44.21	0.00	47.56	-	-	-	-	-	-	-
	Chlorosis Rating (V4)	1 to 9	5.25	3.39	7.17	12.10	8.76	26.08	6.49	46.57	-	-	-	-	-	-	-
	Yield	bu ac^{-1}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NDVI (V2)	-1 to 1	0.24	0.16	0.32	0.00	1.81	26.95	11.62	59.62	-	-	-	-	-	-	-
	NDVI (V4)	-1 to 1	0.27	0.19	0.38	0.17	0.89	29.09	5.40	64.47	-	-	-	-	-	-	-
RYS011	Chlorosis Rating (V2)	1 to 9	6.73	5.29	7.65	1.51	0.00	29.98	0.00	68.51	-	-	-	-	-	-	-
	Chlorosis Rating (V4)	1 to 9	6.15	3.87	7.97	2.91	1.74	28.54	0.24	66.58	-	-	-	-	-	-	-
	Yield	bu ac^{-1}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NDVI (V2)	-1 to 1	0.67	0.18	0.35	2.55	18.45	22.82	0.00	56.19	-	-	-	-	-	-	-
	NDVI (V4)	-1 to 1	0.29	0.19	0.40	3.23	0.00	26.97	0.00	69.80	-	-	-	-	-	-	-
RYS012	Chlorosis Rating (V2)	1 to 9	6.29	4.40	7.49	0.10	3.71	31.81	7.17	57.21	-	-	-	-	-	-	-
	Chlorosis Rating (V4)	1 to 9	4.85	2.63	7.06	7.13	4.31	23.27	0.00	65.28	-	-	-	-	-	-	-
	Yield	bu ac^{-1}				-	-	-	-	-	-	-	-	-	-	-	-
	NDVI (V2)	-1 to 1	0.28	0.25	0.30	9.75	14.60	8.61	0.00	67.04	-	-	-	-	-	-	-
	NDVI (V4)	-1 to 1	0.29	0.20	0.35	1.43	5.15	21.20	0.00	72.22	-	-	-	-	-	-	-
RYS013	Chlorosis Rating (V2)	1 to 9	6.81	4.98	7.52	0.84	0.00	29.06	3.57	66.52	-	-	-	-	-	-	-
	Chlorosis Rating (V4)	1 to 9	5.26	3.50	6.87	8.50	7.11	20.22	0.00	64.17	-	-	-	-	-	-	-
	Yield	bu ac^{-1}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NDVI (V2)	-1 to 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NDVI (V4)	-1 to 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
RYS014	Chlorosis Rating (V2)	1 to 9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Chlorosis Rating (V4)	1 to 9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Yield	bu ac^{-1}	42.55	34.73	48.25	-	-	-	-	-	42.95	11.27	11.18	0.81	5.48	8.36	19.94

Table 4: Pearson's correlations between mean yield and iron deficiency chlorosis severity measures across populations and environments. Yield is measured in units of bushels per acre. Chlorosis is measured using a 1 to 9 scale, where a value of 1 indicates no chlorosis. NDVI (normalized difference vegetative index) values range from -1 to 1 with 1 indicating healthy plants.

Variable	by Variable	*r*
Rating V2	NDVI V2	-0.79****
Rating V4	NDVI V4	-0.89****
Rating V4	Rating V2	0.84****
NDVI V4	NDVI V2	0.86****
Yield	NDVI V2	-0.55****
Yield	NDVI V4	-0.61****
Yield	Rating V2	0.56***
Yield	Rating V4	0.54***

*** Significance at p-value < 0.001

**** Significance at p-value < 0.0001

Table 5: Combined CIM results of bi-parental and joint connected models. Five hundread and one thousand permutation tests with a genome-wide significance of ($\alpha = 0.05$) were conducted to determine significance thresholds for the bi-parental and joint connect models, respectively. Traits were analyzed within and across environments. Population type listed with population identification code for bi-parental populations or 'NPM' (network population mapping) for the connected network of populations. Linkage group/chomosome number (LG/Chr), position of peak LOD score (cM), 1-LOD support interval (cM), peak LOD score, proportion of variation explained by the QTL (R2 %), and additive effects of the parental haplotypes of the QTL are reported. A positive additive effect indicates the parental haplotype increases the trait value, and a negative additive effect indicates the parental haplotype decreases the trait value. Additive effects of the

							Additive Effect of Parental Haplotype							
Population(s)	Trait	LG/Chr	LOD Peak Position	1-LOD Support Interval (cM)	LOD Score	R^2 (%)	03DL052038	03KL015763	04KL108888	04KL109378	04KL109428	04KL111519	04KL111531	WW221162
NPM	RatingV2|Across	A1/5	7.1	7.1 - 17.2	3.4	17.8	-0.18	0.18	-0.18	0.18	0.18	0.18	0.18	0.18
RYS004	RatingV2|Across	A1/5	18.2	17.0 - 22.5	4.2	16.2	-0.21	-	-	0.21	-	-	-	-
RYS004	RatingV2|Fort Dodge	A1/5	18.2	17.0 - 22.5	4.2	16.2	-0.21	-	-	0.21	-	-	-	-
RYS004	RatingV2|Ogden	A1/5	18.2	17.0 - 22.5	4.2	16.2	-0.21	-	-	0.21	-	-	-	-
RYS008	RatingV2|Across	A1/5	18.2	17.2 - 23.1	5.3	23.7	-0.44	-	-	-	-	-	0.44	-
RYS008	RatingV2|Fort Dodge	A1/5	18.2	17.2 - 23.1	5.3	23.7	-0.44	-	-	-	-	-	0.44	-
RYS008	RatingV2|Ogden	A1/5	18.2	17.2 - 23.1	5.3	23.7	-0.44	-	-	-	-	-	0.44	-
RYS014	Yield|Brookings (2010)	A1/5	29.0	28.3 - 36.2	6.0	4.5	-	-	-	-0.46	-	0.46	-	-
RYS002	Yield|Owatonna (2010)	A1/5	42.6	23.9 - 46.1	4.8	15.0	-	-	-2.46	-	2.46	-	-	-
RYS009	Yield|Brookings (2009)	A1/5	166.3	153.9 - 166.8	6.3	4.7	-	-	-	-	-	-0.73	-	0.73
RYS009	Yield|Brookings (2009)	A2/8	24.0	0.0 - 26.1	6.1	3.0	-	-	-	-	-	0.51	-	-0.51
RYS003	RatingV4|Ogden	A2/8	49.0	43.0 - 95.6	6.9	10.3	-	-	0.32	-	-	-	-	-0.32
RYS003	NDVIV4|Ogden	A2/8	148.8	94.6 - 163.3	7.9	46.5	-	-	-0.043	-	-	-	-	0.043
RYS003	Yield|Brookings (2010)	A2/8	159.8	94.1 - 163.2	4.4	5.3	-	-	-0.71	-	-	-	-	0.71
NPM	NDVIV2|Across	A2/8	185.1	185.1 - 185.1	4.0	36.7	-0.013	-0.013	-0.013	0.013	-0.013	-0.013	0.013	0.013
RYS003	NDVIV4|Ogden	A2/8	189.1	184.9 - 216.7	8.8	46.8	-	-	-0.089	-	-	-	-	0.089
RYS002	RatingV4|Across	A2/8	196.8	94.1 - 215.6	5.0	47.2	-	-	-0.80	-	0.80	-	-	-
RYS002	RatingV4|Fort Dodge	A2/8	196.8	94.1 - 215.6	5.0	47.2	-	-	-0.80	-	0.80	-	-	-
RYS002	RatingV4|Ogden	A2/8	196.8	94.1 - 215.6	5.0	47.2	-	-	-0.80	-	0.80	-	-	-
NPM	NDVIV2|Across	B1/11	3.0	3.0 - 22.2	3.6	57.0	0.037	-0.037	0.037	0.037	0.037	0.037	0.037	0.037
NPM	NDVIV2|Ogden	B1/11	3.0	3.0 - 22.2	3.2	70.9	0.020	-0.020	0.020	0.020	0.020	0.020	0.020	0.020
RYS009	Yield|Thorndale (2010)	B1/11	105.7	29.1 - 127.3	8.0	32.2	-	-	-	-	-	-1.31	-	1.31
RYS003	NDVIV2|Ogden	B1/11	142.1	126.6 - 180.9	9.0	17.9	-	-	0.023	-	-	-	-	-0.023
RYS003	NDVIV2|Fort Dodge	B1/11	169.1	126.7 - 180.8	9.4	42.9	-	-	0.036	-	-	-	-	-0.036
RYS003	RatingV4|Ogden	B2/14	45.4	36.1 - 64.4	11.4	22.6	-	-	-0.61	-	-	-	-	0.61
NPM	Yield|Owatonna (2009)	C1/4	4.4	4.0 - 5.1	6.5	47.6	3.36	3.36	3.36	-3.36	3.36	3.36	3.36	3.36
NPM	RatingV4|Ogden	C1/4	5.1	4.1 - 5.1	3.0	24.6	-0.06	-0.06	-0.06	-0.42	0.48	-0.06	-0.06	-0.06
RYS004	Yield|Owatonna (2009)	C1/4	36.4	4.0 - 87.5	21.9	89.9	9.54	-	-	-9.537	-	-	-	-
RYS004	Yield|Across	C1/4	69.4	3.8 - 87.2	5.0	18.9	1.68	-	-	-1.683	-	-	-	-
NPM	Yield|Owatonna (2009)	C1/4	87.8	5.1 - 87.8	4.7	43.7	3.09	-3.09	-3.09	-3.09	-3.09	-3.09	-3.09	-3.09
NPM	Yield|Stanton (2009)	C1/4	87.8	87.8 - 87.8	3.8	51.2	2.33	-2.33	-2.33	-2.33	-2.33	-2.33	-2.33	-2.33
NPM	NDVIV2|Ogden	C1/4	121.8	117.1 - 146.0	3.5	36.2	0.037	-0.031	0.037	0.037	-0.006	-0.006	0.037	0.037
NPM	NDVIV2|Across	C1/4	125.6	117.1 - 130.2	*4.4*	41.1	0.033	-0.031	0.033	0.033	-0.002	-0.002	0.033	0.033

Table 5: Continued.

							Additive Effect of Parental Haplotype							
Population(s)	Trait	LG/Chr	LOD Peak Position	1-LOD Support Interval (cM)	LOD Score	R^2 (%)	03DL052038	03KL015763	04KL108888	04KL109378	04KL109428	04KL111519	04KL111531	WW221162
RYS009	Yield|Owatonna (2009)	C2/6	151.2	150.9 - 172.0	3.9	8.3	-	-	-	-	-	-0.87	-	0.87
NPM	Yield|Stanton (2010)	C2/6	170.5	165.9 - 173.4	2.8	15.9	-2.15	1.38	0.77	0.77	0.77	1.38	1.38	1.38
NPM	Yield|Thorndale (2009)	C2/6	172.3	165.9 - 173.8	3.3	15.1	1.57	-1.22	-0.35	-0.35	-0.35	-1.22	-1.22	-0.35
RYS008	Yield|Across	C2/6	182.1	182.1 - 184.0	15.3	34.2	-1.45	-	-	-	-	-	1.446	-
RYS008	Yield|Brookings (2009)	C2/6	182.1	182.1 - 184.0	7.7	23.8	-1.44	-	-	-	-	-	1.444	-
RYS008	Yield|Nevada (2009)	C2/6	182.1	182.1 - 184.0	9.0	26.8	-4.11	-	-	-	-	-	4.109	-
RYS008	Yield|Nevada (2010)	C2/6	182.1	182.1 - 184.0	5.0	17.7	-1.81	-	-	-	-	-	1.807	-
RYS008	Yield|Owatonna (2009)	C2/6	182.1	182.1 - 184.0	15.6	42.8	-2.14	-	-	-	-	-	2.145	-
RYS008	Yield|Stanton (2010)	C2/6	182.1	182.1 - 184.0	11.5	32.5	-3.08	-	-	-	-	-	3.085	-
RYS008	Yield|Thorndale (2009)	C2/6	182.1	182.1 - 184.0	7.1	22.0	-1.80	-	-	-	-	-	1.804	-
RYS004	Yield|Thorndale (2009)	C2/6	184.9	178.0 - 190.1	9.7	21.8	-1.91	-	-	1.913	-	-	-	-
RYS004	Yield|Brookings (2009)	C2/6	184.9	184.9 - 185.1	9.0	22.5	-2.66	-	-	2.665	-	-	-	-
RYS004	Yield|Nevada (2009)	C2/6	185.1	183.7 - 187.4	6.3	13.5	-2.26	-	-	2.255	-	-	-	-
RYS004	Yield|Across	C2/6	185.1	184.3 - 187.4	10.2	14.9	-1.62	-	-	1.616	-	-	-	-
RYS004	Yield|Nevada (2010)	C2/6	186.1	184.8 - 187.4	7.4	17.4	-2.79	-	-	2.791	-	-	-	-
RYS004	Yield|Stanton (2009)	C2/6	186.1	184.8 - 187.4	15.6	31.5	-3.76	-	-	3.758	-	-	-	-
NPM	Yield|Across	C2/6	187.0	183.4 - 199.6	8.2	38.1	-1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45
NPM	Yield|Brookings (2009)	C2/6	187.0	183.4 - 209.7	7.7	33.5	-1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.55
NPM	Yield|Owatonna (2009)	C2/6	187.0	185.1 - 208.0	7.8	33.7	-2.51	2.51	2.51	2.51	2.51	2.51	2.51	2.51
NPM	Yield|Nevada (2010)	C2/6	187.0	185.1 - 208.0	3.7	20.4	-1.78	1.78	1.78	1.78	1.78	1.78	1.78	1.78
NPM	Yield|Thorndale (2009)	C2/6	187.0	185.1 - 209.7	8.7	36.2	-1.85	1.85	1.85	1.85	1.85	1.85	1.85	1.85
NPM	Yield|Owatonna (2010)	C2/6	187.0	187.0 - 208.0	4.5	23.1	-1.89	1.89	1.89	1.89	1.89	1.89	1.89	1.89
NPM	Yield|Nevada (2009)	C2/6	191.2	183.4 - 209.7	7.8	30.1	-4.44	-0.08	-0.08	-0.08	-0.08	-0.08	4.51	-0.08
NPM	Yield|Stanton (2010)	C2/6	191.2	187.0 - 209.7	7.8	37.0	-2.33	-1.49	-1.49	-1.49	-1.49	-1.49	3.82	-1.49
RYS008	Yield|Owatonna (2009)	C2/6	194.7	186.8 - 218.0	9.2	47.2	2.20	-	-	-	-	-	-2.198	-
RYS003	RatingV2|Ogden	C2/6	197.7	170.7 - 240.7	8.0	33.7	-	-	0.43	-	-	-	-	-0.43
RYS008	Yield|Brookings (2009)	C2/6	217.7	186.8 - 218.0	6.2	15.4	-1.20	-	-	-	-	-	1.197	-
RYS003	NDVIV2|Fort Dodge	D1a/1	82.7	49.5 - 94.6	11.3	41.6	-	-	0.038	-	-	-	-	-0.038
RYS003	NDVIV2|Fort Dodge	D1a/1	101.6	94.6 - 153.9	11.2	41.6	-	-	0.038	-	-	-	-	-0.038
RYS003	NDVIV4|Ogden	D1a/1	106.6	94.6 - 153.7	7.1	2.6	-	-	-0.041	-	-	-	-	0.041

