



Loci underlying resistance to manganese toxicity mapped in a soybean recombinant inbred line population of essex x forrest

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Abstract

Resistance to manganese toxicity is associated with some soybean (*Glycine max*) cultivars grown on acidic soils or in hydroponics. Previously random amplified polymorphic DNA (RAPD) markers had seemed to identify 4 quantitative trait loci (QTL), regions that might underlie resistance to manganese toxicity in a recombinant inbred line (RIL) population derived from 'Essex' x 'Forrest'. Our objective was to identify microsatellite markers linked to these, or additional, QTL for resistance to manganese toxicity in a separate assay. Two hundred and forty microsatellite markers and 100 RILs were used to construct a map. The response of five plants per genotype to manganese was measured by leaf chlorosis (scored from 0–5) and root necrosis (scored from 0–5) from 7–28 days after treatment with 125 μ M of manganese in hydroponics. The experiment was repeated. ANOVA and MapMaker/QTL were used to identify regions underlying the responses. Three genomic regions on different linkage groups were found to contain QTL for resistance to necrosis during manganese toxicity. The regions located on linkage groups C2 (BARC_Satt291), I (BARC_Satt239) and G (OP_OEO2) were each significantly associated ($P < 0.005$, $R^2 = 20\%$) with root necrosis at 7 days after treatment. The regions all derived the beneficial allele from Essex. One of the previously identified RAPD associated root necrosis QTL was identified in this new study. However, no QTL for leaf chlorosis were detected ($P < 0.005$) and none of the RAPD identified leaf chlorosis QTL could be identified. We conclude that root and leaf resistance to manganese toxicity are environmentally sensitive quantitative traits determined by separate loci of different number and magnitude of effect.

Introduction

Soilscientists, plant breeders, and agronomists are challenged, by the increasing demand for agricultural lands throughout the world, to improve the productivity of problem soils (Reich et al., 1981). The acid soils of temperate Southern US, pose serious problems in terms of phytotoxic aluminum (Al; Bianchi-Hall et al., 2000) and to a lesser degree manganese (Mn; Nable et al., 1988) that undermine crop yield-potential and,

thus, attempts to implement sustainable agriculture systems (Reich et al., 1981).

In dicots manganese toxicity symptoms occur as leaf interveinal chlorosis and root necrosis (Heenan and Campbell, 1981; Nable et al., 1988; Kilo and Lightfoot, 1996). In soybeans (*Glycine max* L. Merr.) mechanisms of manganese tolerance are partially located in the shoots, as shown by reciprocal root stockscion grafts of tolerant and non-tolerant genotypes (Heenan and Campbell, 1981). The critical toxicity concentrations of manganese in leaf tissue differ to a

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great extent between plant species, as well as cultivars of species such as soybean (Horst, 1983, 1988).

Leaf and root inhibition of soybean plants may limit yields in the Southern United States. Root growth inhibition may cause increased susceptibility to water deficit (Goldman et al., 1989; Specht et al., 2001). Water deficits account for about 50 percent of soybean yield loss in United States (Specht et al., 2001). Lime is widely applied to increase the pH of acidic soils and thereby reduce the effects of manganese and aluminum toxicities. Soybean cultivars with improved manganese toxicity resistance might protect yields in acid soils, particularly in years when spring rainfall precludes the application of lime (Kilo and Lightfoot, 1996).

Although drought tolerance is a desirable trait; it is not an explicit selection criterion in most breeding programs (Specht et al., 2001). Many breeders consider selection for wide adaptation to be a coincident means of selecting for drought tolerance (Rosielle and Hamblin, 1981; Specht et al., 2001). However, genotype by water ($G \times W$) interactions exist (Korte et al., 1983; Kadhem et al., 1985; Specht et al., 2001), suggesting that selection for a specific adaptation might offer a more direct means of genetically improving drought tolerance (Specht et al., 2001).

