Genotype × Environment Interactions within Iron Deficiency Chlorosis-Tolerant Soybean Genotypes

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ABSTRACT

Iron deficiency chlorosis (IDC) reduces total soybean [*Glycine max* (L.) Merr.] production in the USA by several million metric tonnes each year. Although IDC-tolerant soybean varieties are available, variety screening is difficult and does not always provide reproducible results. More robust screening methods are needed, but it is unclear how soil properties impact varietal responses to IDC. This study evaluated genotype by environment (G × E) interactions among nine IDC-tolerant soybean genotypes and one moderately tolerant genotype in IDC prone environments in Minnesota to determine which environmental factors most affect this putative G × E effect. Although visual severity of IDC within environments varied from moderate to severe over time, relative genotypic responses were similar, indicating that the timing of IDC ratings may not be critically important in ranking varieties for IDC. Across all environments a highly significant G × E interaction for yield was discovered, with soil factors creating a larger impact than year to year variation. Moreover, G × E interactions were found for visual IDC scores even when significant effects on yield were not found, indicating that variety screening based on IDC scores requires multiple locations to be predictive. Seed quality characteristics, as measured by protein and oil concentrations, were significantly affected by genotype, environment, and their interaction; however, these traits are likely indirectly affected by IDC through its effect on yield. A linear model based on three common soil chemical factors was developed to help predict future IDC severity by soil analysis alone.

Iron deficiency chlorosis is a common and economically important yield-limiting factor for soybean produced in the North Central USA. Yield losses have been estimated to cost producers at least $120 million annually (Hansen et al., 2004). As soybean production has expanded throughout high pH regions of western Minnesota and the Dakotas, the scope of this problem has grown. Although IDC only occurs when soil pH $>$ 7.2, many high pH soils (often termed calcareous soils) do not display IDC symptoms (Hansen et al., 2004). Soil properties associated with IDC have been studied for decades; however, clear determination of which factors affect IDC and their relative importance is yet unknown. Several soil factors have been noted as contributors of IDC symptoms including soil carbonates (Inskeep and Bloom, 1987; Morris et al., 1990), specifically HCO_3^-, (Inskeep and Bloom, 1984; Coulombe et al., 1984a), ionic strength of the soil solution, as measured by electrical conductivity (EC) (Franzen and Richardson, 2000; Morris et al., 1990), iron (Fe) oxide concentration in the soil (Morris et al., 1990), and diethylenetriaminepentaacetate (DTPA)-extractable Fe, chromium (Cr), soluble salts, and soil water content (Hansen et al., 2003).

Many management strategies have been studied to reduce the severity of IDC. Foliar sprays (Goos and Johnson, 2000; Penas et al., 1990; Randall, 1981), Fe seed treatments (Wiersma, 2005; Goos and Johnson, 2001; Karkosh et al., 1988), increased seeding rates (S.L. Naeve, unpublished data, 2002; Goos and Johnson, 2000; Penas et al., 1990), and companion crops (S.L. Naeve, unpublished data, 2002) have all shown limited promise as management practices. Variety selection, however, appears to be the most reasonable method available to farmers for reducing losses from IDC (Goos and Johnson 2001, 2000). Producer respondents to a 2002 survey of IDC and IDC management practices in Minnesota overwhelmingly chose variety selection as a primary management practice (Hansen et al., 2003).

Iron deficiency chlorosis varies spatially and temporally. Within production fields, symptoms can vary from severe to nonexistent within a meter due in part to soil heterogeneity. Chlorotic areas can grow and shrink in unpredictable patterns throughout the growing season, and severity of IDC within a single field can vary from year to year. The unpredictable nature of this disorder makes screening of lines and varieties difficult (Diers et al., 1991; Niebur and Fehr, 1981). Many state variety testing programs do not evaluate entries for IDC tolerance. Of those states that do screen, often only one environment is used. Due to soil heterogeneity, IDC screening is conducted with hill plots or in microplots and uses visual IDC scores only. Yield testing under IDC conditions is not usually performed by soybean-breeding programs or cultivar evaluation trials in the USA.

