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Iron-deficiency chlorosis evaluation of soybean with tissue culture

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Summary. The objectives of this study were: (i) to develop a tissue culture technique for the evaluation of Fe efficiency in soybean, and (ii) to compare the laboratory technique with field Fe chlorosis scores. Nineteen genotypes that had low and high levels of Fe efficiency were evaluated in the laboratory and at five field locations. Friable callus was induced from epicotyl sections, weighed, and placed on two different modified Murashige and Skoog media; one low in α-naphthaleneacetic acid and the other low in Fe. Callus growth was rated as lack of growth compared to respective controls. As an example, Fe-inefficient cultivars ('Asgrow A3205' and 'Pride B216') had significantly reduced growth compared to Fe-efficient germ plasm lines ('A11' and 'A14'). Correlation between the laboratory and field chlorosis rating was highest for the low auxin medium ($r^2 = 0.78$), although correlation for the low Fe medium was also significant ($r^2 = 0.72$). These results show that in vitro evaluation for Fe efficiency can be a useful tool for plant breeders.

Key words: *Glycine max* (L.) Merr. – Iron-deficiency chlorosis – Tissue culture

Introduction

Iron-deficiency chlorosis (IDC) of soybean [*Glycine max* (L.) Merr.] grown on calcareous soils can cause economically important yield loss (Fehr and Trimble 1982). Chaney et al. (1972) showed that ferrous (Fe^{2+}) iron was the principle form absorbed by soybeans. Therefore, IDC is most likely to occur on alkaline soils where insoluble ferric (Fe^{3+}) iron predominates (Olsen and Brown 1980). Landsberg (1984) further demonstrated that auxin produced by chlorotic leaves may be a 'signal' to initiate the Fe deficiency response and increase Fe uptake by the roots.

There are two approaches to the control of IDC, chemical and genetic. Chemical strategies include foliar treatment, in-furrow placement, and seed treatment. Randall (1977) showed that a foliar application of chelated Fe is effective in reducing yield losses. Frank and Fehr (1983) found that a band of H_2SO_4 directly under the seed was also effective in reducing yield losses related to Fe chlorosis. Karkosh et al. (1988) evaluated seed treatment as a means to reduce yield loss associated with IDC and found that seed treated with the Fe chelate, Fe-EDDHA [ethylenediamine-di(o-hydroxyphenylacetate)], could be economically useful for genotypes that had a moderate level of Fe efficiency. Fe-inefficient genotypes did not benefit from the treatment when grown on calcareous soil.

The second approach to control IDC is the development of cultivars that can utilize the iron in calcareous soils. Weiss (1943) determined that soybeans differ in their ability to use Fe when grown on calcareous soil and that Fe efficiency was conditioned by a single dominant allele (*Fe*). More recently, de Cianzio and Fehr (1982) reported that Fe efficiency is governed by a major allele plus modifiers and should be treated as a quantitative trait. Cultivars that have high levels of Fe efficiency have been developed (Fehr and Bahrenfuss 1980; Fehr et al. 1984; Jessen et al. 1988).

Under a genetic improvement scheme, a breeder must evaluate for improved resistance to IDC in the field on an alkaline soil (de Cianzio et al. 1979). A nutrient-solution system developed by Coulombe (1984) has been used to evaluate soybean genotypes for Fe chlorosis, and Jessen et al. (1986) reported good correlation ($r^2 = 0.76$) between nutrient-solution chlorosis scores and field

chlorosis ratings. Advantages of the nutrient-solution technique are that it provides a repeatable environment and can be used throughout the year. However, nutrient solution evaluation is expensive and cannot handle the large numbers required in most plant breeding programs (Jessen et al. 1986). An alternative to the nutrient-solution system would be tissue culture, which may be less expensive and not space limited, therefore more genotypes could be evaluated.

The objectives of this study were: (i) to develop a tissue culture technique for the evaluation of Fe efficiency in soybean, and (ii) to compare the laboratory technique with field Fe chlorosis scores.

