Identifying Quantitative Trait Loci Associated with Iron Deficiency Chlorosis in Soybean

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INTRODUCTION

Soybean cultivars developed for the Northern Plain Area are usually susceptible to iron deficiency chlorosis (IDC), a physiological disease characterized by interveinal yellowness of newly developed leaves on plants grown in fields with high pH, calcareous soil. Genotypic variation in the resistance to IDC exists in soybean germplasm and the natural genes could be used to breed varieties with an enhanced resistance to the abiotic stress. A major challenge to the breeding effort is the low efficiency in phenotypic selection for the resistance to IDC, as we lack knowledge about the number, genomic positions and genetic effects of the natural genes and their distribution in soybean germplasm. Thus, the objectives of this research were to identify quantitative trait loci (QTL) associated with the resistance to IDC under field conditions and to pyramid favorable alleles at major QTL in the genetic background of local cultivars.

MATERIALS & METHODS

Mapping population & phenotypic evaluation

A population of 201 recombinant inbred lines (RILs), which were developed from a cross between the local cultivar Surge (*Glycine max*) and the wild soybean accession PI468916 (*G. soja*), were grown in a selected field with the soil pH 8.4 on Larson Farm in Brookings, SD in summers of 2012, 2013 and 2014. The field experiments were conducted using a randomized complete block design with 4 replications. Seeds were sown in 30×70-cm^2 plots in mid-May and five seedlings kept in a plot to evaluate plant responses at time points from early July (V3 stage) to mid-August. The plants were evaluated by 2 or 3 researchers in a 1-5 scale, with 1 and 5 being most resistant and susceptible, respectively (Fig. 1A). Plot means were used for genetic analysis and QTL mapping.

Genetic analysis, linkage map construction, and QTL mapping

The field data from the RIL population at a time point were used to partition the phenotypic variance (V_p) into the genotypic (V_g) and environmental (V_e) components using two-way ANOVA to estimate the broad-sense heritability ($h^2=V_g/V_p$). The 201 RILs were genotyped with 180 microsatellite markers and the genotyping data used to construct a framework linkage map. The genetic map and phenotypic data were used to map QTL by composite interval mapping. One-way ANOVA was used to confirm the QTL and two-way ANOVA used to identify epistatic interactions between the confirmed QTL.

RESULTS

Phenotypic variation & heritability estimate

The RIL lines varied from completely resistant (1) to extremely susceptible (5) to IDC under the field conditions (Fig. 1A). The RIL population displayed approximately normal distributions for the visual scores at each time point (Fig. 1B), indicating that the field stress level was appropriate to distinguish genotypic variation in the resistance to IDC.