Results of genotype by environment (G × E) studies with IDC have been mixed. Niebur and Fehr (1981) evaluated genotypic responses to calcareous and noncalcareous environments with the addition of foliar-applied chelated Fe to remove the confounding effect of IDC itself. They concluded that selection for yield, quality, and harvest traits can be made among IDC-tolerant lines in noncalcareous environments. Jessen et al. (1988) and Coulombe et al. (1984b) found field IDC scores to be highly correlated with analogous scores from nutrient solution systems. Both systems used increased HCO_3^- concentration in nutrient solutions to

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Abbreviations: ANOVA, analysis of variance; DTPA, diethylenetriaminepentaacetate; EC, electrical conductivity; G × E, genotype by environment; IDC, iron deficiency chlorosis.

increase IDC severity. Charlson et al. (2003), however, found visual IDC scores among F2:4 derived lines to vary substantially when grown at two locations with calcareous soils. The authors found three single sequence repeat markers to be associated with IDC scores in each environment. However, each environment was associated with three distinct markers, clearly demonstrating a strong G × E interaction down to the molecular level. Morris et al. (1990) examined three genotypes with a broad range of IDC tolerance on calcareous soils from Texas in a greenhouse experiment. They found a differential response to individual soil factors by genotype. A better understanding of G × E effects among highly tolerant genotypes on IDC prone soils is needed.

While variety selection is the primary tool for managing IDC, Minnesota soybean producers are increasingly concerned about the quality of variety ratings for IDC. Producers often report that varieties perform differently on their farms than stated by seed companies or public variety testing programs, which implies that a G × E interaction may be at play. Varieties may be tested across too few years, as soybean varieties are hastily brought to market, or perhaps varieties are simply screened for IDC tolerance in too few locations. Development of more robust testing strategies is essential.

The objectives of this study were to investigate whether (i) IDC scores can be recorded at any time during the early season, or if genotypes recover from IDC at different rates, (ii) a G × E interaction for soybean yield is present among IDC-tolerant varieties produced on a range of IDC prone soils, (iii) year or environment has greater influence on IDC tolerance, (iv) IDC scores are good predictors of genotypic performance across a range of IDC prone environments, and (v) IDC affects soybean seed protein and oil concentration directly, or through a complex G × E interaction. A sixth objective was to develop an IDC index based on common soil chemical parameters to predict IDC severity. These objectives were tested by growing nine IDC tolerant and one moderately tolerant commercial soybean variety on 11 different IDC prone soils over 3 yr and examining the resulting G × E interactions.

MATERIALS AND METHODS

Thirteen field locations in western Minnesota were selected for studies in 2002 through 2004 in four counties–Wilken, Grant, Swift, and Chippewa. Soils in the area are derived from lacustrine deposits and glacial tills. Locations were identified based on occurrence of IDC in previous soybean crops as observed by farmer cooperators. Location selection criteria included the identification of sites with a range of soil types and soil chemical properties. Composite soil samples (50, 0–15 cm cores) were collected from these georeferenced locations at the time of planting. Samples were oven-dried at 40°C, ground (<2 mm) and analyzed by standard soil tests for available nutrients (Dahnke, 1988), EC by 1:1 soil/water method (USDA, 1954), DTPA-extractable Fe (Lindsay and Norvell, 1978) and calcium carbonate ($CaCO_3$) equivalent (Williams, 1949) (Table 1). No additional fertilizer was applied to any of the locations. Locations were numbered based on geographic location, from northeast to southwest. Locations 1, 2, and 3 were located in the same 130-ha production field in 2002 to 2004. Research plots in this single location had identical rotational history and soil fertility and herbicide programs in each of the 3 yr. Due to a continuous 3-yr rotation (wheat [*Triticum aestivum* L.] followed by sugarbeet [*Beta vulgaris* L.] followed by soybean), these locations were not positioned on exactly the same area of the field each year, but moved on a North-South line within 1 km of one another. The term 'location' is used herein to describe the physical location and associated chemical soil properties, while the term 'environment' is used to describe the combination of location characteristics and year effects experienced by a crop.

Ten commercial soybean genotypes, Asgrow AG0801, Asgrow AG1401, Dekalb DKB09-52, Pioneer 90B51, Pioneer 91B03, Pioneer 91B52, Prairie Brand 810, Stine 0806-4, Stine 1007-4, and Stine 1346-4, were chosen based on IDC ratings made by the University of Minnesota Soybean Variety Testing Program in 2001 (Orf et al., 2002). Nine highly tolerant and one moderately tolerant genotype (Stine 1007-4) were selected. Asgrow AG0801 was selected as a reference genotype as it has been widely grown and generally known to be the most IDC-tolerant, high-yielding, glyphosate-tolerant genotype in its maturity range.

Soybean genotypes were planted in a randomized complete block design in four 76-cm rows with a small-plot, cone-type research drill. Plots measured 7.6 m in length. Plots were seeded at 457 000 seeds ha^{-1}. All locations were planted be-

Table 1. Selected location characteristics, 2002 to 2004.