Table 5: Continued.

							Additive Effect of Parental Haplotype							
Population(s)	Trait	LG/Chr	LOD Peak Position	1-LOD Support Interval (cM)	LOD Score	R^2 (%)	03DL052038	03KL015763	04KL108888	04KL109378	04KL109428	04KL111519	04KL111531	WW221162
RYS003	RatingV4|Ogden	D1b/2	27.5	26.4 - 41.1	8.7	13.1	-	-	0.48	-	-	-	-	-0.48
RYS003	NDVIV2|Fort Dodge	D1b/2	31.5	26.1 - 40.8	10.7	46.2	-	-	-0.042	-	-	-	-	0.042
RYS003	RatingV4|Across	D1b/2	39.5	25.7 - 40.3	6.6	16.1	-	-	-0.38	-	-	-	-	0.38
RYS003	NDVIV2|Across	D1b/2	53.7	43.7 - 87.7	7.6	47.2	-	-	0.028	-	-	-	-	-0.028
RYS003	RatingV4|Across	D1b/2	63.7	43.3 - 87.5	8.1	18.9	-	-	-0.41	-	-	-	-	0.41
RYS003	NDVIV2|Fort Dodge	D1b/2	75.7	43.5 - 89.7	8.3	47.3	-	-	0.036	-	-	-	-	-0.036
RYS007	NDVIV4|Across	D1b/2	94.9	99.1 - 99.5	6.5	44.3	0.020	-	-	-	-0.020	-	-	-
RYS007	NDVIV4|Ogden	D1b/2	99.5	99.0 - 109.4	9.3	45.6	0.023	-	-	-	-0.023	-	-	-
RYS007	NDVIV4|Fort Dodge	D1b/2	101.5	99.4 - 109.1	6.5	42.8	0.021	-	-	-	-0.021	-	-	-
RYS003	NDVIV4|Across	D1b/2	105.8	103.4 - 108.7	7.6	35.3	-	-	-0.008	-	-	-	-	0.008
NPM	RatingV4|Ogden	D1b/2	108.4	106.9 - 108.4	2.9	12.6	-0.22	-0.22	0.22	0.22	0.22	-0.22	-0.22	-0.22
NPM	RatingV4|Fort Dodge	D1b/2	108.4	107.9 - 108.4	2.8	12.2	-0.21	-0.21	0.21	0.21	0.21	-0.21	-0.21	-0.21
RYS007	NDVIV4|Across	D1b/2	108.4	108.4 - 138.3	4.7	29.9	-0.015	-	-	-	0.015	-	-	-
RYS003	RatingV2|Ogden	D2/17	121.4	113.0 - 134.2	8.3	5.4	-	-	0.37	-	-	-	-	-0.37
RYS003	RatingV2|Ogden	D2/17	166.1	153.7 - 172.8	11.6	20.6	-	-	0.40	-	-	-	-	-0.40
RYS009	Yield|Brookings (2009)	E/15	64.2	44.6 - 80.3	6.1	2.3	-	-	-	-	-	-0.29	-	0.29
RYS002	Yield|Brookings (2010)	E/15	157.9	157.1 - 168.6	3.9	12.7	-	-	-1.07	-	1.07	-	-	-
RYS002	Yield|Brookings (2009)	F/13	104.3	57.1 - 128.3	5.0	21.5	-	-	1.98	-	-1.98	-	-	-
RYS002	Yield|Brookings (2009)	F/13	144.2	128.0 - 154.7	5.4	35.8	-	-	1.76	-	-1.76	-	-	-
RYS003	NDVIV4|Ogden	F/13	181.2	170.9 - 197.9	7.8	2.1	-	-	-0.043	-	-	-	-	0.043
RYS008	Yield|Stanton (2009)	F/13	195.2	127.5 - 207.0	5.2	33.7	-2.15	-	-	-	-	-	2.155	-
RYS008	Yield|Thorndale (2010)	F/13	203.2	127.4 - 207.0	5.0	45.2	-1.87	-	-	-	-	-	1.868	-
NPM	Yield|Brookings (2009)	F/13	226.9	137.7 - 226.9	3.9	25.3	-1.27	1.27	1.27	1.27	1.27	1.27	1.27	1.27
RYS009	Yield|Stanton (2009)	G/18	0.0	0.0 - 25.8	4.6	15.2	-	-	-	-	-	1.48	-	-1.48
NPM	Yield|Brookings (2010)	G/18	23.2	20.9 - 23.2	3.0	9.4	-0.10	-0.74	-0.74	-0.74	-0.74	-0.10	-0.74	0.84
RYS009	Yield|Brookings (2009)	G/18	181.0	180.7 - 199.2	6.1	3.5	-	-	-	-	-	-0.39	-	0.39
RYS008	NDVIV2|Across	H/12	94.5	67.2 - 116.9	6.2	28.3	0.018	-	-	-	-	-	-0.018	-
RYS008	NDVIV2|Fort Dodge	H/12	94.5	67.2 - 116.9	6.1	28.7	0.018	-	-	-	-	-	-0.018	-
RYS008	NDVIV2|Ogden	H/12	94.5	67.2 - 116.9	6.3	27.7	0.018	-	-	-	-	-	-0.018	-
RYS008	NDVIV2|Across	H/12	155.4	143.3 - 166.0	4.6	30.3	0.018	-	-	-	-	-	-0.018	-
RYS004	NDVIV2|Fort Dodge	H/12	172.4	143.1 - 200.5	5.3	17.3	0.021	-	-	-0.021	-	-	-	-
RYS009	Yield|Brookings (2009)	I/20	82.7	82.4 - 94.6	6.0	0.7	-	-	-	-	-	-0.15	-	0.15
RYS003	NDVIV4|Ogden	I/20	84.6	67.4 - 98.6	7.0	4.3	-	-	0.051	-	-	-	-	-0.051
NPM	NDVIV2|Across	I/20	118.8	118.8 - 120.0	4.6	46.4	0.016	-0.016	0.016	0.016	0.016	0.016	0.016	0.016
NPM	NDVIV2|Ogden	I/20	118.8	118.8 - 120.0	3.8	42.1	0.016	-0.016	0.016	0.016	0.016	0.016	0.016	0.016
RYS003	NDVIV4|Fort Dodge	J/16	56.0	39.9 - 164.3	7.1	40.3	-	-	0.038	-	-	-	-	-0.038
NPM	RatingV2|Ogden	J/16	80.4	80.4 - 174.8	2.8	93.8	-2.66	1.15	1.15	1.15	1.15	1.15	1.51	1.15
RYS002	Yield|Owatonna (2010)	J/16	111.3	64.1 - 141.7	5.9	5.8	-	-	3.10	-	-3.10	-	-	-
NPM	Yield|Owatonna (2010)	J/16	147.5	147.5 - 164.9	6.0	31.1	-2.31	-2.31	2.31	2.31	-2.31	2.31	-2.31	2.31

Table 5: Continued.

Population(s)	Trait	LG/Chr	LOD Peak Position	1-LOD Support Interval (cM)	LOD Score	R^2 (%)	Additive Effect of Parental Haplotype							
							03DL052038	03KL015763	04KL108888	04KL109378	04KL109428	04KL111519	04KL111531	WW221162
RYS009	Yield|Thorndale (2010)	K/9	51.3	42.3 - 163.5	12.3	33.0	-	-	-	-	-	-1.28	-	1.28
RYS003	Yield|Stanton (2009)	K/9	99.6	75.3 - 106.8	3.8	6.8	-	-	-1.35	-	-	-	-	1.35
RYS003	Yield|Stanton (2009)	K/9	112.0	105.5 - 145.6	3.9	7.3	-	-	-1.33	-	-	-	-	1.33
NPM	NDVIV4|Across	K/9	190.7	190.7 - 191.7	3.4	42.4	-0.019	0.019	-0.019	-0.019	0.019	0.019	0.019	0.019
RYS002	RatingV2|Across	L/19	54.8	54.4 - 72.9	5.5	13.1	-	-	-0.34	-	0.34	-	-	-
RYS002	RatingV2|Fort Dodge	L/19	54.8	54.4 - 72.9	5.5	13.1	-	-	-0.34	-	0.34	-	-	-
RYS002	RatingV2|Ogden	L/19	54.8	54.4 - 72.9	5.5	13.1	-	-	-0.34	-	0.34	-	-	-
NPM	RatingV2|Ogden	L/19	98.0	90.4 - 102.3	2.89	19.50	-0.20	0.20	-0.20	0.20	0.20	0.20	0.20	0.20
NPM	RatingV2|Across	L/19	98.0	97.9 - 102.3	5.06	27.62	-0.24	0.24	-0.24	0.24	0.24	0.24	0.24	0.24
RYS004	RatingV2|Across	L/19	99.8	34.1 - 99.9	4.36	17.71	-0.22	-	-	0.22	-	-	-	-
RYS004	RatingV2|Fort Dodge	L/19	99.8	34.1 - 99.9	4.36	17.71	-0.22	-	-	0.22	-	-	-	-
RYS004	RatingV2|Ogden	L/19	99.8	34.1 - 99.9	4.36	17.71	-0.22	-	-	0.22	-	-	-	-
NPM	NDVIV2|Across	L/19	160.9	157.2 - 163.0	5.4	18.3	0.011	-0.011	0.011	0.011	0.011	0.011	-0.011	0.011
NPM	NDVIV2|Ogden	L/19	160.9	157.2 - 164.3	4.6	20.8	0.010	-0.010	0.010	0.010	0.010	0.010	-0.010	0.010
NPM	NDVIV2|Fort Dodge	L/19	160.9	157.2 - 164.3	3.9	18.4	0.010	-0.010	0.010	0.010	0.010	0.010	-0.010	0.010
RYS009	Yield|Owatonna (2009)	L/19	161.4	159.5 - 164.9	5.1	13.4	-	-	-	-	-	1.14	-	-1.14
RYS009	Yield|Brookings (2010)	L/19	161.4	161.4 - 164.6	7.6	15.4	-	-	-	-	-	1.01	-	-1.01
NPM	NDVIV2|Ogden	M/7	6.7	5.4 - 8.2	3.2	13.4	-0.001	-0.009	0.010	0.010	0.010	0.010	-0.001	0.010
RYS004	NDVIV4|Fort Dodge	M/7	16.4	5.1 - 24.51	6.0	30.1	-0.012	-	-	0.012	-	-	-	-
RYS008	Yield|Owatonna (2010)	M/7	37.9	33.7 - 51.8	6.3	20.3	-2.17	-	-	-	-	-	2.165	-
RYS002	RatingV2|Across	M/7	68.4	58.0 - 77.7	5.2	42.3	-	-	0.44	-	-0.44	-	-	-
RYS002	RatingV2|Fort Dodge	M/7	68.4	58.0 - 77.7	5.2	42.3	-	-	0.44	-	-0.44	-	-	-
RYS002	RatingV2|Ogden	M/7	68.4	58.0 - 77.7	5.2	42.3	-	-	0.44	-	-0.44	-	-	-
RYS009	Yield|Stanton (2009)	M/7	141.9	130.4 - 143.9	8.9	30.2	-	-	-	-	-	2.21	-	-2.21
RYS009	Yield|Brookings (2009)	M/7	147.3	147.2 - 241.1	7.4	7.5	-	-	-	-	-	0.68	-	-0.68
RYS009	Yield|Stanton (2009)	M/7	159.3	146.6 - 240.1	7.8	57.2	-	-	-	-	-	3.01	-	-3.01
RYS009	Yield|Owatonna (2010)	N/3	38.1	26.9 - 43.3	4.7	15.8	-	-	-	-	-	-1.50	-	1.50
NPM	Yield|Brookings (2010)	N/3	289.8	295.9 - 301.8	4.1	10.1	-0.59	0.00	-0.04	0.52	-0.29	-0.84	0.10	1.14
NPM	RatingV2|Across	N/3	295.9	295.9 - 300.4	3.2	17.0	-0.21	0.20	-0.02	-0.28	0.20	0.03	0.09	-0.01
NPM	RatingV2|Ogden	N/3	295.9	295.9 - 300.4	3.9	23.3	-0.22	0.24	0.00	-0.33	0.23	-0.04	0.09	0.04
NPM	RatingV2|Fort Dodge	N/3	295.9	295.9 - 300.4	4.1	27.3	-0.30	0.16	0.00	-0.38	0.32	-0.07	0.19	0.09
RYS009	Yield|Brookings (2010)	N/3	299.8	293.1 - 301.9	4.4	10.0	-	-	-	-	-	-0.85	-	0.85