Soybean cultivars 'Essex' (Smith and Camper, 1973) and 'Forrest' (Hartwig and Epps, 1973) and their derived recombinant inbred line (RILs) populations (Njiti et al., 1998) contrast for many traits including: yield, phytoestrogen content, trigonelline content, resistance to Mn toxicity; resistance to sudden death syndrome (SDS); and resistance to soybean cyst nematode (SCN) (Kilo and Lightfoot, 1996; Njiti et al., 2002; Iqbal et al., 2001; Meksem et al., 1999, 2001a, b; Cho et al., 2002; Yuan et al., 2002; Kassem et al., 2003a, b). The preliminary genetic map of Essex and Forrest genome based on one hundred and eighty one random amplified polymorphic DNA (RAPD) and RFLP markers anchored by a few microsatellite markers allowed these 6 traits to be used to identify 23 Quantitative Trait Loci (QTL) in adapted germplasm. However, errors in RAPD maps are likely to have caused some errors in assigning QTL. The objective of this study was to use only high quality scores generated by two hundred and forty microsatellite markers to identify QTL that underlie resistance to manganese toxicity.

Material and methods

Plant material

This study involved the F_5 -derived recombinant inbred line (RILs) population from Essex \times Forrest ($E \times F$, $n = 100$). The parents Essex and Forrest contrast for resistance to manganese toxicity (Kilo and Lightfoot, 1996). The EXF derived RILs ($F_{5:16}$) have been described extensively (Meksem et al., 1999; 2001a, b; Iqbal et al., 2001; Njiti et al., 2002; Kassem et al., 2003a, b).

Assay of Mn toxicity

The following experiment was repeated during the period September to November of 1998. Seeds were germinated in a sand soil mix (50:50). After 14 days the plants were transplanted into 25 L buckets containing Hoagland solution. The solution was continuously aerated and the buckets covered by Styrofoam rings. Eight plants per bucket were transferred into holes on the Styrofoam rings supported by foam corks. The plants were divided into a treated and control group with four replicates per genotype (eight plants) and arranged in a randomized complete block. The hydroponics solution was adjusted to exactly 125 micro molar manganese. The manganese was 125 mM $MnSO_4$ in 25 mL of water that was added to all the treated 25 L buckets (Kilo and Lightfoot, 1996), water alone was added to the control buckets. The pH of the solution was maintained at 6.7 ± 0.1 by daily inspection and adjustment when necessary with 100 mM sodium hydroxide. Quantitative traits measured included leaf chlorosis (Chlo1-4) and root necrosis (Necro1-4) at 7, 14, 21 and 28 days, respectively. A scoring chart had previously been developed (Kilo and Lightfoot, 1996) in which, the responses of the plants were recorded using a visual rating of 0.0–5.0 for quantitative determination of qualitative symptoms. A score of 0.0 indicated a complete absence of any symptoms, while a score of 5.0 indicated very severe, lethal symptoms (Kilo and Lightfoot, 1996). In a severe test at 28 days a score of 2.0 would be like Essex, while a score of 4.0 would be like Forrest (Figure 1). In this experiment the Essex means on that chart were 1.03 ± 0.12 (Chlo4), 1.01 ± 0.09 (Chlo3), 0.95 ± 0.08 (Necro1) and the means for Forrest ($n = 8$) were 2.15 ± 0.14 (Chlo4), 1.93 ± 0.12 (Chlo3), 1.27 ± 0.09 (Necro1).



Fig. 1. Leaf chlorosis 28 days after treatment and root necrosis 7 days after treatment. Shown are Essex (score 2) and Forrest (score 4) leaves (A) and roots (B).

DNA isolation

The RILs were grown in the greenhouse; 3 grams of leaves were collected from 5–6 two weeks old seedlings and immediately frozen in liquid nitrogen (after Iqbal et al., 2001). The leaves were ground in liquid nitrogen into a very fine powder and DNA was extracted. DNA concentration was measured by a flourometer and diluted to 15–30 ng/ μ L for further use in PCR reactions.