Location	Year	Environment	Soil series†	pH	EC‡	$CaCO_3$§	OM	Olsen-P	K	Mn	Zn	Cu	Fe
					$S\ m^{-1}$	$g\ kg^{-1}$		$mg\ kg^{-1}$					
46°19′ N, 96°24′ W	2002	1	Elmsville vf sal	8.2	0.11	58	41	17	147	4.3	1.7	0.6	6.3
46°20′ N, 96°24′ W	2003	2	Elmsville vf sal	8.3	0.06	49	40	20	134	3.5	1.2	0.5	4.6
46°20′ N, 96°24′ W	2004	3	Elmsville vf sal	8.2	0.08	20	34	10	102	3.8	1.6	0.5	5.1
46°17′ N, 96°24′ W	2002	4	Hamerly-Lindaas scl	8.0	0.10	25	39	22	187	4.4	1.6	1.0	5.2
46°16′ N, 96°24′ W	2003	5	Wheatville sl	8.3	0.03	70	35	11	152	4.5	1.1	1.0	7.2
46°14′ N, 96°27′ W	2004	6	Aazdahl cl	7.8	0.05	24	38	17	170	7.7	1.5	0.8	12.3
45°58′ N, 96°12′ W	2002	7	Roliss l	7.9	0.09	54	ND#	5	180	ND	ND	ND	13.4
45°50′ N, 96°11′ W	2003	8	Roliss l	8.5	0.02	49	21	10	98	6.6	2.2	0.4	6.4
45°15′ N, 95°42′ W	2004	9	Bearden scl	8.3	0.12	141	49	25	186	5.2	2.8	0.9	5.9
45°10′ N, 95°27′ W	2002	10	Colvin scl	8.3	0.04	184	ND	21	274	ND	ND	ND	7.7
44°56′ N, 95°20′ W	2003	11	Colvin-Spicer scl	8.0	0.21	102	56	13	138	6.4	1.9	1.0	7.7
44°55′ N, 95°19′ W	2003	12	Colvin-Spicer scl	7.9	0.13	44	68	27	170	4.5	3.0	1.0	9.9
44°55′ N, 95°18′ W	2002	13	Colvin-Spicer scl	8.0	0.22	86	59	18	239	6.0	1.7	1.1	5.9

† vf = very fine, sa = sandy loam, scl = silty clay loam, sl = silt loam, cl = clay loam, l = loam.

‡ EC = Electrical conductivity.

§ $CaCO_3$ = calcium carbonate equivalents.

¶ OM = Organic matter.

ND = Not determined.

tween 16 and 28 May each year. Weeds were controlled by hand weeding and applications of glyphosate [N(phosphonomethyl)glycine] at 0.84 kg a.e. ha^{-1} two times at each location as needed.

Visual IDC scores were recorded, where 1 = green and 5 = severe chlorosis with some necrosis (Cianzio et al., 1979). In 2002, visual IDC scores were recorded on 21 June, 3 July, 12 July, 24 July, and 3 August (IDC Scores 1, 2, 3, 4, and 5, respectively). In 2003, visual scores were recorded on 1 July, 16 July, and 29 July (IDC Scores 2, 3, and 4, respectively). In 2004, visual scores were recorded on 1 and 20 July (IDC Scores 2 and 4, respectively). Relative leaf chlorophyll concentrations were measured with a Minolta SPAD-502 chlorophyll meter (Minolta, Ramsey, NJ) by examining five fully expanded leaves in 2002. Relative leaf chlorophyll concentrations were correlated with visual ratings ($r^2 = -0.93$) and were not reported here. Similarity of these methods has been demonstrated by others (Hansen et al., 2003; Cianzio et al., 1979); therefore, chlorophyll concentrations were not recorded in 2003 or 2004.

The two center rows of each plot were harvested with a small-plot combine. Grain mass and moisture concentration were recorded in-field, and a 1-kg subsample was retained for further analysis. Protein and oil analysis was conducted by near infrared spectroscopy. A Foss full scanning 6500 monochrometer (Foss North America, Eden Prairie, MN) fitted with equations developed by the University of Minnesota using ISIscan software (Infrasoft Intl. LLC., State College, PA) and validated by Caltest, LLC. (Clifton Park, NY) was used. To ensure the availability of an appropriate sample, only plots yielding >300 kg ha^{-1} were used for protein and oil analysis.