Table 5: Continued.

Population(s)	Trait	LG/Chr	LOD Peak Position	1-LOD Support Interval (cM)	LOD Score	R^2 (%)	Additive Effect of Parental Haplotype							
							03DL052038	03KL015763	04KL108888	04KL109378	04KL109428	04KL111519	04KL111531	WW221162
NPM	Yield|Brookings (2009)	O/10	137.4	132.6 - 141.4	10.9	73.2	-1.55	-1.74	-1.74	4.01	-0.72	4.01	-1.74	-1.74
NPM	Yield|Thorndale (2009)	O/10	143.0	141.4 - 147.8	22.1	42.9	-3.20	-3.20	-3.20	0.97	0.97	0.97	2.23	2.23
NPM	Yield|Owatonna (2010)	O/10	143.0	141.4 - 147.8	21.4	43.0	-4.09	-4.09	-4.09	3.90	3.90	3.90	0.19	0.19
NPM	Yield|Nevada (2009)	O/10	143.0	141.4 - 149.4	9.0	23.7	-3.98	-3.98	-3.98	3.55	3.55	3.55	0.43	0.43
NPM	Yield|Stanton (2009)	O/10	144.6	141.4 - 146.2	34.8	54.0	-4.84	-4.84	-4.84	2.17	2.17	2.17	2.67	2.67
NPM	Yield|Owatonna (2009)	O/10	144.6	141.4 - 146.2	52.4	65.3	-6.66	-6.66	-6.66	2.74	2.74	2.74	3.92	3.92
NPM	Yield|Across	O/10	144.6	141.4 - 147.8	28.7	53.4	-2.97	-2.97	-2.97	1.28	1.28	1.28	-2.97	1.70
NPM	Yield|Brookings (2010)	O/10	144.6	141.4 - 147.8	18.6	39.9	-2.17	-2.17	-2.17	0.53	0.53	0.53	1.64	1.64
NPM	Yield|Stanton (2010)	O/10	144.6	141.4 - 149.4	8.3	22.5	-2.63	-2.63	-2.63	0.91	0.91	0.91	1.72	1.72
NPM	Yield|Nevada (2010)	O/10	146.2	141.4 - 149.4	28.6	47.6	-4.99	-4.99	-4.99	3.23	3.23	3.23	1.77	1.77
NPM	Yield|Brookings (2009)	O/10	166.8	149.8 - 168.1	9.6	34.5	-2.24	-2.24	-2.24	2.11	2.11	0.13	0.13	0.13
NPM	Yield|Across	O/10	168.1	166.8 - 170.5	24.5	55.8	-1.97	-1.97	-1.97	1.97	1.97	1.97	-1.97	1.97
NPM	Yield|Nevada (2009)	O/10	168.1	166.8 - 170.5	5.8	30.3	-3.26	-3.26	-3.26	3.26	3.26	-3.26	-3.26	-3.26
NPM	Yield|Owatonna (2010)	O/10	170.5	168.1 - 176.1	10.8	70.2	-1.74	-5.03	-1.74	1.58	5.20	-1.74	-1.74	-1.74
NPM	Yield|Nevada (2010)	O/10	170.5	168.1 - 176.1	18.5	82.2	-1.32	-7.37	-1.32	2.86	5.82	-1.32	-1.32	-1.32
NPM	Yield|Stanton (2009)	O/10	170.5	168.1 - 178.7	25.2	86.6	-1.46	-7.23	-1.46	6.435	2.245	-1.46	-1.46	-1.46
NPM	Yield|Owatonna (2009)	O/10	170.5	168.1 - 178.7	41.9	91.6	-2.12	-10.18	-2.12	9.40	2.91	-2.12	-2.12	-2.12
NPM	Yield|Brookings (2010)	O/10	170.5	168.1 - 178.7	13.1	78.1	0.02	-3.42	0.02	1.58	1.83	0.02	0.02	0.02
NPM	Yield|Thorndale (2009)	O/10	172.9	168.1 - 176.1	14.9	80.9	-0.61	-5.07	-0.61	3.08	2.60	-0.61	-0.61	-0.61
NPM	Yield|Stanton (2010)	O/10	175.4	166.8 - 176.1	8.4	67.3	-0.22	-4.29	-0.22	-0.55	5.06	-0.22	-0.22	-0.22

FIGURES

Figure 1A-1D: Images of IDC hill plots. Images taken of the 2009 IDC evaluation trial in Ogden, IA (1A-1B), and Nevada, IA (1C) at plant growth stages V2 and V4. V2 and V4 stage soybeans failed to express chlorosis in both locations. 1B image shows 03DL052038 (center left) and KE107 (center right), which are tolerant and susceptible checks, respectively. Images depict a lack of chlorotic variation among individuals in the thirteen segregating RIL populations. 1D image shows the expected chlorosis phenotype of a tolerant cultivar (top image) and a susceptible cultivar (bottom image).

1A

1B

1C

1D

Figure 2: Depiction of the network of 13 RIL populations. Boxes depict the 8 parents of the 13 populations. Twelve populations evaluated for iron deficiency chlorosis are depicted in blue; one population evaluated for yield is depicted in red; five populations evaluated for iron deficiency chlorosis and yield are depicted in purple.

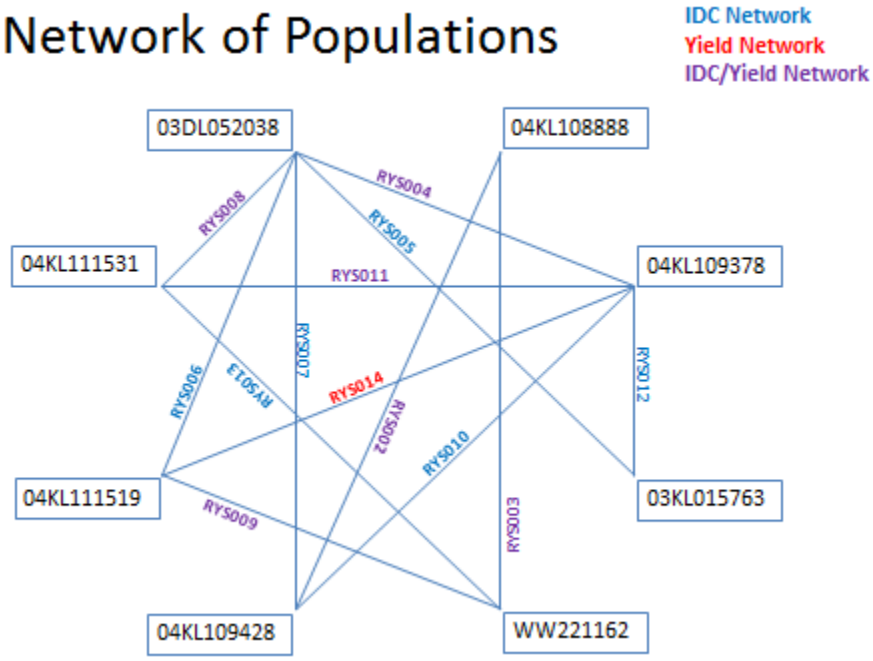


Figure 3: Estimated surface for the Ogden environment based on NDVI values at V4 of the repeated check cultivar of S21-N6.

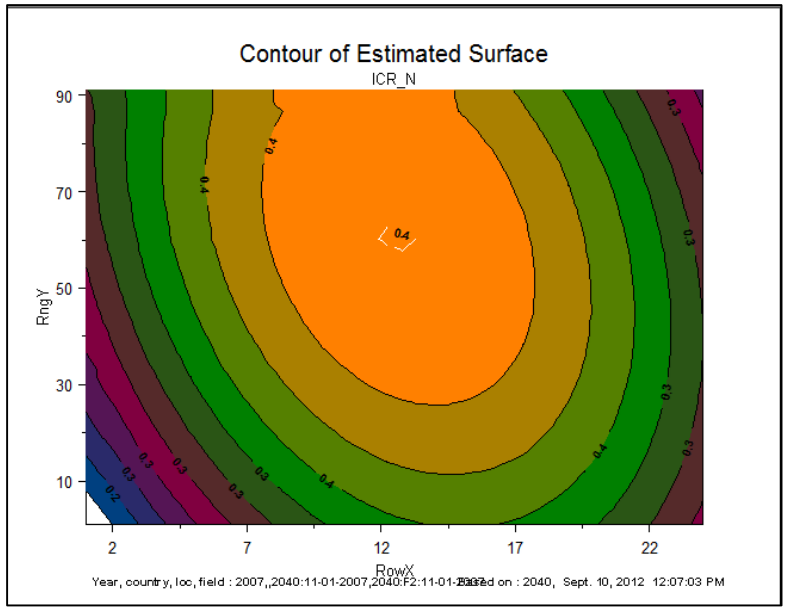


Figure 4: Estimated surface for the Ogden environment based on chlorosis rating at V4 of the repeated check cultivar of S21-N6.

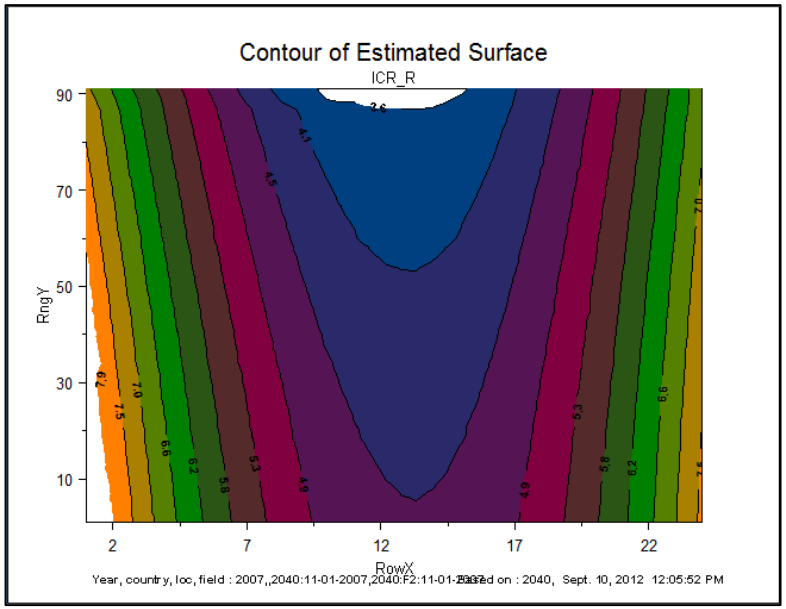


Figure 5: Estimated surface for the Fort Dodge environment based on NDVI values at V2 of the repeated check cultivar of S21-N6. .