Materials and methods

Soybean genotypes, which could be placed into three Fe efficiency classes (efficient, intermediate, and inefficient), were selected for this study based on the Fe chlorosis scores in the Uniform Soybean Tests, Northern States (Wilcox 1988). Germ plasm lines 'A7,' 'A11,' 'A12,' 'A13,' 'A14,' and 'A15' (Fehr et al. 1984; Jessen et al. 1988) and the cultivar Burlison [('Tracy' × 'Pomona') × 'Century'] made up the Fe-efficient class. Cultivars Century (Wilcox et al. 1980), Sturdy {'Evans' × ['Corsoy' × (('Lincoln'^{2} × 'Richland') × PI 180.507))] × Century}, Zane (Walker et al. 1986), and Corsoy 79 (Bernard and Cremeens 1988) comprised the intermediate class. Cultivars BSR 201 (Tachibana et al. 1983), Asgrow A3205 ('NK S1474' × 'A3127'), Pride B216 (Corsoy × 'Wayne'), and the experimental line LN85-6210, a selection from {('Hack' × [(Pride B216 × 'Hodgson')] × ('NK S1492' × 'Pella')}, constituted the Fe-inefficient class. Cultivars Clark (Johnson 1958) and Harosoy (Weiss and Stevenson 1955) were included in the study, since the isolines Clark-*fe* (C^6 × PI 54.619) and 'Harosoy'-*fe* (H^6 × PI 54.619) are known to carry a single recessive allele (*fe*) conditioning Fe-inefficiency (Bernard 1974).

Soybean genotypes were evaluated for 1 year at five field locations [(Humbolt/IA, pH 7.6 (Harps clay loam, mesic Typic Calciaquoll); Poccahantus/IA, pH 7.9 (Knoke silt loam, mesic cumulic Haploquoll); Bechyn/MN, pH 8.0 (Harps clay loam, mesic Typic Calciaquoll); Morgan/MN, pH 7.8 (Canisteo clay loam, mesic Typic Haplaquoll); and, Redwood Falls/MN, pH 7.8 (Canisteo silty clay loam, mesic Typic Haplaquoll)]. All locations produced leaf chlorosis symptoms on intermediate and Fe-inefficient genotypes. A randomized complete-block design was used with four replications per environment. Plots at Morgan and Bechyn were planted as hills with ten seeds per hill, and the remaining locations were planted in 1.5-m rows with a 76-cm row spacing. Plots were rated at V3 (Fehr et al. 1971) to the nearest 0.5 score on the scale of 1 = no yellowing, 2 = slight yellowing, 3 = moderate yellowing, 4 = intense yellowing, and 5 = severe yellowing with some necrosis (de Cianzio et al. 1979). In the analysis, genotypes were considered fixed and locations random.

For laboratory evaluation, seeds were surface-sterilized with 20% v/v Clorox and placed into regeneration tubes containing MS (Murashige and Skoog 1962) basal salts agar medium without hormones, and then put into a standard chamber 28°/19 °C (day/night) with a 16-h photoperiod (light from cool-white fluorescent lamps; Sylvania, Fall River/MA; ca. 80 μmol photons·m^{-2}·s^{-1}. Callus was initiated by removing epicotyl sections from 12-day-old seedlings and placing them onto 4MSII medium (Barwale et al. 1986).

Table 1. Analysis of variance for field iron chlorosis scores

Sources of variation	*df*	Mean squares	*F*-value
Locations	4	14.38	36.0*
Blocks/locations	15	0.40	
Genotypes	18	29.37	30.9**
Locations × genotypes	72	0.95	4.8**
Pooled error	270	0.20	

** Significant at 0.01 probability level

In order to develop an in vitro assay for the IDC response, initial evaluation with various media using callus of A7 (Fe-efficient) and Pride B216 (Fe-inefficient) was done. Components (auxin and Fe levels) in the callus growth medium were adjusted until the observed differences in growth between A7 and Pride B216 were maximized, i.e., A7 would remain green and produce good callus growth, while Pride B216 became chlorotic and callus growth was limited. Two different media were then selected for Fe efficiency evaluation, a low auxin (LA) and a low iron (LI) medium, both modifications of 4MSII. For the LA medium, FeEDTA was decreased from 100 μ*M* to 50 μ*M* and NAA was reduced to 0.02 μ*M*. In contrast, the LI medium contained 0.2 μ*M* NAA and 10 μ*M* FeEDTA. Control for LA contained 50 μ*M* FeEDTA and 0.2 μ*M* NAA, and control for LI contained 100 μ*M* FeEDTA. There were three plates (replicates) per treatment arranged in a completely randomized design. Five 40-mg (approximately) pieces of friable callus were placed onto each plate from the initiation callus. For the analysis, callus growth was measured (g) and rated as the percent growth relative to the respective control after 30 days. The experiment was repeated twice and means were calculated after testing for homogeneity of variance.

Results

For the field Fe-chlorosis scores, the genotype × location interaction was highly significant (Table 1). Increased chlorosis scores for the Fe-efficient lines at Morgan/MN were due to high-soluble salts (2.6 mΩ cm^{-1} versus 0.4 mΩ cm^{-1} at Bechyn/MN) and contributed to the interaction. Correlations between Morgan and other locations were high (Table 2) since soybean genotype rankings for Morgan compared to other locations remained relatively unchanged. LA medium had a higher correlation with field chlorosis scores than LI at all locations except Morgan (Table 2). The scores from the LI medium, like the Morgan location, generally did not differentiate between Fe-efficient and intermediate genotypes, resulting in higher correlation for LI at this location.