A critical element of this research was the development of a yield-based measure of IDC severity for each environment. This value allowed the creation of a mathematical model relating soil properties to IDC that could be applied to other environments. Iron deficiency chlorosis severity was determined to be best estimated by the yield of the most susceptible genotype (Stine 1007-4) relative to the yield of the most IDC-tolerant genotype (Asgrow AG0801). This value best represents actual IDC severity for each environment because it uses actual grain yields and includes measures of both IDC severity and overall yield potential. A step-wise regression was used to create a linear model for location soil properties that best correlated with the observed IDC severity of each environment. The regressional analysis used all soil properties shown in Table 1 vs. IDC severity of each environment to create a best fit model. The resulting linear model provides an IDC index value for any soil based on common soil chemical factors.

Visual IDC scores as well as seed yield and quality values from all 10 genotypes were used in all statistical analyses and results reported here except in the creation of the IDC index described above, which used only yields of the most susceptible and tolerant genotypes. All analysis of variance (ANOVA), correlation, and regression analyses were performed with GLM, CORR, and REG procedures, respectively, using SAS v. 9.1 (SAS Institute, Cary, NC). Environment and genotype within environment means were compared with Duncan's multiple range test. Sources of variation, means, and correlations were declared significant at $P \leq 0.05$. During stepwise regression a significance level of 0.15 was used for selection and removal from the regression model.

RESULTS

All locations had adequate soil phosphorus (P) and potassium (K) fertility, except the location corresponding with Environment 7 which had a low soil test P level (Table 1). Although previous research has not provided perfect causal relationships between individual soil properties and soybean yields under IDC conditions, the majority of IDC research with soybean has pointed to one or more factors (pH, calcium carbonates, salts, or DTPA-extractable Fe) as the primary drivers of this disorder. In the research described here, all locations had calcareous soils with pH > 7.8. Calcium carbonates were often high (>40 mg kg^{-1}) and several locations had EC levels that were >0.10 S m^{-1}. Based on these soil factors, all of these locations would be considered to be at medium to high risk for IDC in soybean (Hansen et al., 2003).

Temporal Changes in Visual Iron Deficiency Chlorosis Severity

Although all locations were at risk of developing IDC based on previous history and soil test values, severity of IDC symptoms varied greatly among environments. Visual IDC ratings were recorded in early and late July (Scores 2 and 4, respectively) in all environments (Table 2). Three environments produced uniformly green soybean genotypes at the early June screening date (Score 2 = 1.0). By late July, two additional environments had average scores of 1.0 and one more had an average score of 1.1. Two environments were severely chlorotic at the time of Score 2 with average scores of ≥3.0, and three environments had average scores of ≥3.0 at the time of Score 4.

In 2002, visual IDC ratings were recorded at five times during the season from 21 June through 3 August for five environments (Fig. 1). Average IDC scores for these sample dates indicate that the temporal variation in IDC symptoms varied slightly within and among environments. Symptoms tended to be most severe through the month of July and improved thereafter. An ANOVA for environmental, genotypic, and date of IDC Score indicated that while there was an environment by date of IDC Score interaction, there was not a genotype by date or genotype by date by environment interaction (Table 3). This suggests that although genotypic scores tended to change over time, the relative tolerance of genotypes within environments does not change.

Relative Importance of Year and Soil Properties to Environment

Because Environments 1, 2, and 3 occurred in the same production field in 2002, 2003, and 2004, respectively, they offered a unique opportunity to examine G × E interactions for IDC while minimizing the soil component of E. Soil chemical properties varied only slightly within this location over years (Table 1), so that environmental effects were driven primarily by changes in weather patterns (Fig. 2). Average air temperature at this location was only 16.2°C (15 May through 14 October) in 2004, whereas it was 18.0°C in both 2002 and 2003. Although 2004 was much cooler overall, 2003 was dryer. Total rainfall for the same period was 268 mm in 2003 compared with 342 and 371 mm in 2002 and 2004. Although visual IDC scores and yields were moderate

Table 2. Visual iron deficiency chlorosis (IDC) severity scores, seed yields, seed protein and oil concentrations, and IDC index values for 13 environments. Environment mean yields, yields of tolerant and susceptible genotypes, and their relative yield are provided. Seed protein and oil concentrations for environmental means, as well as those of tolerant and susceptible varieties are also provided. Iron deficiency chlorosis index values were calculated for each site from soil properties by step-wise regression analysis using relative yield values.