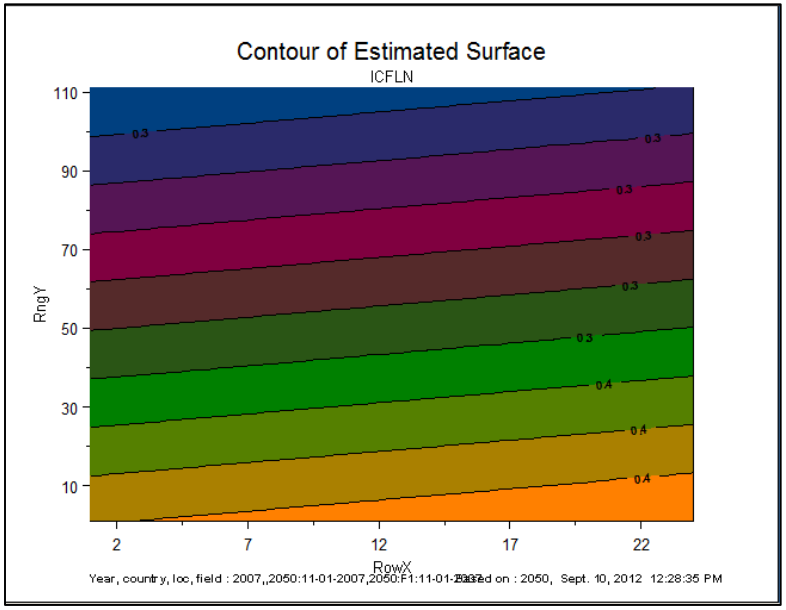


Figure 6: Estimated surface for the Fort Dodge environment based on chlorosis rating at V2 of the repeated check cultivar of S21-N6.

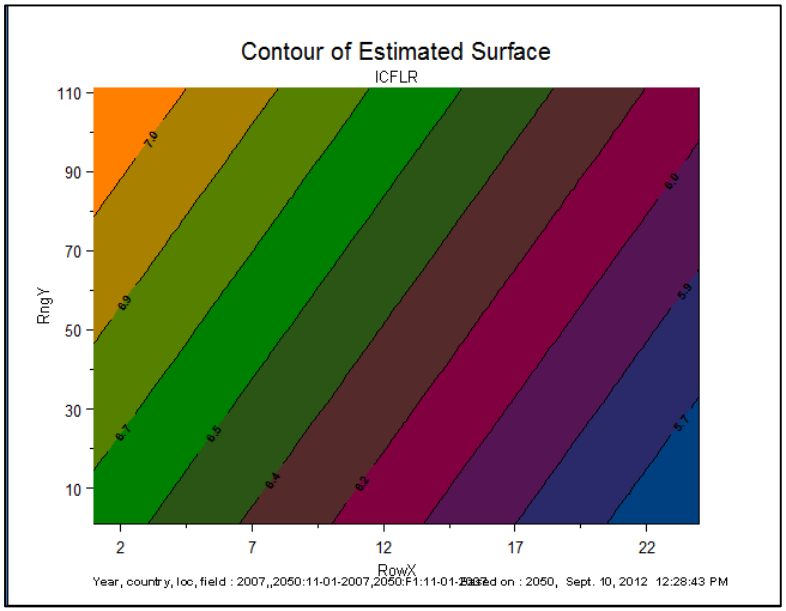


Figure 7: Estimated surface for the Fort Dodge environment based on chlorosis rating at V4 of the repeated check cultivar of S21-N6.

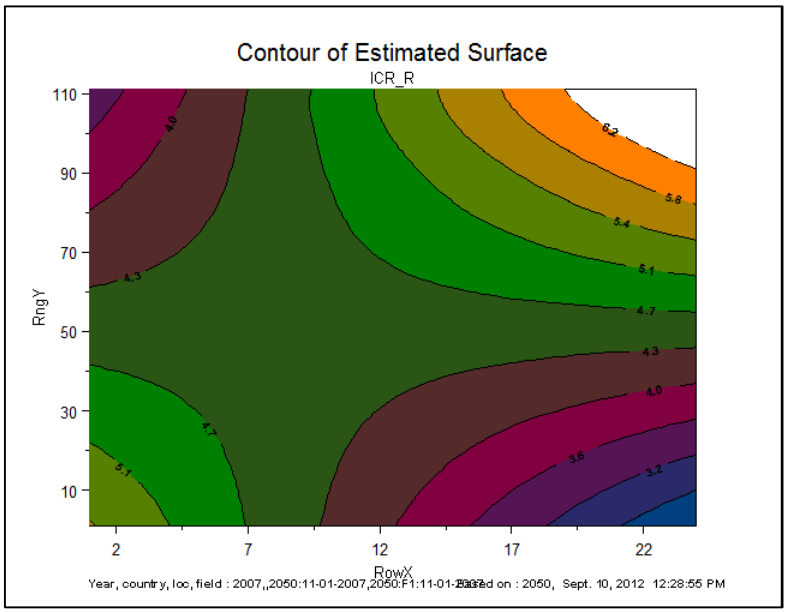
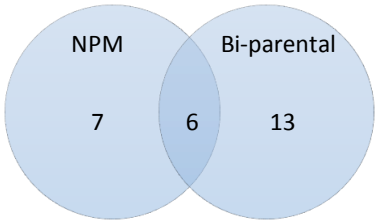
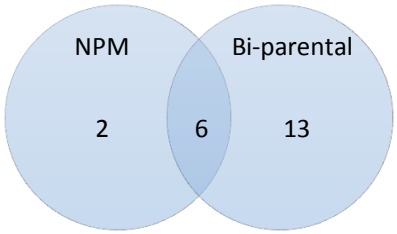


Figure 8 (A-B): Venn diagram depicting the number of chromosomal regions associated with IDC tolerance/susceptibility (A) and yield (B) using the connected and/or bi-parental models.

A:



B:



:

APPENDIX

Appendix A: Correlations between mean yield and iron deficiency chlorosis severity measures across environments by population. Yield is measured in units of bushels per acre. Chlorosis is measured using a 1 to 9 scale, where a value of 1 indicates no chlorosis. NDVI (normalized difference vegetative index) values range from -1 to 1 with 1 indicating healthy plants.

Population	Variable	by Variable	*r*
RYS002	Yield	NDVI V2	-0.12
RYS002	Yield	NDVI V4	-0.13
RYS002	Yield	Rating V2	0.35*
RYS002	Yield	Rating V4	0.322
RYS003	Yield	NDVI V2	-0.40
RYS003	Yield	NDVI V4	-0.12
RYS003	Yield	Rating V2	0.123
RYS003	Yield	Rating V4	0.131
RYS004	Yield	NDVI V2	0.125
RYS004	Yield	NDVI V4	0.104
RYS004	Yield	Rating V2	-0.06
RYS004	Yield	Rating V4	-0.16
RYS008	Yield	NDVI V2	-0.35*
RYS008	Yield	NDVI V4	-0.39*
RYS008	Yield	Rating V2	0.289
RYS008	Yield	Rating V4	0.40*
RYS009	Yield	NDVI V2	-0.01
RYS009	Yield	NDVI V4	-0.11
RYS009	Yield	Rating V2	0.167
RYS009	Yield	Rating V4	0.125

* Significance at p-value < 0.05

Appendix B: Correlations between mean NDVI at V2 and V4 and chlorosis severity rating at V2 and V4 across environments by population. Chlorosis is measured using a 1 to 9 scale, where a value of 1 indicates no chlorosis. NDVI (normalized difference vegetative index) values range from -1 to 1 with 1 indicating healthy plants.

Population	Variable	by Variable	*r*
RYS002	Rating V2	NDVI V2	-0.66***
RYS002	Rating V4	NDVI V4	-0.79***
RYS002	NDVI V4	NDVI V2	0.55***
RYS002	Rating V4	Rating V2	0.84***
RYS003	Rating V2	NDVI V2	-0.76***
RYS003	Rating V4	NDVI V4	-0.58***
RYS003	NDVI V4	NDVI V2	0.68***
RYS003	Rating V4	Rating V2	0.76***
RYS004	Rating V2	NDVI V2	-0.47***
RYS004	Rating V4	NDVI V4	-0.52***
RYS004	NDVI V4	NDVI V2	0.69***
RYS004	Rating V4	Rating V2	0.77***
RYS005	Rating V2	NDVI V2	-0.45***
RYS005	Rating V4	NDVI V4	-0.67***
RYS005	NDVI V4	NDVI V2	0.63***
RYS005	Rating V4	Rating V2	0.62***
RYS006	Rating V2	NDVI V2	-0.66***
RYS006	Rating V4	NDVI V4	-0.58***
RYS006	NDVI V4	NDVI V2	0.69***
RYS006	Rating V4	Rating V2	0.77***
RYS007	Rating V2	NDVI V2	-0.65***
RYS007	Rating V4	NDVI V4	-0.81***
RYS007	NDVI V4	NDVI V2	0.61***
RYS007	Rating V4	Rating V2	0.84***
RYS008	Rating V2	NDVI V2	-0.6***
RYS008	Rating V4	NDVI V4	-0.69***
RYS008	NDVI V4	NDVI V2	0.72***
RYS008	Rating V4	Rating V2	0.87***

Appendix B Continued

Population	Variable	by Variable	*r*
RYS009	Rating V2	NDVI V2	-0.48***
RYS009	Rating V4	NDVI V4	-0.66***
RYS009	NDVI V4	NDVI V2	0.71***
RYS009	Rating V4	Rating V2	0.78***
RYS010	Rating V2	NDVI V2	-0.62***
RYS010	Rating V4	NDVI V4	-0.81***
RYS010	NDVI V4	NDVI V2	0.61***
RYS010	Rating V4	Rating V2	0.83***
RYS011	Rating V2	NDVI V2	-0.67***
RYS011	Rating V4	NDVI V4	-0.8***
RYS011	NDVI V4	NDVI V2	0.77***
RYS011	Rating V4	Rating V2	0.81***
RYS012	Rating V2	NDVI V2	-0.73***
RYS012	Rating V4	NDVI V4	-0.84***
RYS012	NDVI V4	NDVI V2	0.75***
RYS012	Rating V4	Rating V2	0.85***
RYS013	Rating V2	NDVI V2	-0.61***
RYS013	Rating V4	NDVI V4	-0.87***
RYS013	NDVI V4	NDVI V2	0.74***
RYS013	Rating V4	Rating V2	0.73***

*** Significance at p-value < 0.001

Appendix C: Correlations between mean NDVI at V2 and V4 and chlorosis severity rating at V2 and V4 across populations and within environments. Chlorosis is measured using a 1 to 9 scale, where a value of 1 indicates no chlorosis. NDVI (normalized difference vegetative index) values range from -1 to 1 with 1 indicating healthy plants.

Variable	by Variable	*r*
Rating V2 Fort Dodge	NDVI V2 Fort Dodge	-0.73***
Rating V4 Fort Dodge	NDVI V4 Fort Dodge	-0.82***
Rating V4 Fort Dodge	Rating V2 Fort Dodge	0.85***
NDVI V4 Fort Dodge	NDVI V2 Fort Dodge	0.79***
Rating V2 Ogden	NDVI V2 Ogden	-0.75***
Rating V4 Ogden	NDVI V4 Ogden	-0.86***
Rating V4 Ogden	Rating V2 Ogden	0.81***
NDVI V4 Ogden	NDVI V2 Ogden	0.82***
Rating V2 Fort Dodge	Rating V2 Ogden	0.92***
NDVI V2 Fort Dodge	NDVI V2 Ogden	0.87***
Rating V4 Fort Dodge	Rating V4 Ogden	0.93***
NDVI V4 Fort Dodge	NDVI V4 Ogden	0.92***

*** Significance at p-value < 0.001

Appendix D: Correlations between mean NDVI at V2 and V4 and chlorosis severity rating at V2 and V4 within populations and within environments. Chlorosis is measured using a 1 to 9 scale, where a value of 1 indicates no chlorosis. NDVI (normalized difference vegetative index) values range from -1 to 1 with 1 indicating healthy plants.