Microsatellite amplifications

Microsatellites markers from all 20 linkage groups were selected from the soybean genetic map (Cregan et al., 1999). The primer pairs were purchased from Research Genetics, Inc. (Huntsville, AL). Amplifications were carried out in a PE 9600 thermal cycler (Foster City, CA; after Iqbal et al., 2001). Two negative controls (with no template DNA) along with the two parents DNA as positive controls were run in all the amplifications. After PCR, the amplification products were electrophoresed on 5% (w/v) acrylamide gel that was used to expose X-ray film. The recombinant inbred lines were classified by parental genotypes for each marker.

Data analysis

To detect genomic regions associated with resistance to manganese toxicity, the recombinant inbred lines were classified as Essex (E) type or Forrest (F) type for each marker. Marker data were compared with

resistance to manganese toxicity by a one-way analysis of variance (ANOVA) performed with SAS (SAS Institute Inc., Cary, N.C., Wang et al., 1994). The probability of association of each marker with the trait (resistance to Mn toxicity) was determined and a significant association was declared if $P \leq 0.005$, to maximize the detection of associations (Lander and Botstein, 1989). A value of $P \leq 0.0005$ is suggested by an approximate Bonferroni correction ($P < 0.05/100$) for the set of about 100 independent (unlinked or > 10 cM apart) DNA markers (from the 240 mapped). However, allowing for locations where gaps are greater than 10 cM in the map we also accept $P < 0.005$ as a significant association if the interval was flanking a single marker. Precedents with first pass mapping other traits (Hnetkovsky et al., 1996; Chang et al., 1997; Njiti et al., 1997) have shown these criteria to be valid during saturation mapping of the intervals inferred at marginal P values (Meksem et al., 2001a; Njiti et al., 2002; Yuan et al., 2002).

Mapping quantitative trait loci

A linkage map was created using MAPMAKER-EXP 3.0 (Lander et al., 1987). Map distances between linked markers were calculated in centimorgans (cM) to construct a linkage map (heterogenous lines were excluded). The recombinant inbred line (ri-self) genetic model was used. The \log_{10} of the odds ratio (LOD) for grouping markers was set at 2.0, maximum distance was 30 cm. Conflicts were resolved in favor of the highest LOD score after checking the raw data for errors. Marker order within groups was determined by comparing the likelihood of many map orders. A maximum likelihood map was computed with error detection. Groups were assigned to linkage groups by assuming all microsatellite markers anchor to single loci in soybean (Cregan et al., 1999).

The map and resistance to Mn toxicity data were simultaneously analyzed with Mapmaker/QTL 1.1 (Paterson et al., 1991) using the F_2 -back-cross genetic model for trait segregation (Webb et al., 1995). Quantitative trait loci were inferred when LOD scores are equal or greater than 2.0 at some point in each interval since this was found empirically to be equivalent to a single marker $P \leq 0.005$, the criterion used in one-way ANOVA. The positions of the QTL were inferred from the interval peak LOD score.

The microsatellite markers used in this study have been mapped (Cregan et al., 1999) in other soybean populations. Therefore, markers were anchored on the

linkage groups on the basis of their expected locations. Anchored markers are important for the comparison of both known QTL locations from several populations (Mansur et al., 1996) and positions of dominant markers from earlier studies (Kilo and Lightfoot, 1996).

Results

Polymorphism and linkage

A total of 600 microsatellite markers covering all the 20 linkage groups of soybean were tested for polymorphism between Essex and Forrest. Two hundred forty microsatellite markers were polymorphic between the two parents. Each was scored in the RIL population with two to three replicates. Two hundred and one markers formed 22 linkage groups that represented each of the 20 known linkage groups. On average 9.55 markers were placed on each linkage group. The actual number of markers ranged from 2 for linkage group J to 21 on LG G. The total map encompassed 2823.1 cm with an average of 26.4 cm between loci excluding the 39 unlinked markers. This coverage is comparable to the expected recombination distance of about 3000 cm encompassing 20 linkage groups of soybean (Cregan et al., 1999).