	All genotypes†					Tolerant genotype‡			Susceptible genotype§				
Environment	IDC score 2¶#	IDC score 4††	Grain yield	Protein conc.	Oil conc.	Grain yield	Protein	Oil	Grain yield	Protein conc.	Oil conc.	Relative yield‡‡	Index value§§
			kg ha^{-1}	g kg^{-1}		kg ha^{-1}	g kg^{-1}		kg ha^{-1}	g kg^{-1}			
1	3.0 b	2.7 b	1760 e	399 c	215 d	2210 cdef	377 cd	217 cd	1080 d	415 ab	204 d	0.465	0.578
2	1.4 g	1.5 d	1480 fg	419 a	210 e	1630 f	406 ab	213 d	610 edf	426 ab	211 cd	0.436	0.914
3	2.3 de	1.9 c	1600 ef	415 ab	210 e	1620 f	410 ab	199 e	1170 de	439 a	228 ab	0.726	0.789
4	1.2 h	1.0 e	2450 c	372 f	216 d	2520 bcd	366 d	212 d	2410 b	373 d	211 cd	0.993	0.722
5	1.7 f	1.1 e	2160 d	387 d	227 b	2360 bcde	381 c	223 bc	1770 c	389 cd	225 bc	0.813	0.745
6	1.0 h	1.0 e	2080 d	414 b	207 f	1940 def	412 ab	199 e	2130 bc	414 abc	205 d	1.102	1.277
7	1.0 h	1.0 e	3050 a	410 b	209 ef	2660 bc	402 b	204 e	3050 a	406 bc	208 d	1.185	1.083
8	1.0 h	1.0 e	2420 c	391 d	228 b	2580 bcd	384 c	220 bcd	2390 bc	401 bc	224 bc	0.935	0.838
9	2.9 b	3.2 a	758 h	414 b	208 ef	779 g	417 a	203 e	198 f	423 ab	202 d	0.133	0.056
10	2.5 cd	2.7 b	1400 fg	400 c	216 d	2560 bcd	387 c	215 cd	183 f	415 ab	205 d	0.065	0.101
11	3.9 a	3.3 a	1260 g	380 e	220 c	1720 ef	376 cd	227 ab	391 ef	410 bc	210 cd	0.163	0.188
12	2.2 e	1.0 e	2800 b	378 e	236 a	2900 ab	376 cd	233 a	2630 ab	366 d	242 a	0.907	0.835
13	2.5 c	3.2 a	2410 c	388 d	221 c	3540 a	372 cd	217 cd	341 f	417 ab	208 d	0.098	0.146

† Environmental mean of 10 genotypes (*n* = 40).
‡ Tolerant = yield, protein, and oil concentration of AG0801 (*n* = 4).
§ Susceptible = yield, protein, and oil concentration of 1007–4 (*n* = 4).
¶ Score 2 was recorded between 1 July and 8 July in each environment.
# Within the same column, means followed by the same letter are not significantly different (*P* = 0.05).
†† Score 4 was recorded between 20 July and 29 July in each environment.
‡‡ Relative yield = fractional yield of susceptible relative to tolerant genotype.
§§ IDC Index value.

and relatively stable across these environments over the 3-yr study period (Table 2), the ANOVA for these three environments (Table 4, three environments at one location) indicated there was a strong genotype effect on soybean yield without a significant G × E interaction. Environment itself was nearly significant (*P* = 0.0572).

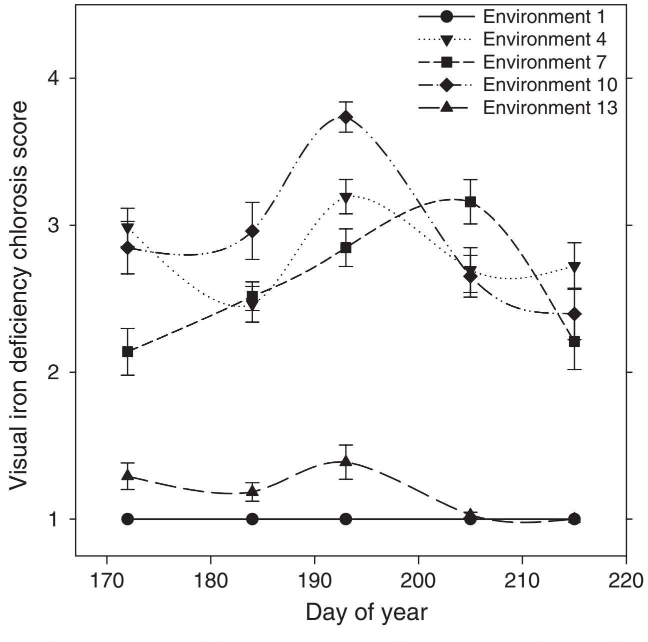

Fig. 1. Mean iron deficiency chlorosis (IDC) scores over time for five western Minnesota environments studied in 2002. Values represent visual chlorosis scores (Cianzio et al., 1979) where 1 = green and 5 = severe chlorosis with some necrosis for each environment, and are means of 10 genotypes and four replications ± SE (*n* = 40). Scores were recorded on 21 June, 3 July, 12 July, 24 July, and 3 August.