Population	Variable	by Variable	*r*
RYS002	Rating V2 Fort Dodge	NDVI V2 Fort Dodge	-0.58***
RYS002	Rating V4 Fort Dodge	NDVI V4 Fort Dodge	-0.78***
RYS002	Rating V4 Fort Dodge	Rating V2 Fort Dodge	0.85***
RYS002	NDVI V4 Fort Dodge	NDVI V2 Fort Dodge	0.61***
RYS002	Rating V2 Ogden	NDVI V2 Ogden	-0.58***
RYS002	Rating V4 Ogden	NDVI V4 Ogden	-0.78***
RYS002	Rating V4 Ogden	Rating V2 Ogden	0.85***
RYS002	NDVI V4 Ogden	NDVI V2 Ogden	0.61***
RYS002	Rating V2 Fort Dodge	Rating V2 Ogden	1.00***
RYS002	NDVI V2 Fort Dodge	NDVI V2 Ogden	1.00***
RYS002	Rating V4 Fort Dodge	Rating V4 Ogden	1.00***
RYS002	NDVI V4 Fort Dodge	NDVI V4 Ogden	1.00***
RYS003	Rating V2 Fort Dodge	NDVI V2 Fort Dodge	-0.58***
RYS003	Rating V4 Fort Dodge	NDVI V4 Fort Dodge	-0.54***
RYS003	Rating V4 Fort Dodge	Rating V2 Fort Dodge	0.72***
RYS003	NDVI V4 Fort Dodge	NDVI V2 Fort Dodge	0.67***
RYS003	Rating V2 Ogden	NDVI V2 Ogden	-0.75***
RYS003	Rating V4 Ogden	NDVI V4 Ogden	-0.59***
RYS003	Rating V4 Ogden	Rating V2 Ogden	0.89***
RYS003	NDVI V4 Ogden	NDVI V2 Ogden	0.71***
RYS003	Rating V2 Fort Dodge	Rating V2 Ogden	0.65***
RYS003	NDVI V2 Fort Dodge	NDVI V2 Ogden	0.82***
RYS003	Rating V4 Fort Dodge	Rating V4 Ogden	0.91***
RYS003	NDVI V4 Fort Dodge	NDVI V4 Ogden	0.27

Appendix D Continued

Population	Variable	by Variable	*r*
RYS004	Rating V2 Fort Dodge	NDVI V2 Fort Dodge	-0.41***
RYS004	Rating V4 Fort Dodge	NDVI V4 Fort Dodge	-0.65***
RYS004	Rating V4 Fort Dodge	Rating V2 Fort Dodge	0.78***
RYS004	NDVI V4 Fort Dodge	NDVI V2 Fort Dodge	0.69***
RYS004	Rating V2 Ogden	NDVI V2 Ogden	-0.4***
RYS004	Rating V4 Ogden	NDVI V4 Ogden	-0.68***
RYS004	Rating V4 Ogden	Rating V2 Ogden	0.8***
RYS004	NDVI V4 Ogden	NDVI V2 Ogden	0.69***
RYS004	Rating V2 Fort Dodge	Rating V2 Ogden	1.00***
RYS004	NDVI V2 Fort Dodge	NDVI V2 Ogden	0.67***
RYS004	Rating V4 Fort Dodge	Rating V4 Ogden	0.97***
RYS004	NDVI V4 Fort Dodge	NDVI V4 Ogden	0.93***
RYS005	Rating V2 Fort Dodge	NDVI V2 Fort Dodge	-0.51***
RYS005	Rating V4 Fort Dodge	NDVI V4 Fort Dodge	-0.74***
RYS005	Rating V4 Fort Dodge	Rating V2 Fort Dodge	0.71***
RYS005	NDVI V4 Fort Dodge	NDVI V2 Fort Dodge	0.66***
RYS005	Rating V2 Ogden	NDVI V2 Ogden	-0.51***
RYS005	Rating V4 Ogden	NDVI V4 Ogden	-0.75***
RYS005	Rating V4 Ogden	Rating V2 Ogden	0.71***
RYS005	NDVI V4 Ogden	NDVI V2 Ogden	0.67***
RYS005	Rating V2 Fort Dodge	Rating V2 Ogden	1.00***
RYS005	NDVI V2 Fort Dodge	NDVI V2 Ogden	1.00***
RYS005	Rating V4 Fort Dodge	Rating V4 Ogden	0.99***
RYS005	NDVI V4 Fort Dodge	NDVI V4 Ogden	0.99***

Appendix D Continued

Population	Variable	by Variable	*r*
RYS006	Rating V2 Fort Dodge	NDVI V2 Fort Dodge	-0.53***
RYS006	Rating V4 Fort Dodge	NDVI V4 Fort Dodge	-0.66***
RYS006	Rating V4 Fort Dodge	Rating V2 Fort Dodge	0.84***
RYS006	NDVI V4 Fort Dodge	NDVI V2 Fort Dodge	0.68***
RYS006	Rating V2 Ogden	NDVI V2 Ogden	-0.53***
RYS006	Rating V4 Ogden	NDVI V4 Ogden	-0.65***
RYS006	Rating V4 Ogden	Rating V2 Ogden	0.82***
RYS006	NDVI V4 Ogden	NDVI V2 Ogden	0.68***
RYS006	Rating V2 Fort Dodge	Rating V2 Ogden	1.00***
RYS006	NDVI V2 Fort Dodge	NDVI V2 Ogden	1.00***
RYS006	Rating V4 Fort Dodge	Rating V4 Ogden	0.95***
RYS006	NDVI V4 Fort Dodge	NDVI V4 Ogden	1.00***
RYS007	Rating V2 Fort Dodge	NDVI V2 Fort Dodge	-0.53***
RYS007	Rating V4 Fort Dodge	NDVI V4 Fort Dodge	-0.74***
RYS007	Rating V4 Fort Dodge	Rating V2 Fort Dodge	0.86***
RYS007	NDVI V4 Fort Dodge	NDVI V2 Fort Dodge	0.59***
RYS007	Rating V2 Ogden	NDVI V2 Ogden	-0.56***
RYS007	Rating V4 Ogden	NDVI V4 Ogden	-0.76***
RYS007	Rating V4 Ogden	Rating V2 Ogden	0.85***
RYS007	NDVI V4 Ogden	NDVI V2 Ogden	0.66***
RYS007	Rating V2 Fort Dodge	Rating V2 Ogden	0.87***
RYS007	NDVI V2 Fort Dodge	NDVI V2 Ogden	0.78***
RYS007	Rating V4 Fort Dodge	Rating V4 Ogden	0.92***
RYS007	NDVI V4 Fort Dodge	NDVI V4 Ogden	0.97***

Appendix D Continued

Population	Variable	by Variable	*r*
RYS008	Rating V2 Fort Dodge	NDVI V2 Fort Dodge	-0.64***
RYS008	Rating V4 Fort Dodge	NDVI V4 Fort Dodge	-0.77***
RYS008	Rating V4 Fort Dodge	Rating V2 Fort Dodge	0.87***
RYS008	NDVI V4 Fort Dodge	NDVI V2 Fort Dodge	0.8***
RYS008	Rating V2 Ogden	NDVI V2 Ogden	-0.64***
RYS008	Rating V4 Ogden	NDVI V4 Ogden	-0.77***
RYS008	Rating V4 Ogden	Rating V2 Ogden	0.87***
RYS008	NDVI V4 Ogden	NDVI V2 Ogden	0.80***
RYS008	Rating V2 Fort Dodge	Rating V2 Ogden	1.00***
RYS008	NDVI V2 Fort Dodge	NDVI V2 Ogden	1.00***
RYS008	Rating V4 Fort Dodge	Rating V4 Ogden	1.00***
RYS008	NDVI V4 Fort Dodge	NDVI V4 Ogden	1.00***
RYS009	Rating V2 Fort Dodge	NDVI V2 Fort Dodge	-0.54***
RYS009	Rating V4 Fort Dodge	NDVI V4 Fort Dodge	-0.78***
RYS009	Rating V4 Fort Dodge	Rating V2 Fort Dodge	0.84***
RYS009	NDVI V4 Fort Dodge	NDVI V2 Fort Dodge	0.66***
RYS009	Rating V2 Ogden	NDVI V2 Ogden	-0.51***
RYS009	Rating V4 Ogden	NDVI V4 Ogden	-0.63***
RYS009	Rating V4 Ogden	Rating V2 Ogden	0.79***
RYS009	NDVI V4 Ogden	NDVI V2 Ogden	0.72***
RYS009	Rating V2 Fort Dodge	Rating V2 Ogden	0.97***
RYS009	NDVI V2 Fort Dodge	NDVI V2 Ogden	1.00***
RYS009	Rating V4 Fort Dodge	Rating V4 Ogden	0.84***
RYS009	NDVI V4 Fort Dodge	NDVI V4 Ogden	0.71***

Appendix D Continued

Population	Variable	by Variable	*r*
RYS010	Rating V2 Fort Dodge	NDVI V2 Fort Dodge	-0.67***
RYS010	Rating V4 Fort Dodge	NDVI V4 Fort Dodge	-0.82***
RYS010	Rating V4 Fort Dodge	Rating V2 Fort Dodge	0.87***
RYS010	NDVI V4 Fort Dodge	NDVI V2 Fort Dodge	0.71***
RYS010	Rating V2 Ogden	NDVI V2 Ogden	-0.71***
RYS010	Rating V4 Ogden	NDVI V4 Ogden	-0.85***
RYS010	Rating V4 Ogden	Rating V2 Ogden	0.81***
RYS010	NDVI V4 Ogden	NDVI V2 Ogden	0.76***
RYS010	Rating V2 Fort Dodge	Rating V2 Ogden	1.00***
RYS010	NDVI V2 Fort Dodge	NDVI V2 Ogden	0.94***
RYS010	Rating V4 Fort Dodge	Rating V4 Ogden	0.94***
RYS010	NDVI V4 Fort Dodge	NDVI V4 Ogden	0.97***
RYS011	Rating V2 Fort Dodge	NDVI V2 Fort Dodge	-0.71***
RYS011	Rating V4 Fort Dodge	NDVI V4 Fort Dodge	-0.80***
RYS011	Rating V4 Fort Dodge	Rating V2 Fort Dodge	0.88***
RYS011	NDVI V4 Fort Dodge	NDVI V2 Fort Dodge	0.81***
RYS011	Rating V2 Ogden	NDVI V2 Ogden	-0.68***
RYS011	Rating V4 Ogden	NDVI V4 Ogden	-0.84***
RYS011	Rating V4 Ogden	Rating V2 Ogden	0.87***
RYS011	NDVI V4 Ogden	NDVI V2 Ogden	0.80***
RYS011	Rating V2 Fort Dodge	Rating V2 Ogden	1.00***
RYS011	NDVI V2 Fort Dodge	NDVI V2 Ogden	0.88***
RYS011	Rating V4 Fort Dodge	Rating V4 Ogden	1.00***
RYS011	NDVI V4 Fort Dodge	NDVI V4 Ogden	0.96***

Appendix D Continued

Population	Variable	by Variable	*r*
RYS012	Rating V2 Fort Dodge	NDVI V2 Fort Dodge	-0.72***
RYS012	Rating V4 Fort Dodge	NDVI V4 Fort Dodge	-0.84***
RYS012	Rating V4 Fort Dodge	Rating V2 Fort Dodge	0.85***
RYS012	NDVI V4 Fort Dodge	NDVI V2 Fort Dodge	0.76***
RYS012	Rating V2 Ogden	NDVI V2 Ogden	-0.67***
RYS012	Rating V4 Ogden	NDVI V4 Ogden	-0.84***
RYS012	Rating V4 Ogden	Rating V2 Ogden	0.88***
RYS012	NDVI V4 Ogden	NDVI V2 Ogden	0.76***
RYS012	Rating V2 Fort Dodge	Rating V2 Ogden	0.94***
RYS012	NDVI V2 Fort Dodge	NDVI V2 Ogden	1.00***
RYS012	Rating V4 Fort Dodge	Rating V4 Ogden	1.00***
RYS012	NDVI V4 Fort Dodge	NDVI V4 Ogden	1.00***
RYS013	Rating V2 Fort Dodge	NDVI V2 Fort Dodge	-0.67***
RYS013	Rating V4 Fort Dodge	NDVI V4 Fort Dodge	-0.81***
RYS013	Rating V4 Fort Dodge	Rating V2 Fort Dodge	0.78***
RYS013	NDVI V4 Fort Dodge	NDVI V2 Fort Dodge	0.82***
RYS013	Rating V2 Ogden	NDVI V2 Ogden	-0.70***
RYS013	Rating V4 Ogden	NDVI V4 Ogden	-0.81***
RYS013	Rating V4 Ogden	Rating V2 Ogden	0.78***
RYS013	NDVI V4 Ogden	NDVI V2 Ogden	0.82***
RYS013	Rating V2 Fort Dodge	Rating V2 Ogden	0.98***
RYS013	NDVI V2 Fort Dodge	NDVI V2 Ogden	1.00***
RYS013	Rating V4 Fort Dodge	Rating V4 Ogden	1.00***
RYS013	NDVI V4 Fort Dodge	NDVI V4 Ogden	1.00***

*** Significance at p-value < 0.001

Appendix E: Results of CIM for the RYS002 population created by crossing female parent 04KL108888 and male parent 04KL109428. Five hundred permutation tests with a genome-wide significance of ($\alpha = 0.05$) were conducted to determine significance thresholds. Traits were analyzed within and across environments. Linkage group (LG), position of peak LOD score (cM), 1-LOD support interval (cM), peak LOD score, proportion of variation explained by the QTL (R2 %), and additive effect of the QTL are reported. The sign of the additive effect references the male parent, where a positive additive effect indicates the male parent allele inscreases the trait value, and a negative additive effect indicates the male parent allele decreases the trait value.