Genomic regions associated with resistance to root necrosis

Root necrosis was only analyzed at 7 days because by 14–28 days the population parents could not be distinguished. Trait distribution was normal (data not shown) and the broad sense heritability values were calculated as 61%. Three chromosomal regions on different linkage groups were found to contain QTL that influence resistance to root necrosis and were anchored by microsatellite markers. The first region located on linkage group C2 and identified by the microsatellite markers BARC_SATT305 and BARC_SATT291 was significantly ($P = 0.0026$, $R^2 = 27\%$) associated with necrosis at 7 days after treatment and derived the beneficial allele from Forrest. The interval containing the QTL spanned about 11 cm and had a peak LOD score of 2.7 (Figure 2) and explained about 31% of variation in resistance to Mn toxicity (Table 1). The second region was located on linkage group I and was identified by the microsatellite marker BARC_SATT239. It was significantly ($P = 0.0037$, $R^2 = 23\%$) associated with

necrosis at 14 days after treatment and derived the beneficial allele from Forrest. The interval containing the QTL spanned about 20 cm and had a peak LOD score of 2.1 (Figure 2) and explained about 27% of variation in resistance to Mn toxicity (Table 1).

A third region was detected, but only by using one of the RAPD markers linked (17.3 cm) to a microsatellite marker Satt324 (Kilo and Lightfoot, 1996). The RAPD marker, OE02₁₀₀₀, on linkage group G was significantly ($P = 0.0004$, $R^2 = 59\%$) associated with root necrosis and explained about 20.6% of variation in resistance to Mn toxicity. The region derived the beneficial allele from Essex (Kilo and Lightfoot, 1996). The three regions jointly explained about 58% of the total variation in mean resistance to Mn toxicity and showed no significant non-additive interactions.

Resistance to leaf chlorosis

Leaf chlorosis was not evident until 21 days after treatment. Trait distributions were normal (not shown) and broad sense heritability values were calculated as 35% for chlorosis at 21 days (Chloro3) and 70% for chlorosis at 28 days (Chloro4). No significant loci conditioning variation in leaf chlorosis could be detected. Four loci had previously been detected with RAPD markers $P < 0.005$ so we relaxed the level of significance to $P < 0.05$ to try to detect these with linked satellite markers (Table 1). Two loci could then be detected. One of these, located on linkage group B2 and identified by the microsatellite marker BARC_SATT318, was significantly associated with chlorosis at 21 days after treatment ($P = 0.045$, $R^2 = 14\%$) and chlorosis 28 days after treatment ($P = 0.019$, $R^2 = 18\%$), and derived the beneficial allele from Essex. The interval containing the QTL spanned about 13.1 cm and had a peak LOD score of 2.5 (Table 1, Figure 2) and may explain up to 35% of variation in resistance to Mn toxicity (Table 1). The second region located on linkage group D2 and identified by the microsatellite marker BARC_Sat_092 was significantly associated ($P = 0.05$, $R^2 = 11.1\%$) with chlorosis at 28 days after treatment and derived the beneficial allele from Forrest. The interval containing the QTL spanned about 10 cm and had a peak LOD score of 2.2 (Table 1, Figure 2) and may explain up to 35% of the variation for resistance to manganese toxicity (Table 1).