In contrast with the effect on yield, there was a strong G × E interaction for visual IDC scores and quality traits across these three environments at one location.

Table 4 also shows an ANOVA of IDC scores, yield, and quality trait effects across all environments (13 environments). The main effects of environment and genotype were significant for all dependant variables examined, as was their interaction. To further analyze whether genotypic responses to stresses induced by IDC may be stable across environments with similar soil properties, environments with IDC index values within the range of those found in locations 1, 2, and 3 (i.e., locations 4 and 5) were analyzed as a group with environments 1 to 3 to examine G × E interactions. Similar to the results from the single location analysis (Table 4, three environments at one location) and in contrast to the full analysis (13 environments), this combined analysis (five environments) showed no G × E interaction for seed yield. As in other analyses, quality factors and visual IDC ratings did show interactive effects between genotype and environment. When environments were grouped and analyzed based on severity of visual IDC

Table 3. Significance of environment, score date, genotype and their interactions on visual iron deficiency chlorosis (IDC) scores within five environments (1, 4, 7, 10, and 13) in 2002.

	Effects
Source of variation	IDC score
Environment (E)	<0.0001
Date of score recording (D)	<0.0001
E × D	<0.0001
Genotype (G)	<0.0001
G × E	<0.0001
G × D	0.7301
G × E × D	0.9001

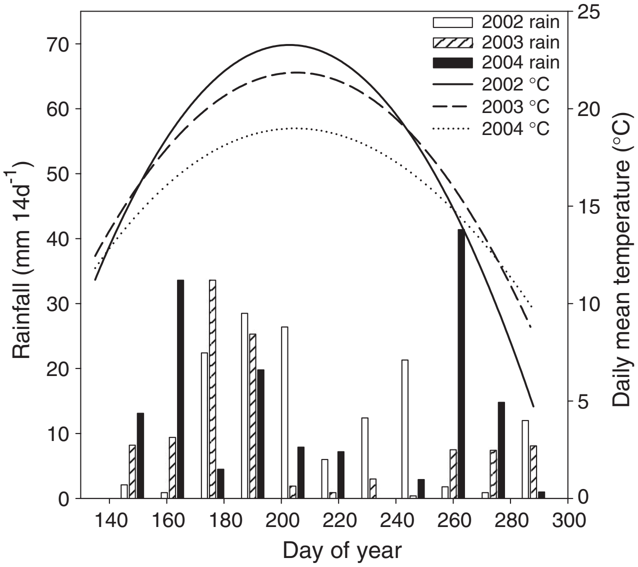

Fig. 2. Rainfall and daily mean temperatures for Environments 1, 2, and 3. Rainfall is represented as a summation of daily rainfall over 14-d periods beginning 15 May and ending 14 October. Daily mean temperatures are represented by third order regression curves drawn to best fit temperature data over the same period.

scores alone, all combinations of environments resulted in significant G × E effects for all dependant variables (data not shown).

When analyzed across all genotypes and environments, soybean yield, seed quality traits, and visual IDC scores tended to correlate with one another in a predictable manner (Table 5). Protein and oil were positively and negatively correlated with seed yield, respectively, while these components were negatively correlated with one another. The sum of protein plus oil negatively correlated with seed yield. Iron deficiency chlorosis Score 2 and Score 4 were both negatively correlated with seed yield, but Score 4 appeared to predict seed yield effects of IDC slightly better. Although protein and oil were correlated with yield, as were the visual IDC scores, Score 2 and Score 4 correlated with soybean protein and oil relatively poorly (albeit significantly in three of four instances).

Examining correlations within environments and genotypes provides additional information about IDC's potential effects on yield and quality traits (Table 5). Of 10 environments exhibiting IDC symptoms at the time of IDC Score 2, only five showed a significant ($P < 0.05$) negative correlation between yield and Score 2. This number increased to seven of seven environments exhibiting significant IDC symptoms at the time of Score 4. Likewise, 8 of 10 genotypes showed significant correlations between Score 2 and yield. This number increased to 10 at the time of Score 4. Score 4 was significantly correlated with protein and oil concentration when analyzed across environments and genotypes, however, when analyzed within these, the relationship appears less concrete. Score 4 was positively correlated with seed protein concentration in only 2 of 13 environments and 1 of 10 genotypes.