Trait	LG	LOD Peak Position (cM)	1-LOD Support Interval (cM)	LOD Score	R^2 (%)	Additive Effect
RatingV2|Ogden	L/19	54.8	54.4 - 72.9	5.5	13.1	0.34
RatingV2|Ogden	M/7	68.4	58.0 - 77.7	5.2	42.3	-0.44
RatingV2|Fort Dodge	L/19	54.8	54.4 - 72.9	5.5	13.1	0.34
RatingV2|Fort Dodge	M/7	68.4	58.0 - 77.7	5.2	42.3	-0.44
RatingV2|Across	L/19	54.8	54.4 - 72.9	5.5	13.1	0.34
RatingV2|Across	M/7	68.4	58.0 - 77.7	5.2	42.3	-0.44
RatingV4|Ogden	A2/8	196.8	94.1 - 215.6	5.0	47.2	0.80
RatingV4|Fort Dodge	A2/8	196.8	94.1 - 215.6	5.0	47.2	0.80
RatingV4|Across	A2/8	196.8	94.1 - 215.6	5.0	47.2	0.80
Yield|Brookings (2009)	F/13	104.3	57.1 - 128.3	5.0	21.5	-1.98
Yield|Brookings (2009)	F/13	144.2	128.0 - 154.7	5.4	35.8	-1.76
Yield|Brookings (2010)	E/15	157.9	157.1 - 168.6	3.9	12.7	1.07
Yield|Owatonna (2010)	A1/5	42.6	23.9 - 46.1	4.8	15.0	2.46
Yield|Owatonna (2010)	J/16	111.3	64.1 - 141.7	5.9	5.8	-3.10

Appendix F: Results of CIM for RYS003 population created by crossing female parent 04KL108888 and male parent WW221162. Five hundred permutation tests with a genome-wide significance of ($\alpha = 0.05$) were conducted to determine significance thresholds. Traits were analyzed within and across environments. Linkage group (LG), position of peak LOD score (cM), 1-LOD support interval (cM), peak LOD score, proportion of variation explained by the QTL (R2 %), and additive effect of the QTL are reported. The sign of the additive effect references the male parent, where a positive additive effect indicates the male parent allele inscreases the trait value, and a negative additive effect indicates the male parent allele decreases the trait value.

Trait	LG	LOD Peak Position (cM)	1-LOD Support Interval (cM)	LOD Score	R^2 (%)	Additive Effect
NDVIV2|Ogden	B1/11	142.1	126.6 - 180.9	9.0	17.9	-0.023
NDVIV2|Fort Dodge	B1/11	169.1	126.7 - 180.8	9.4	42.9	-0.036
NDVIV2|Fort Dodge	D1a/1	82.7	49.5 - 94.6	11.3	41.6	-0.038
NDVIV2|Fort Dodge	D1a/1	101.6	94.6 - 153.9	11.2	41.6	-0.038
NDVIV2|Fort Dodge	D1b/2	31.5	26.1 - 40.8	10.7	46.2	0.042
NDVIV2|Fort Dodge	D1b/2	75.7	43.5 - 89.7	8.3	47.3	-0.036
NDVIV2|Across	D1b/2	53.7	43.7 - 87.7	7.6	47.2	-0.028
NDVIV4|Ogden	A2/8	148.8	94.6 - 163.3	7.9	46.5	0.043
NDVIV4|Ogden	A2/8	189.1	184.9 - 216.3	8.8	46.8	0.089
NDVIV4|Ogden	D1a/1	106.6	94.6 - 153.7	7.1	2.6	0.041
NDVIV4|Ogden	F/13	181.2	170.9 - 197.9	7.8	2.1	0.043
NDVIV4|Ogden	I/20	84.6	67.4 - 98.6	7.0	4.3	-0.051
NDVIV4|Fort Dodge	J/16	56.0	39.9 - 164.3	7.1	40.3	-0.038
NDVIV4|Across	D1b/2	105.8	103.4 - 108.7	7.6	35.3	0.008
RatingV2|Ogden	C2/6	197.7	170.7 - 240.7	8.0	33.7	-0.43
RatingV2|Ogden	D2/17	121.4	113.0 - 134.2	8.3	5.4	-0.37
RatingV2|Ogden	D2/17	166.1	153.7 - 172.8	11.6	20.6	-0.40
RatingV4|Ogden	A2/8	49.0	43.0 - 95.6	6.9	10.3	-0.32
RatingV4|Ogden	B2/14	45.4	36.1 - 64.4	11.4	22.6	0.61
RatingV4|Ogden	D1b/2	27.5	26.4 - 41.1	8.7	13.1	-0.48
RatingV4|Across	D1b/2	39.5	25.7 - 40.3	6.6	16.1	0.38
RatingV4|Across	D1b/2	63.7	43.3 - 87.5	8.1	18.9	0.41
Yield|Stanton (2009)	K/9	99.6	75.3 - 106.8	3.8	6.8	1.35
Yield|Stanton (2009)	K/9	112.0	105.5 - 145.6	3.9	7.3	1.33
Yield|Brookings (2010)	A2/8	159.8	94.1 - 163.2	4.4	5.3	0.71

Appendix G: Results of CIM for RYS004 population created by crossing female parent 04KL109378 and male parent 03DL052038. Five hundred permutation tests with a genome-wide significance of ($\alpha = 0.05$) were conducted to determine significance thresholds. Traits were analyzed within and across environments. Linkage group (LG), position of peak LOD score (cM), 1-LOD support interval (cM), peak LOD score, proportion of variation explained by the QTL (R2 %), and additive effect of the QTL are reported. The sign of the additive effect references the male parent, where a positive additive effect indicates the male parent allele inscreases the trait value, and a negative additive effect indicates the male parent allele decreases the trait value.

Trait	LG	LOD Peak Position (cM)	1-LOD Support Interval (cM)	LOD Score	R^2 (%)	Additive Effect
NDVIV2|Fort Dodge	H/12	172.4	143.1 - 200.5	5.3	17.3	0.021
NDVIV4|Fort Dodge	M/7	16.4	5.1 - 24.5	6.0	30.1	-0.012
RatingV2|Ogden	A1/5	18.2	17.0 - 22.5	4.2	16.2	-0.211
RatingV2|Ogden	L/19	99.8	34.1 - 99.9	4.4	17.7	-0.220
RatingV2|Fort Dodge	A1/5	18.2	17.0 - 22.5	4.2	16.2	-0.211
RatingV2|Fort Dodge	L/19	99.8	34.1 - 99.9	4.4	17.7	-0.220
RatingV2|Across	A1/5	18.2	17.0 - 22.5	4.2	16.2	-0.211
RatingV2|Across	L/19	99.8	34.1 - 99.9	4.4	17.7	-0.220
Yield|Brookings (2009)	C2/6	184.9	184.9 - 185.1	9.0	22.5	-2.665
Yield|Stanton (2009)	C2/6	186.1	184.8 - 187.4	15.6	31.5	-3.758
Yield|Owatonna (2009)	C1/4	36.4	4.0 - 87.5	21.9	89.9	9.537
Yield|Thorndale (2009)	C2/6	184.9	178.0 - 190.1	9.7	21.8	-1.913
Yield|Nevada (2009)	C2/6	185.1	183.7 - 187.4	6.3	13.5	-2.255
Yield|Nevada (2010)	C2/6	186.1	184.8 - 187.4	7.4	17.4	-2.791
Yield|Across	C1/4	69.4	3.8 - 87.2	5.0	18.9	1.683
Yield|Across	C2/6	185.1	184.3 - 187.4	10.2	14.9	-1.616

Appendix H: Results of CIM for RYS005 population created by crossing female parent 04KL015763 and male parent 03DL052038. Five hundred permutation tests with a genome-wide significance of ($\alpha = 0.05$) were conducted to determine significance thresholds. Traits were analyzed within and across environments. Linkage group (LG), position of peak LOD score (cM), 1-LOD support interval (cM), peak LOD score, proportion of variation explained by the QTL (R2 %), and additive effect of the QTL are reported. The sign of the additive effect references the male parent, where a positive additive effect indicates the male parent allele inscreases the trait value, and a negative additive effect indicates the male parent allele decreases the trait value.

Trait	LG	LOD Peak Position (cM)	1-LOD Support Interval (cM)	LOD Score	R^2 (%)	Additive Effect

No significant QTLs detected

Appendix I: Results of CIM for RYS006 population created by crossing female parent 04KL111519 and male parent 03DL052038. Five hundred permutation tests with a genome-wide significance of ($\alpha = 0.05$) were conducted to determine significance thresholds. Traits were analyzed within and across environments. Linkage group (LG), position of peak LOD score (cM), 1-LOD support interval (cM), peak LOD score, proportion of variation explained by the QTL (R2 %), and additive effect of the QTL are reported. The sign of the additive effect references the male parent, where a positive additive effect indicates the male parent allele inscreases the trait value, and a negative additive effect indicates the male parent allele decreases the trait value.

Trait	LG	LOD Peak Position (cM)	1-LOD Support Interval (cM)	LOD Score	R^2 (%)	Additive Effect

No significant QTLs detected

Appendix J: Results of CIM for RYS007 population created by crossing female parent 04KL109428 and male parent 03DL052038. Five hundred permutation tests with a genome-wide significance of ($\alpha = 0.05$) were conducted to determine significance thresholds. Traits were analyzed within and across environments. Linkage group (LG), position of peak LOD score (cM), 1-LOD support interval (cM), peak LOD score, proportion of variation explained by the QTL (R2 %), and additive effect of the QTL are reported. The sign of the additive effect references the male parent, where a positive additive effect indicates the male parent allele inscreases the trait value, and a negative additive effect indicates the male parent allele decreases the trait value.

Trait	LG	LOD Peak Position (cM)	1-LOD Support Interval (cM)	LOD Score	R^2 (%)	Additive Effect
NDVIV4|Ogden	D1b/2	99.5	99.0 - 109.4	9.3	45.6	0.023
NDVIV4|Fort Dodge	D1b/2	101.5	99.4 - 109.1	6.5	42.8	0.021
NDVIV4|Across	D1b/2	94.9	19.1 - 99.5	6.5	44.3	0.020
NDVIV4|Across	D1b/2	108.4	108.4 - 138.3	4.7	29.9	-0.015

Appendix K: Results of CIM for RYS008 population created by crossing female parent 04KL111531 and male parent 03DL052038. Five hundred permutation tests with a genome-wide significance of ($\alpha = 0.05$) were conducted to determine significance thresholds. Traits were analyzed within and across environments. Linkage group (LG), position of peak LOD score (cM), 1-LOD support interval (cM), peak LOD score, proportion of variation explained by the QTL (R2 %), and additive effect of the QTL are reported. The sign of the additive effect references the male parent, where a positive additive effect indicates the male parent allele inscreases the trait value, and a negative additive effect indicates the male parent allele decreases the trait value.

Trait	LG	LOD Peak Position (cM)	1-LOD Support Interval (cM)	LOD Score	R^2 (%)	Additive Effect
NDVIV2|Ogden	H/12	94.5	67.2 - 116.9	6.3	27.7	0.018
NDVIV2|Fort Dodge	H/12	94.5	67.2 - 116.9	6.1	28.7	0.018
NDVIV2|Across	H/12	94.5	67.2 - 116.9	6.2	28.3	0.018
NDVIV2|Across	H/12	155.4	143.3 - 166.0	4.6	30.3	0.018
RatingV2|Ogden	A1/5	18.2	17.2 - 23.1	5.3	23.7	-0.440
RatingV2|Fort Dodge	A1/5	18.2	17.2 - 23.1	5.3	23.7	-0.440
RatingV2|Across	A1/5	18.2	17.2 - 23.1	5.3	23.7	-0.440
Yield|Brookings (2009)	C2/6	182.1	182.1 - 184.0	7.7	23.8	-1.444
Yield|Brookings (2009)	C2/6	217.7	186.8 - 218.0	6.2	15.4	-1.197
Yield|Stanton (2009)	F/13	195.2	127.5 - 207.0	5.2	33.7	-2.155
Yield|Owatonna (2009)	C2/6	182.1	182.1 - 184.0	15.6	42.8	-2.145
Yield|Owatonna (2009)	C2/6	194.7	186.8 - 218.0	9.2	47.2	2.198
Yield|Thorndale (2009)	C2/6	182.1	182.1 - 184.0	7.1	22.0	-1.804
Yield|Nevada (2009)	C2/6	182.1	182.1 - 184.0	9.0	26.8	-4.109
Yield|Stanton (2010)	C2/6	182.1	182.1 - 184.0	11.5	32.5	-3.085
Yield|Owatonna (2010)	M/7	37.9	33.7 - 51.8	6.3	20.3	-2.165
Yield|Thorndale (2010)	F/13	203.2	127.4 - 207.0	5.0	45.2	-1.868
Yield|Nevada (2010)	C2/6	182.1	182.1 - 184.0	5.0	17.7	-1.807
Yield|Across	C2/6	182.1	182.1 - 184.0	15.3	34.2	-1.446

Appendix L: Results of CIM for RYS009 population created by crossing female parent WW221162 and male parent 04KL111519. Five hundred permutation tests with a genome-wide significance of ($\alpha = 0.05$) were conducted to determine significance thresholds. Traits were analyzed within and across environments. Linkage group (LG), position of peak LOD score (cM), 1-LOD support interval (cM), peak LOD score, proportion of variation explained by the QTL (R2 %), and additive effect of the QTL are reported. The sign of the additive effect references the male parent, where a positive additive effect indicates the male parent allele inscreases the trait value, and a negative additive effect indicates the male parent allele decreases the trait value.