Discussion

Results for Clark-*fe* on LA medium failed to be related with field scores, however, on LI medium Clark-*fe* had a

Table 2. Correlation coefficients for lab evaluation correlated with Fe-field chlorosis scores and correlations between locations

	LA	Humbolt IA	Pocahantus IA	Redwood Falls MN	Morgan MN	Bechyn MN	Over locations
LI[a]	0.46*	0.72**	0.71**	0.69**	0.73**	0.70**	0.72**
LA[b]		0.75**	0.78**	0.77**	0.67**	0.77**	0.78**
Humbolt			0.99**	0.98**	0.89**	0.96**	0.99**
Pocahantus				0.99**	0.92**	0.97**	0.99**
Redwood Falls					0.90**	0.96**	0.99**
Morgan						0.89**	0.93**
Bechyn							0.98**

*,** Significant at the 0.05 and 0.01 probability levels, respectively
[a] LI, low iron medium
[b] LA, low auxin medium

Table 3. Comparison of averaged Fe-field chlorosis scores and tissue culture scores for 19 soybean genotypes evaluated

Entry	Field[a]	LA[b]		LI[c]	
		Lab[d]	Control[e]	Lab[d]	Control[e]
	Score[f]	%	Grams	%	Grams
A14	1.3	54	7.9	37	7.2
A13	1.3	55	6.4	12	6.4
A12	1.3	54	5.7	20	5.8
A11	1.5	57	5.7	49	6.4
A15	1.6	28	6.7	47	6.8
A7	1.8	59	6.8	17	7.1
Burlison	2.6	28	6.3	19	6.2
Century	3.0	32	4.3	21	5.9
Sturdy	3.4	8	4.6	19	5.8
Corsoy 79	3.6	18	5.8	18	6.7
Zane	3.8	31	4.8	8	5.6
BSR 201	3.9	7	4.0	5	6.4
A3205	4.2	14	6.1	7	5.9
LN85-6210	4.2	13	4.8	15	6.2
Pride B216	4.4	16	4.9	12	6.3
Clark	2.9	49	6.0	17	6.6
Clark-fe	4.6	38	5.1	9	7.2
Harosoy	3.8	3	7.4	13	7.6
Harosoy-fe	4.7	8	8.6	4	6.1
Mean	3.1	30	5.9	18	6.4
LSD (0.5)	0.6	8	1.5	6	1.8

[a] Averaged over five field locations
[b] LA, low auxin medium (50 μM FeEDTA, 0.02 μM NAA)
[c] LI, low iron medium (10 μM FeEDTA, 0.2 μM NAA)
[d] %increase in fresh weight relative to control, averaged for two experiments
[e] Control fresh weight in rams for callus (per plate)
[f] Score based on the scale of 1=no yellowing to 5=severe yellowing, with some necrosis

closer relationship to field scores (Table 3). The (*fe*) gene in the Clark isoline is not believed to be the gene that is expressed in the other Fe-inefficient genotypes evaluated (R.L. Bernard, personal communication). Therefore, the Fe uptake mechanism present in the Clark and Harosoy isolines (having come from PI 54.619) may be different from that of the other genotypes tested. The correlation between LA and LI was only 0.46. This suggests that the two laboratory methods may be evaluating different Fe uptake mechanisms.

Differences in Fe requirements for soybean cell lines in culture have previously been demonstrated by Sain and Johnson (1984), who reported that PI 54.619 cell suspension cultures required a higher concentration of iron as FeEDTA than Hawkeye (Fe-efficient). In working with various media, it was observed that auxin deletion slowed callus growth and increased chlorosis more in the Fe-inefficient genotypes. When the LA medium was used, a closer relationship ($r^2=0.78$) to field scores was found than with the LI medium. This result agrees with an Fe uptake mechanism described by Landsberg (1984) in which the presence of auxin is important for Fe uptake in plants.

We have successfully used tissue culture to differentiate between Fe-efficient and Fe-inefficient genotypes. Using tissue culture techniques, soybean breeders can delete Fe-inefficient lines while continuing to evaluate the more Fe-efficient lines in a field nursery, and single plant selection of highly Fe-efficient lines from a segregating population would also be possible. Using the LA technique, a soybean breeder could delete genotypes that had less than 50% growth relative to control and retain most of the Fe-efficient lines in a breeding program.

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