A Predictive Iron Deficiency Chlorosis Index

The best measure of IDC severity of individual environments was determined to be the yield of the most IDC susceptible genotype (Stine 1007-4) relative to the yield of the most tolerant (Asgrow AG0801) (Table 2). This IDC severity measure removed much of the influence of non-IDC yield affecting factors that would have been present when using yields of individual genotypes or mean yields of all genotypes. A predictive IDC index was calculated for each environment by using this relative yield measure as the dependant variable and soil chemical factors as the independent variables through a step-wise regression. Soluble salts, $CaCO_3$, and DTPA-extractable Fe were found to best describe the variation in the IDC severity between environments by the following relationship:

$$I = 0.77 - 2.25EC - 0.00572CaCO_3 + 0.0615Fe$$

where I = the IDC severity index, EC = soluble salts concentration as measured by electrical conductivity (S m^{-1}), $CaCO_3$ = calcium carbonate equivalents (g kg^{-1}), and Fe = DTPA-extractable Fe (mg kg^{-1}). Calcium carbonate

Table 4. Significance of environment, genotype, and their interaction on seed yield, seed protein concentration, oil concentration, sum of protein and oil concentrations, and two visual iron deficiency chlorosis (IDC) scores.

Source of variation	Effects Yield	Protein	Oil	P + O†	IDC score 2	IDC score 4
			3 environments at 1 location‡			
Environment (E)	**0.0572**	**<0.0001**	**0.0003**	**<0.0001**	**<0.0001**	**<0.0001**
Genotype (G)	**0.003**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
G × E	**0.2316**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0003**	**0.0364**
			13 environments§			
Environment (E)	**<0.0001**	**<0.0001**	**0.004**	**0.001**	**<0.0001**	**<0.0001**
Genotype (G)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
V × E	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
			5 environments¶			
Environment (E)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Genotype (G)	**0.0006**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
G × E	**0.0747**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

† P + O, the numerical sum of protein and oil concentrations.

‡ Environments 1, 2, and 3 were combined for this ANOVA. These environments were located in the same production field in 2002, 2003, and 2004.

§ Environments 1 to 13 were combined for this ANOVA. These environments represent all environments examined.

¶ Environments 1, 2, 3, 4, and 5 were combined for this ANOVA. These five environments had similar IDC severity ratings (between 0.58 and 0.79) as shown in Table 2.

Table 5. Correlation matrix for yield, seed protein concentration, oil concentration, the sum of protein and oil concentrations, and two visual iron deficiency chlorosis (IDC) scores across environments and genotypes and within environments and genotypes.

	Protein	Oil	P + O†	IDC score 2	IDC score 4
Yield					
Correlation‡	−0.3504	0.3772	−0.1895	−0.5153	−0.6939
Significance§	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Significant environments¶	4−, 1+	7+, 1−	3−, 1+	5−, 1+	7−
Significant genotypes††	6−	7+	3−	8−	10−
Protein (P)					
Correlation	–	−0.6524	0.842	−0.1057	0.1158
Significance	–	<0.0001	<0.0001	0.0249	0.0138
Significant environments	–	11−, 1+	13+	0	2+
Significant genotypes	–	10+	10+	0	1+
Oil (O)					
Correlation	–	–	−0.1407	0.0712	−0.17
Significance	–	–	0.0027	0.1315	0.0003
Significant environments	–	–	3−, 1+	1+, 1−	4−, 1+
Significant genotypes	–	–	2−	1+	2−
P + O					
Correlation	–	–	–	−0.0874	0.0303
Significance	–	–	–	0.0638	0.5201
Significant environments	–	–	–	1+	1+
Significant genotypes	–	–	–	1−	1+
IDC score 2					
Correlation	–	–	–	–	0.7896
Significance	–	–	–	–	<0.0001
Significant environments	–	–	–	–	8+
Significant genotypes	–	–	–	–	10+

† P + O, the numerical sum of protein and oil concentrations.

‡ Pearson correlation coefficient for independent variables examined across all environments and genotypes.

§ Probability of a larger *r* value for independent variables examined across all environments and genotypes.

¶ Number of significant ($P \leq 0.05$) environments (of 13) when variables were examined by environment. The + and – symbols represent the number of environments where the two independent variables were positively and negatively correlated, respectively.

†† Number of significant ($P \leq 0.05$) genotypes (of 10) when variables were examined by genotypes. The + and – symbols represent the number of environments where the two independent variables were positively and negatively correlated, respectively.

equivalent alone provided an r^2 of 0.51, while the second step combined $CaCO_3$ and DTPA-extractable Fe concentrations to produce an r^2 of 0.67. Together $CaCO_3$, DTPA-extractable Fe concentrations and EC produced a highly significant r^2 of 0.77 ($P < 0.0001$).