Trait	LG	LOD Peak Position (cM)	1-LOD Support Interval (cM)	LOD Score	R^2 (%)	Additive Effect
Yield|Brookings (2009)	A1/5	166.3	153.9 - 166.8	6.3	4.7	-0.73
Yield|Brookings (2009)	A2/8	24.0	0.0 - 26.1	6.1	3.0	0.51
Yield|Brookings (2009)	E/15	64.2	44.6 - 80.3	6.1	2.3	-0.29
Yield|Brookings (2009)	G/18	181.0	180.7 - 199.2	6.1	3.5	-0.39
Yield|Brookings (2009)	I/20	82.7	82.4 - 94.6	6.0	0.7	-0.15
Yield|Brookings (2009)	M/7	147.3	147.2 - 241.1	7.4	7.5	0.68
Yield|Stanton (2009)	G/18	0.0	0.0 - 25.8	4.6	15.2	1.48
Yield|Stanton (2009)	M/7	141.9	130.4 - 143.9	8.9	30.2	2.21
Yield|Stanton (2009)	M/7	159.3	146.6 - 240.1	7.8	57.2	3.01
Yield|Owatonna (2009)	C2/6	151.2	150.9 - 172.0	3.9	8.3	-0.87
Yield|Owatonna (2009)	L/19	161.4	159.5 - 164.9	5.1	13.4	1.14
Yield|Brookings (2010)	L/19	161.4	161.4 - 164.6	7.6	15.4	1.01
Yield|Brookings (2010)	N/3	299.8	293.1 - 301.9	4.4	10.0	-0.85
Yield|Owatonna (2010)	N/3	38.1	26.9 - 43.3	4.7	15.8	-1.50
Yield|Thorndale (2010)	B1/11	105.7	29.1 - 127.3	8.0	32.2	-1.31
Yield|Thorndale (2010)	K/9	51.3	42.3 - 163.5	12.3	33.0	-1.28

Appendix M: Results of CIM for RYS010 population created by crossing female parent 04KL109378 and male parent O4KL109428. Five hundred permutation tests with a genome-wide significance of ($\alpha = 0.05$) were conducted to determine significance thresholds. Traits were analyzed within and across environments. Linkage group (LG), position of peak LOD score (cM), 1-LOD support interval (cM), peak LOD score, proportion of variation explained by the QTL (R2 %), and additive effect of the QTL are reported. The sign of the additive effect references the male parent, where a positive additive effect indicates the male parent allele inscreases the trait value, and a negative additive effect indicates the male parent allele decreases the trait value.

Trait	LG	LOD Peak Position (cM)	1-LOD Support Interval (cM)	LOD Score	R^2 (%)	Additive Effect

No significant QTLs detected

Appendix N: Results of CIM for RYS011 population created by crossing female parent 04KL111531 and male parent 04KL109378. Five hundred permutation tests with a genome-wide significance of ($\alpha = 0.05$) were conducted to determine significance thresholds. Traits were analyzed within and across environments. Linkage group (LG), position of peak LOD score (cM), 1-LOD support interval (cM), peak LOD score, proportion of variation explained by the QTL (R2 %), and additive effect of the QTL are reported. The sign of the additive effect references the male parent, where a positive additive effect indicates the male parent allele inscreases the trait value, and a negative additive effect indicates the male parent allele decreases the trait value.

Trait	LG	LOD Peak Position (cM)	1-LOD Support Interval (cM)	LOD Score	R^2 (%)	Additive Effect

No significant QTLs detected

Appendix O: Results of CIM for RYS012 population created by crossing female parent 04KL109378 and male parent 03KL015763. Five hundred permutation tests with a genome-wide significance of ($\alpha = 0.05$) were conducted to determine significance thresholds. Traits were analyzed within and across environments. Linkage group (LG), position of peak LOD score (cM), 1-LOD support interval (cM), peak LOD score, proportion of variation explained by the QTL (R2 %), and additive effect of the QTL are reported. The sign of the additive effect references the male parent, where a positive additive effect indicates the male parent allele inscreases the trait value, and a negative additive effect indicates the male parent allele decreases the trait value.

Trait	LG	LOD Peak Position (cM)	1-LOD Support Interval (cM)	LOD Score	R^2 (%)	Additive Effect

No significant QTLs detected

Appendix P: Results of CIM for RYS013 population created by crossing female parent 04KL111531 and male parent WW221162. Five hundred permutation tests with a genome-wide significance of ($\alpha = 0.05$) were conducted to determine significance thresholds. Traits were analyzed within and across environments. Linkage group (LG), position of peak LOD score (cM), 1-LOD support interval (cM), peak LOD score, proportion of variation explained by the QTL (R2 %), and additive effect of the QTL are reported. The sign of the additive effect references the male parent, where a positive additive effect indicates the male parent allele inscreases the trait value, and a negative additive effect indicates the male parent allele decreases the trait value.

Trait	LG	LOD Peak Position (cM)	1-LOD Support Interval (cM)	LOD Score	R^2 (%)	Additive Effect

No significant QTLs detected

Appendix Q: Results of CIM for RYS014 population created by crossing female parent 04KL109378 and male parent O4KL111519. Five hundred permutation tests with a genome-wide significance of ($\alpha = 0.05$) were conducted to determine significance thresholds. Traits were analyzed within and across environments. Linkage group (LG), position of peak LOD score (cM), 1-LOD support interval (cM), peak LOD score, proportion of variation explained by the QTL (R2 %), and additive effect of the QTL are reported. The sign of the additive effect references the male parent, where a positive additive effect indicates the male parent allele inscreases the trait value, and a negative additive effect indicates the male parent allele decreases the trait value.

Trait	LG	LOD Peak Position (cM)	1-LOD Support Interval (cM)	LOD Score	R^2 (%)	Additive Effect
Yield|Brookings (2010)	A1/5	29.0	28.3 - 36.2	6.0	4.5	0.46

Appendix R: Results of CIM for the network of 13 population analyzed using the connected model. One thousand permutation tests with a genome-wide significance of ($\alpha = 0.05$) were conducted to determine significance thresholds. Traits were analyzed within and across environments. Linkage group (LG), position of peak LOD score (cM), 1-LOD support interval (cM), peak LOD score, proportion of variation explained by the QTL (R2 %), and additive effects of the parental haplotypes of the QTL are reported.

						Additive Effect of Parental Haplotype							
Trait	LG	LOD Peak Position	1-LOD Support Interval (cM)	LOD Score	R^2 (%)	03DL052038	03KL015763	04KL108888	04KL109378	04KL109428	04KL111519	04KL111531	WW221162
NDVIV2|Across	A2/8	185.1	185.1 - 185.1	4.0	36.7	-0.013	-0.013	-0.013	0.013	-0.013	-0.013	0.013	0.013
NDVIV2|Across	B1/11	3.0	3.0 - 22.2	3.6	57.0	0.037	-0.037	0.037	0.037	0.037	0.037	0.037	0.037
NDVIV2|Across	C1/4	125.6	117.1 - 130.2	4.4	41.1	0.033	-0.031	0.033	0.033	-0.002	-0.002	0.033	0.033
NDVIV2|Across	I/20	118.8	118.8 - 120.0	4.6	46.4	0.016	-0.016	0.016	0.016	0.016	0.016	0.016	0.016
NDVIV2|Across	L/19	160.9	157.2 - 163.0	5.4	18.3	0.011	-0.011	0.011	0.011	0.011	0.011	-0.011	0.011
NDVIV4|Across	K/9	190.7	190.7 - 191.7	3.4	42.4	-0.019	0.019	-0.019	-0.019	0.019	0.019	0.019	0.019
RatingV2|Across	A1/5	7.1	7.1 - 17.2	3.4	17.8	-0.18	0.18	-0.18	0.18	0.18	0.18	0.18	0.18
RatingV2|Across	L/19	98.0	97.9 - 102.3	5.1	27.6	-0.24	0.24	-0.24	0.24	0.24	0.24	0.24	0.24
RatingV2|Across	N/3	295.9	295.9 - 300.4	3.2	17.0	-0.21	0.20	-0.02	-0.28	0.20	0.03	0.09	-0.01
Yield|Across	C2/6	187.0	183.4 - 199.6	8.2	38.1	-1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45
Yield|Across	O/10	144.6	141.4 - 147.8	28.7	53.4	-2.97	-2.97	-2.97	1.28	1.28	1.28	-2.97	1.70
Yield|Across	O/10	168.1	166.8 - 170.5	24.5	55.8	-1.97	-1.97	-1.97	1.97	1.97	1.97	-1.97	1.97

Appendix R: Continued

Trait	LG	LOD Peak Position	1-LOD Support Interval (cM)	LOD Score	R^2 (%)	Additive Effect of Parental Haplotype							
						03DL052038	03KL015763	04KL108888	04KL109378	04KL109428	04KL111519	04KL111531	WW221162
NDVIV2|Ogden	B1/11	3.0	3.0 - 22.2	3.2	70.9	0.020	-0.020	0.020	0.020	0.020	0.020	0.020	0.020
NDVIV2|Ogden	C1/4	121.8	117.1 - 146.0	3.5	36.2	0.037	-0.031	0.037	0.037	-0.006	-0.006	0.037	0.037
NDVIV2|Ogden	I/20	118.8	118.8 - 120.0	3.8	42.1	0.016	-0.016	0.016	0.016	0.016	0.016	0.016	0.016
NDVIV2|Ogden	L/19	160.9	157.2 - 164.3	4.6	20.8	0.010	-0.010	0.010	0.010	0.010	0.010	-0.010	0.010
NDVIV2|Ogden	M/7	6.7	5.4 - 8.2	3.2	13.4	-0.001	-0.009	0.010	0.010	0.010	0.010	-0.001	0.010
NDVIV2|Fort Dodge	L/19	160.9	157.2 - 164.3	3.9	18.4	0.010	-0.010	0.010	0.010	0.010	0.010	-0.010	0.010
RatingV2|Ogden	J/16	80.4	80.4 - 174.8	2.8	93.8	-2.66	1.15	1.15	1.15	1.15	1.15	1.51	1.15
RatingV2|Ogden	L/19	98.0	90.4 - 102.3	2.9	19.5	-0.20	0.20	-0.20	0.20	0.20	0.20	0.20	0.20
RatingV2|Ogden	N/3	295.9	295.9 - 300.4	3.9	23.3	-0.22	0.24	0.00	-0.33	0.23	-0.04	0.09	0.04
RatingV2|Fort Dodge	N/3	295.9	295.9 - 300.4	4.1	27.3	-0.30	0.16	0.00	-0.38	0.32	-0.07	0.19	0.09
RatingV4|Ogden	C1/4	5.1	4.1 - 5.1	3.0	24.6	-0.06	-0.06	-0.06	-0.42	0.48	-0.06	-0.06	-0.06
RatingV4|Ogden	D1b/2	108.4	106.9 - 108.4	2.9	12.6	-0.22	-0.22	0.22	0.22	0.22	-0.22	-0.22	-0.22
RatingV4|Fort Dodge	D1b/2	108.4	107.9 - 108.4	2.8	12.2	-0.21	-0.21	0.21	0.21	0.21	-0.21	-0.21	-0.21