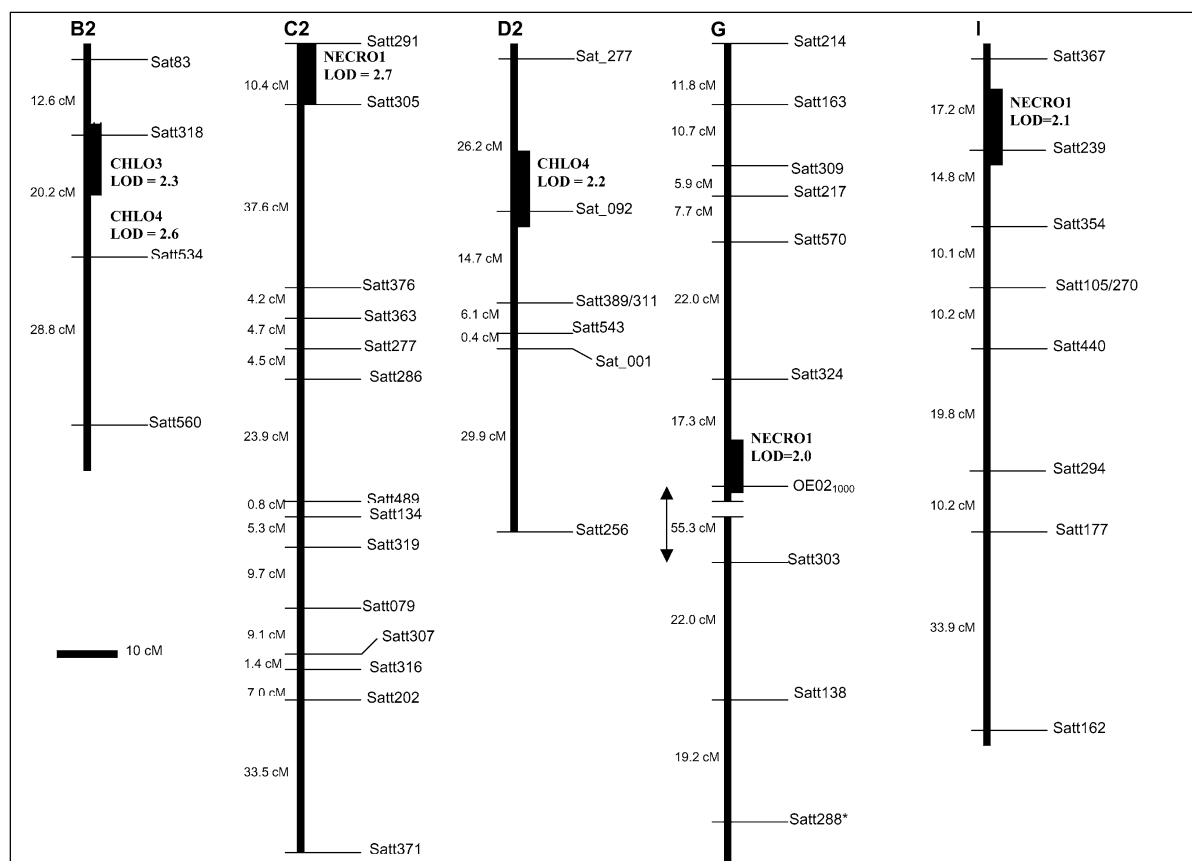


Fig. 2. Locations of DNA markers and the QTL conditioning Manganese toxicity. Linkage groups are shown as dark bars. The QTL were assigned to linkage groups B2, C2, D2, G and I on the soybean genetic map (Cregan et al., 1999). Genetic distances were from the recombinant inbred line function of MAPMAKER/EXP 3.0. The distance representing 10 cm is shown by the dark bar. The estimated position of the interval containing the QTL is shown as a dark bar based on interval mapping using MAPMAKER/QTL 1.1. The QTL LOD score is the peak LOD score of the interval showing association with resistance to Mn toxicity. NECRO1: necrosis at 7 days after treatment. Chloro 3 is chlorosis at 21 days after treatment. Chloro 4 is chlorosis at 28 days after treatment.

Discussion

Manganese toxicity was not severe in this experiment perhaps due to lower light intensity during the autumn of 1998 compared to the previous experiment carried out in summer of 1996. The mild symptom expressions reduced the separation between the parents. However, data from mild symptom expression is often consistent with more severe tests (Hnetkovsky et al., 1997) and may replicate field conditions more accurately than a severe test (Njiti et al., 2001). Three chromosomal regions on different linkage groups were found to contain QTL that influence root resistance to Mn toxicity in this population. Each of the QTL is only moderately associated with the trait, but this is due to the small population size and the mild symptoms observed rather than lack of diversity between

the population parents (Figure 1). The QTL are additive where 3 favorable alleles provide more resistance to necrosis than 2,1, or none (Figure 3). Jointly, the three QTLs explained about 58% of the total variation in root resistance to Mn toxicity. Therefore, additional QTL for resistance to Mn toxicity may be discovered in this population as more DNA markers become available.