DISCUSSION

Iron deficiency chlorosis is an important soybean production problem in the upper Midwest of the USA (Hansen et al., 2004), that can be partially overcome by variety selection (Goos and Johnson, 2001, 2000). However, soybean producers often report that varietal ratings from public testing programs and from the private seed industry are not predictive of performance in their own fields. This may be a result of variety evaluations in too few years or locations. This study set out to examine G × E affects of IDC on soybean to better understand the relative importance of soil and other environmental impacts on genotypic responses to IDC.

Soybean breeders and those evaluating released varieties have been concerned about the timing of visual IDC scoring in IDC screening trials. The apparent lack of a genotype by date of scoring effect in this study indicates that relative genotypic tolerance is not affected by the date of the score. However, this study does show that IDC scores recorded in late July (Score 4) correlate slightly better with soybean yield than those recorded in early July (Score 2). While IDC scores were significantly correlated with yield overall, this relationship was not a perfect one. Varietal screening should include yield testing when possible.

While research studies of IDC have focused on soil factors, anecdotal evidence has indicated that weather patterns play a very large role in this disorder. Farmers complain that IDC is highly variable from year to year. This complicates variety selection for the producer as they are unsure as to the severity of IDC in the upcoming year. Hansen et al. (2003) found soil moisture content to be positively correlated with IDC symptoms in producer fields, indicating that yearly variations in rainfall patterns could affect IDC symptoms. If weather does affect IDC, the larger question becomes: Do weather and genotypic effects interact so that the relative IDC tolerance of genotypes changes over years? Results from one location studied in 3 yr implied that weather effects do not greatly affect the relative performance of genotypes impacted by IDC. Moreover, when the analysis was expanded to include other locations with similar soil properties, a continued lack of G × E interaction indicated that similar soils affect genotypes in the same way even when these soils are a part of distinctly different environments. Conversely, when environments were grouped and analyzed simply by visual IDC severity rather than by soil factors (data not shown), large G × E's were identified. This indicates that visual IDC severity of a location alone is not a good predictor of future varietal performance.

The large G × E interaction for soybean yield found when all environments were analyzed together, and the apparent lack of a year effect from the analysis of one location over 3 yr, indicates that soil chemical properties have a significant effect on not only IDC severity but also on the manner in which individual genotypes may respond to this stress. This solidifies the notion that variety evaluation for IDC must occur on multiple IDC prone locations with varying soil chemical factors. A broad range of soil chemical backgrounds is important when multiple locations are chosen for variety evaluation. Therefore, it is important to use soil-test data (rather than simply historical visual IDC severity) when selecting locations. The importance of evaluating genotypic tolerance over multiple years was not borne out by this work. Alternatively, the apparent lack of a year effect indicated that varieties that yield well in individual IDC prone production fields are likely to continue to perform well for producers over several years.

In support of Froehlich and Fehr (1981) who found that IDC did not affect soybean seed protein or oil concentration, this study was unable to provide evidence that protein and oil concentration are directly impacted by IDC. Large environmental effects on seed quality may be due simply to year effects (temperature and rainfall), while large genotypic effects are likely due to genetic protein and oil potentials rather than IDC tolerance, per se. The highly significant G × E effect on seed quality may imply that IDC is acting to differentially affect genotypic protein and oil concentrations directly, but this study did not identify other indicators of this. Although protein and oil were positively and negatively correlated, respectively, with IDC scores taken in late July, regression coefficients were relatively small (0.12 and -0.17) and few of the individual environments or genotypes showed significant correlations when these factors were analyzed independently. It is likely that protein and oil are simply autocorrelated to score through their relationship with yield.

This study provided an IDC forecasting model based on commonly analyzed soil chemical properties and derived from the relative yield of a moderately IDC tolerant and an IDC-tolerant genotype. While this model does not have general applicability across all moderately tolerant/tolerant genotypic pairs, it does provide a numerical prediction of future IDC severity of an individual location based on three commonly tested soil factors (EC, $CaCO_3$, and DTPA-extractable Fe). A location with an IDC index of >1.0 would not likely be impacted by IDC, as moderately tolerant varieties would be expected to be as productive as highly tolerant ones. Alternatively, a location with an IDC index of <0.5 is likely to exhibit strong IDC symptoms, as a moderately tolerant variety would be expected to yield less than half that of a highly tolerant one. This field should be planted to only the most tolerant genotypes available.

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