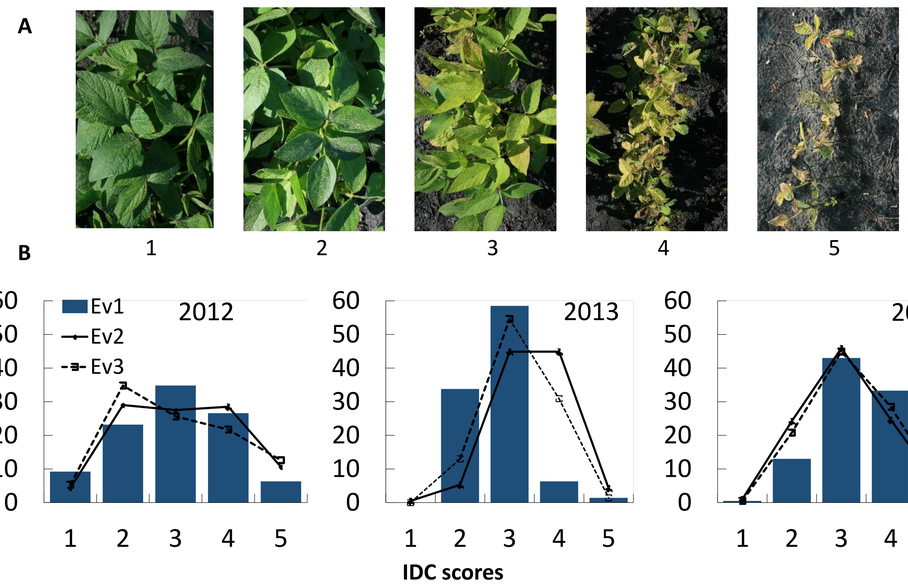

Fig.1 Phenotypic variation for IDC in the RIL population grown in field. A. Plant morphologies varying in the IDC score from 1 to 5 (Cianzio et al., 1979). **B.** Frequency distributions of IDC score. 201 RILs were evaluated at 3 time points (Ev1-3) from early July to mid-August in years 2012 to 2014.

A significant genotypic effect on IDC was detected in the RIL population at all time points across the 3 years. Heritability estimates for the trait varied from 0.25 to 0.70 (Table.1).

Table.1 Variance components and broad sense heritability estimates for IDC

	First evaluation			Second evaluation			Third evaluation		
	2012	2013	2014	2012	2013	2014	2012	2013	2014
V_p	1.43	0.69	0.99	1.44	0.83	0.96	1.51	0.80	1.05
V_g	0.91	0.18	0.53	1.02	0.21	0.51	1.07	0.27	0.58
V_e	0.52	0.51	0.45	0.43	0.62	0.45	0.43	0.54	0.48
h^2	0.63	0.25	0.54	0.70	0.25	0.53	0.71	0.33	0.54

V_p, V_g & V_e: Phenotypic, genotypic & environment variances, respectively; h^2: broad-sense heritability.

Quantitative trait loci associated with IDC

A linkage map covering 2156 cM of the 20 chromosomes (chr) was constructed. A total of 11 QTL were associated with IDC (Fe effic-#) in the RIL population. These QTL were distributed on 8 chrs. (Fig. 2) and contributed 6-16% of the phenotypic variances. Of the 11 loci, 6 were detected at multiple time points/years, including the one on chr. 5 that has the Fe efficiency allele from the wild soybean parent.

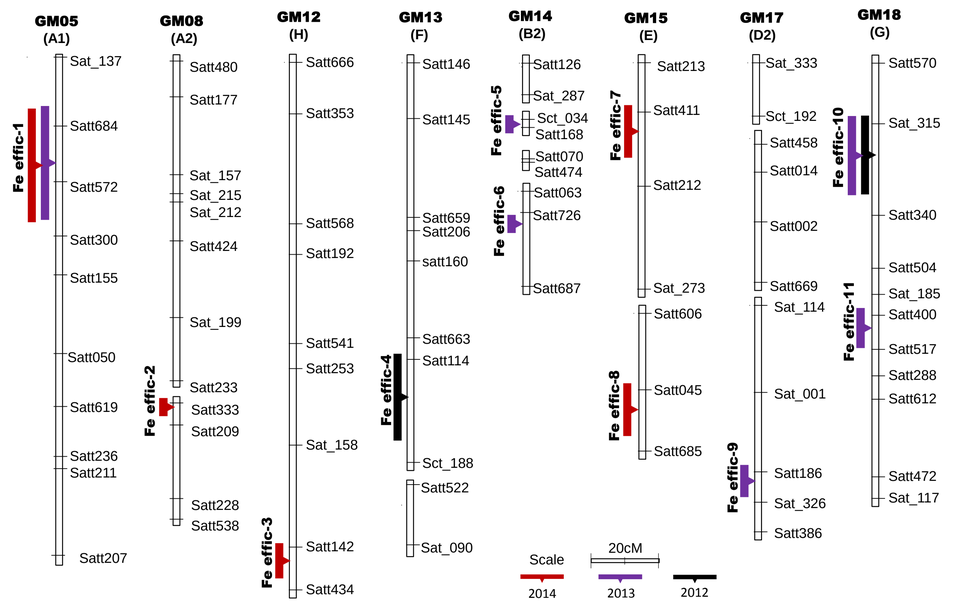

Fig. 2 Chromosomal distribution of QTL for the IDC trait in the RIL population evaluated in 2012-2014