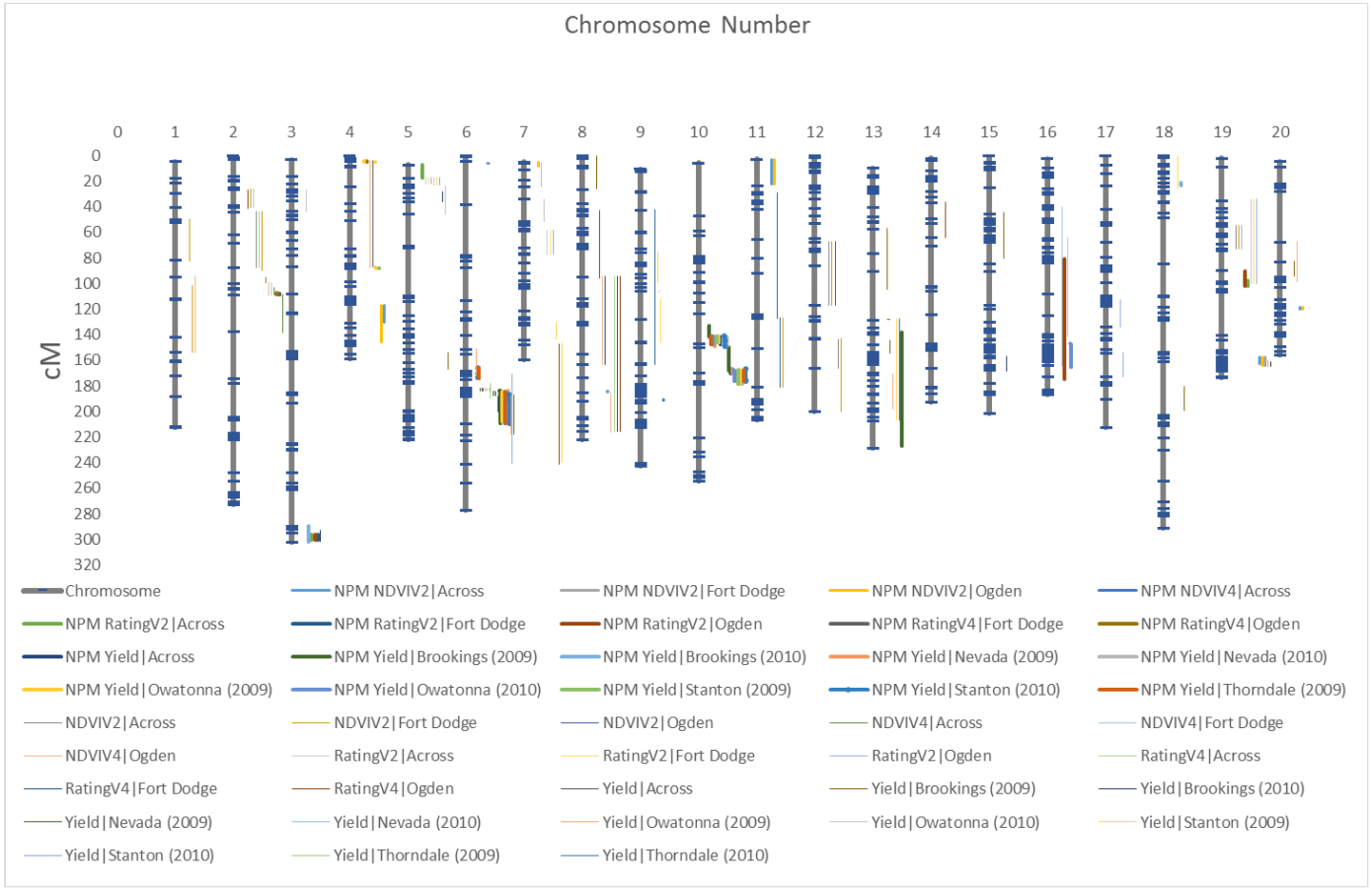
Appendix R: Continued

						Additive Effect of Parental Haplotype							
Trait	LG	LOD Peak Position	1-LOD Support Interval (cM)	LOD Score	R^2 (%)	03DL052038	03KL015763	04KL108888	04KL109378	04KL109428	04KL111519	04KL111531	WW221162
Yield|Brookings (2009)	C2/6	187.0	183.4 - 209.7	7.7	33.5	-1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.55
Yield|Brookings (2009)	F/13	226.9	137.7 - 226.9	3.9	25.3	-1.27	1.27	1.27	1.27	1.27	1.27	1.27	1.27
Yield|Brookings (2009)	O/10	137.4	132.6 - 141.4	10.9	73.2	-1.55	-1.74	-1.74	4.01	-0.72	4.01	-1.74	-1.74
Yield|Brookings (2009)	O/10	166.8	149.8 - 168.1	9.6	34.5	-2.24	-2.24	-2.24	2.11	2.11	0.13	0.13	0.13
Yield|Stanton (2009)	C1/4	87.8	87.8 - 87.8	3.8	51.2	2.33	-2.33	-2.33	-2.33	-2.33	-2.33	-2.33	-2.33
Yield|Stanton (2009)	O/10	144.6	141.4 - 146.2	34.8	54.0	-4.84	-4.84	-4.84	2.17	2.17	2.17	2.67	2.67
Yield|Stanton (2009)	O/10	170.5	168.1 - 178.7	25.2	86.6	-1.46	-7.23	-1.46	6.435	2.245	-1.46	-1.46	-1.46
Yield|Owatonna (2009)	C1/4	4.4	4.0 - 5.1	6.5	47.6	3.36	3.36	3.36	-3.36	3.36	3.36	3.36	3.36
Yield|Owatonna (2009)	C1/4	87.8	5.1 - 87.8	4.7	43.7	3.09	-3.09	-3.09	-3.09	-3.09	-3.09	-3.09	-3.09
Yield|Owatonna (2009)	C2/6	187.0	185.1 - 208.0	7.8	33.7	-2.51	2.51	2.51	2.51	2.51	2.51	2.51	2.51
Yield|Owatonna (2009)	O/10	144.6	141.4 - 146.2	52.4	65.3	-6.66	-6.66	-6.66	2.74	2.74	2.74	3.92	3.92
Yield|Owatonna (2009)	O/10	170.5	168.1 - 178.7	41.9	91.6	-2.12	-10.18	-2.12	9.40	2.91	-2.12	-2.12	-2.12
Yield|Thorndale (2009)	C2/6	172.3	165.9 - 173.8	3.3	15.1	1.57	-1.22	-0.35	-0.35	-0.35	-1.22	-1.22	-0.35
Yield|Thorndale (2009)	C2/6	187.0	185.1 - 209.7	8.7	36.2	-1.85	1.85	1.85	1.85	1.85	1.85	1.85	1.85
Yield|Thorndale (2009)	O/10	143.0	141.4 - 147.8	22.1	42.9	-3.20	-3.20	-3.20	0.97	0.97	0.97	2.23	2.23
Yield|Thorndale (2009)	O/10	172.9	168.1 - 176.1	14.9	80.9	-0.61	-5.07	-0.61	3.08	2.60	-0.61	-0.61	-0.61
Yield|Nevada (2009)	C2/6	191.2	183.4 - 209.7	7.8	30.1	-4.44	-0.08	-0.08	-0.08	-0.08	-0.08	4.51	-0.08
Yield|Nevada (2009)	O/10	143.0	141.4 - 149.4	9.0	23.7	-3.98	-3.98	-3.98	3.55	3.55	3.55	0.43	0.43
Yield|Nevada (2009)	O/10	168.1	166.8 - 170.5	5.8	30.3	-3.26	-3.26	-3.26	3.26	3.26	-3.26	-3.26	-3.26

Appendix R: Continued

						Additive Effect of Parental Haplotype							
Trait	LG	LOD Peak Position	1-LOD Support Interval (cM)	LOD Score	R^2 (%)	03DL052038	03KL015763	04KL108888	04KL109378	04KL109428	04KL111519	04KL111531	WW221162
Yield|Brookings (2010)	G/18	23.2	20.9 - 23.2	3.0	9.4	-0.10	-0.74	-0.74	-0.74	-0.74	-0.10	-0.74	0.84
Yield|Brookings (2010)	N/3	289.8	295.9 - 301.8	4.1	10.1	-0.59	0.00	-0.04	0.52	-0.29	-0.84	0.10	1.14
Yield|Brookings (2010)	O/10	144.6	141.4 - 147.8	18.6	39.9	-2.17	-2.17	-2.17	0.53	0.53	0.53	1.64	1.64
Yield|Brookings (2010)	O/10	170.5	168.1 - 178.7	13.1	78.1	0.02	-3.42	0.02	1.58	1.83	0.02	0.02	0.02
Yield|Stanton (2010)	C2/6	170.5	165.9 - 173.4	2.8	15.9	-2.15	1.38	0.77	0.77	0.77	1.38	1.38	1.38
Yield|Stanton (2010)	C2/6	191.2	187.0 - 209.7	7.8	37.0	-2.33	-1.49	-1.49	-1.49	-1.49	-1.49	3.82	-1.49
Yield|Stanton (2010)	O/10	144.6	141.4 - 149.4	8.3	22.5	-2.63	-2.63	-2.63	0.91	0.91	0.91	1.72	1.72
Yield|Stanton (2010)	O/10	175.4	166.8 - 176.1	8.4	67.3	-0.22	-4.29	-0.22	-0.55	5.06	-0.22	-0.22	-0.22
Yield|Owatonna (2010)	C2/6	187.0	187.0 - 208.0	4.5	23.1	-1.89	1.89	1.89	1.89	1.89	1.89	1.89	1.89
Yield|Owatonna (2010)	J/16	147.5	147.5 - 164.9	6.0	31.1	-2.31	-2.31	2.31	2.31	-2.31	2.31	-2.31	2.31
Yield|Owatonna (2010)	O/10	143.0	141.4 - 147.8	21.4	43.0	-4.09	-4.09	-4.09	3.90	3.90	3.90	0.19	0.19
Yield|Owatonna (2010)	O/10	170.5	168.1 - 176.1	10.8	70.2	-1.74	-5.03	-1.74	1.58	5.20	-1.74	-1.74	-1.74
Yield|Nevada (2010)	C2/6	187.0	185.1 - 208.0	3.7	20.4	-1.78	1.78	1.78	1.78	1.78	1.78	1.78	1.78
Yield|Nevada (2010)	O/10	146.2	141.4 - 149.4	28.6	47.6	-4.99	-4.99	-4.99	3.23	3.23	3.23	1.77	1.77
Yield|Nevada (2010)	O/10	170.5	168.1 - 176.1	18.5	82.2	-1.32	-7.37	-1.32	2.86	5.82	-1.32	-1.32	-1.32

Appendix S: Consensus genetic linkage map, showing the soybean 20 chromosomes colored grey and their (No Suggestions) (cM) lengths. Markers are displayed as blue hash marks. QTLs associated with yield, visual ratings taken at V2 and V4 (RatingV2 and Rating V4), and normalized difference vegetative index taken at V2 and V4 (NDVIV2 and NDVIV4) are displayed as solid lines to the right of chromosomes. Network population mapping (NPM) QTL are represented in thick lines and bi-parental mapping QTL are represented in thin lines.



Appendix T: Variance of the entry mean (V_Y) and narrow-sense heritability on a progeny-mean basis (h^2) Bernardo (2010).

Population	Trait	V_Y	h^2	Population	Trait	V_Y	h^2
RYS002	NDVI (V2)	18.77	0.47	RYS009	NDVI (V2)	19.13	0.48
	NDVI (V4)	18.46	0.49		NDVI (V4)	24.00	0.39
	Chlorosis Rating (V2)	14.99	0.67		Chlorosis Rating (V2)	20.66	0.35
	Chlorosis Rating (V4)	12.51	0.74		Chlorosis Rating (V4)	20.81	0.29
	Yield	1.53	0.86		Yield	1.98	0.75
RYS003	NDVI (V2)	20.55	0.63	RYS010	NDVI (V2)	22.10	0.42
	NDVI (V4)	29.57	0.23		NDVI (V4)	18.86	0.58
	Chlorosis Rating (V2)	22.89	0.53		Chlorosis Rating (V2)	11.89	0.79
	Chlorosis Rating (V4)	16.27	0.57		Chlorosis Rating (V4)	14.89	0.64
	Yield	1.39	0.84		Yield		
RYS004	NDVI (V2)	27.52	0.37	RYS011	NDVI (V2)	20.71	0.57
	NDVI (V4)	13.92	0.36		NDVI (V4)	18.81	0.61
	Chlorosis Rating (V2)	23.39	0.66		Chlorosis Rating (V2)	17.13	0.64
	Chlorosis Rating (V4)	23.08	0.66		Chlorosis Rating (V4)	16.76	0.63
	Yield	1.26	0.81		Yield		
				RYS012			
RYS005	NDVI (V2)	17.56	0.45		NDVI (V2)	14.05	0.62
	NDVI (V4)	21.05	0.39		NDVI (V4)	17.45	0.61
	Chlorosis Rating (V2)	18.49	0.55		Chlorosis Rating (V2)	17.89	0.64
	Chlorosis Rating (V4)	15.77	0.66		Chlorosis Rating (V4)	16.32	0.59
	Yield				Yield		
RYS006	NDVI (V2)	19.38	0.33	RYS013	NDVI (V2)	16.76	0.34
	NDVI (V4)	19.36	0.37		NDVI (V4)	18.05	0.54
	Chlorosis Rating (V2)	18.17	0.54		Chlorosis Rating (V2)	18.42	0.61
	Chlorosis Rating (V4)	19.75	0.39		Chlorosis Rating (V4)	16.04	0.56
	Yield				Yield		
RYS007	NDVI (V2)	21.88	0.27	RYS014	NDVI (V2)		
	NDVI (V4)	20.36	0.51		NDVI (V4)		
	Chlorosis Rating (V2)	18.90	0.58		Chlorosis Rating (V2)		
	Chlorosis Rating (V4)	17.81	0.55		Chlorosis Rating (V4)		
	Yield				Yield	4.58	0.54
RYS008	NDVI (V2)	17.02	0.64				
	NDVI (V4)	16.72	0.64				
	Chlorosis Rating (V2)	13.68	0.72				
	Chlorosis Rating (V4)	15.01	0.63				
	Yield	1.50	0.78				