The RAPD marker, OE02₁₀₀₀, on linkage group G appears to be located in a gap on the microsatellite map. We are currently developing a SCAR from the RAPD to help fill this gap. This RAPD marker previously identified a QTL for SDS resistance on L.G. G (Iqbal et al., 2001). Both QTL may be caused by errors in scoring of a dominant marker in a population with residual 6% heterogeneity, despite scoring the marker in triplicate experiments by independent

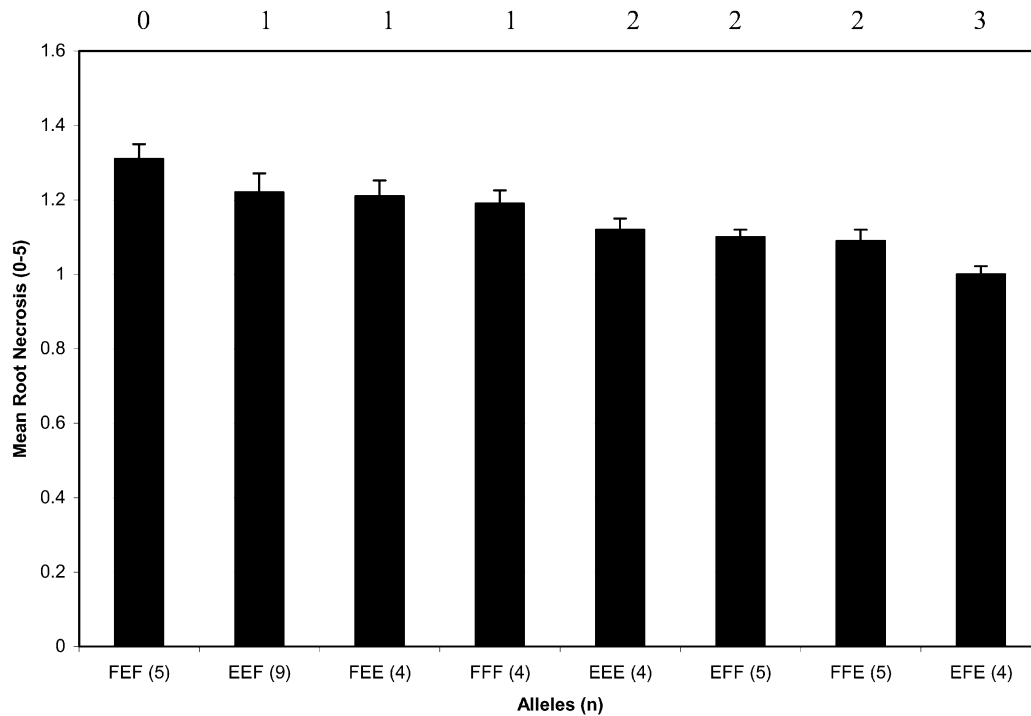


Fig. 3. Allele substitution at the QTL Loci for root necrosis: the loci are shown in each allelic state for the three loci with effect on the trait \pm SEM. Shown are loci SATT305 (C2), OE02₁₀₀₀ (G), SATT239 (I). E indicates the allele is from Essex, F indicates the allele is from Forrest. The number of RILs representing each of the eight genotypes is shown in parentheses. The number above each bar is the number of beneficial QTL predicted by DNA marker linkage.