Epistasis interactions between the detected QTL

A total of 53 pairs of digenic epistasis were detected between the IDC QTL. These epistatic interactions involved 9 loci (Fe effic-2, 3, 4, 5, 7, 8, 9, 10 & 11) and could be divided into three patterns (Fig. 3). The interaction patterns suggest that the effect of one QTL on IDC resistance could be enhanced (I & II) or inverted (III) by the genotype of the other locus in the background.

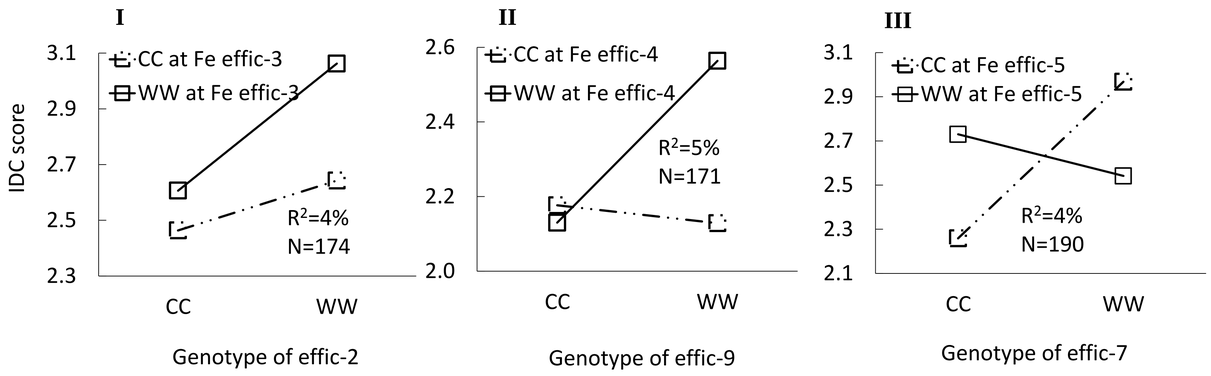

Fig. 3 Three patterns of digenic epistasis between QTL detected in the RIL population. Genotypes for a QTL are represented by alleles from the cultivated (CC) or the wild (WW) soybean line. N is the number of RILs used for the evaluation and R^2 is the proportion of phenotypic variance explained by the epistatic interaction.

Introgression of the allele at *Fe effic-1* from wild into cultivated soybean

Three RILs with the *Fe effic-1* allele based on marker genotypes were crossed with the cultivar Surge. Resulting hybrid F1s were backcrossed with Surge to generate the BC1F1 and advanced segregating lines. Segregation for the marker loci and the IDC and plant morphologies was observed in the offspring lines (Fig. 4). Both marker-assisted and phenotypic selection techniques will be used to advance the recurrent backcross to pyramid beneficial genes for the IDC and other traits in the recipient background.

Fig. 4 Genotypic and phenotypic variations in a BC1F3 line. Gel images showing genotypes of the *Fe effic-1* marker Satt572 (top) or plant morphologies (right). C, allele from the cultivar Surge; W, allele from wild soybean; & H, Heterozygote.

CONCLUSION & DISCUSSION

Heritability for IDC in the RIL population was low to medium (0.25-0.71) in the 3-year experiment.

A total of 11 putative QTL were associated with Fe efficiency. Most (9/11) of the QTL were involved in epistatic interactions to regulate phenotypic variation in the resistance to IDC. One of the two QTL with a relatively large, consistent effect has the Fe-efficient allele from the wild parent, indicating that wild germplasm retains beneficial genes to improve soybean cultivars for resistance to IDC.

Marker-assisted selection is being used to pyramid the Fe-effic QTL alleles, including the one from wild soybean, in the genetic background of the local cultivar "Surge".

REFERENCES

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ACKNOWLEDGMENT

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