Table 1. Markers and intervals associated with resistance to manganese toxicity

Marker	L.G.	Trait	P^a	$(R^2) (\%)^b$	LOD ^c	QTL var. ^d	Allele Mean \pm SEM	
							Essex ^e	Forrest ^f
SATT318	B2	CHLO3	0.045	14.0	2.3	36.3	1.59 \pm 0.13	1.35 \pm 0.04
SATT318	B2	CHLO4	0.019	18.0	2.6	33.9	1.78 \pm 0.20	1.33 \pm 0.08
SATT318	B2	Mean	0.032	16.0	2.5	35.1	1.68 \pm 0.16	1.34 \pm 0.06
SAT_092	D2	CHLO4	0.0500	11.1	2.2	35.1	1.4 \pm 0.09	1.8 \pm 0.17
SATT305	C2	NECRO1	0.0026	27.2	2.7	30.5	1.02 \pm 0.02	1.20 \pm 0.03
SATT291	C2	NECRO1	0.003	23.0	2.1	23.3	1.03 \pm 0.02	1.13 \pm 0.03
OE02 ₁₀₀₀	G	NECRO1	0.0004	59.0	2.0	20.6	1.20 \pm 0.03	1.02 \pm 0.01
SATT239	I	NECRO1	0.0037	30.1	2.1	27.4	1.1 \pm 0.05	1.2 \pm 0.01

^aThe probability of significant association with resistance to root necrosis ($P < 0.005$) and leaf chlorosis ($P < 0.05$).

^bThe R square.

^cLOD is the log10 of the odds ratio that supports evidence for the presence of the QTL at the locus from Mapmaker/QTL

^dThe percent of variation associated with the interval from Mapmaker/QTL

^eThe means for Essex ($n = 8$) were 1.03 \pm 0.12 (Chlo4), 1.01 \pm 0.09 (Chlo3), 0.95 \pm 0.08 (Necro1).

^fThe means for Forrest ($n = 8$) were 2.15 \pm 0.14 (Chlo4), 1.93 \pm 0.12 (Chlo3), 1.27 \pm 0.09 (Necro1).

^gPopulation means were 1.583 (Chlo4), 1.497 (CHLO3), 1.084 (NECRO1)

researchers. We await the SCAR to test this hypothesis with rigor. The QTL for root necrosis on linkage group N detected with RAPD markers (Kilo and Lightfoot, 1996) could not be detected in this experiment despite linkage to satellite markers. Therefore, this locus may be environmentally dependant or an artifact of the first experiments.

The region identified by the microsatellite marker BARC_Satt291 that contained the QTL for resistance to Mn toxicity on linkage group C2 also contains a QTL for yield (Yuan et al., 2002; Kassem et al., 2003a). Independent studies identified and mapped QTLs for reproductive period, seed pod maturity, sudden death syndrome (SDS) and soybean cyst nematode (SCN) resistances, plant height, seed weight and yield to this region (Mansur et al., 1996; Orf et al., 1999a, b; Specht et al., 2001; Yue et al., 2001; Iqbal et al., 2001; Specht et al., 2001; Kassem et al., 2003b).

Among the loci for resistance to leaf chlorosis there were no significant associations by ANOVA but two significant associations by Mapmaker/QTL. This would seem to suggest that leaf chlorosis is a polygenic trait with many loci of small effects contributing. The QTL on linkage group B2 that was associated with leaf chlorosis by Mapmaker/QTL was within a region where no previous studies identified QTL for Mn toxicity. However, QTL for iron efficiency (Lin et al., 2000), resistance to sclerotinia stem rot (Arahana et al., 2001), and SCN (Qiu et al., 1999; Yue and Sleper, 2001) were mapped to this region of linkage group B2.

This study demonstrates that specific loci influencing soybean resistance to Mn toxicity can be identified via molecular markers. In a separate study, RAPDs identified previously 4 loci that might underlie resistance to Mn toxicity on linkage groups A, G, N and unlinked (Kilo and Lightfoot, 1996). We confirmed the presence of one of those QTL (G) and presented here two additional QTL for resistance to Mn toxicity in a soybean recombinant inbred line population derived from the Essex \times Forrest cross. Since few of the RAPD detected loci could be confirmed unequivocally further experimental replication and conversion of the associated RAPD markers to SCARs will be required to test the veracity of these earlier associations.

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