Theor Appl Genet (1997) 94: 971–974

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QTLs associated with chlorimuron ethyl sensitivity in soybean: Effects on seed yield and related traits

Received: 6 August 1996 / Accepted: 28 February 1997

Abstract Soybean, *Glycine max* (L.) Merr., genotypes are known to differ in chlorimuron ethyl sensitivity (CS). Earlier we have reported two putatively independent marker loci linked to two quantitative trait loci (QTLs) controlling CS in a soybean population derived from a cross of PI97100 (sensitive to chlorimuron ethyl) and 'Coker 237' (tolerant to chlorimuron ethyl). The objective of the present study was to quantify the association of the two marker loci with seed yield and related traits in this soybean population following application of chlorimuron ethyl. Phenotypic data were collected for 111 F_2-derived lines of the cross grown in replicated plots at Athens, G.A., in 1994 and 1995, and at Blackville, S.C., in 1995. The two CS marker loci explained as much as 50% of the genetic variation in seed yield and seed number m^{-2}, but had no association with seed weight, plant height, lodging, seed protein, and seed oil. There were no epistatic interactions between the two marker loci for any of the traits. The marker locus (cr168-1 on USDA linkage group E) linked to the major CS QTL explained between 13 and 23% of the variation in seed yield. The Coker 237 allele at this locus was associated with decreased CS and increased seed yield. The marker locus (Blt015-2 on an unknown linkage group) linked to the minor CS QTL accounted for a maximum of 11% of the variation in seed yield. The Coker 237 allele at this locus was associated with an increase in CS and a decrease in seed yield. The association of the two marker loci with seed number m^{-2} strongly resembled their association with seed yield. Seed yield had a strong positive correlation ($r = 0.74 - 0.94$) with seed number m^{-2}, and the effect of chlorimuron ethyl on seed yield was due mainly to its effect on seed number m^{-2} rather than seed weight.

Key words Soybean · *Glycine max* · QTLs · RFLP · Chlorimuron ethyl · Seed yield

Communicated by A. L. Kahler

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Introduction

Soybean genotypes are known to differ in their sensitivity to chlorimuron ethyl (Lloyd and Wax 1984). Chlorimuron ethyl (2-[[[[(4-chloro-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino]sulphonyl]benzoic acid), trade name Classic, was introduced in 1984 as a herbicide for soybean (Beyer et al. 1988) and belongs to the sulphonylurea group of herbicides. The high herbicidal activity and low mammalian toxicity make these herbicides highly attractive for weed control. However, because of crop sensitivity very few sulphonylurea herbicides can be used to their full potential for weed control in soybean.

Based on classical genetic studies, the chlorimuron ethyl sensitivity (CS) of soybean was believed to be controlled by a single recessive gene (Pomeranke and Nickell 1988; Sebastian et al. 1989). Sebastian and Chaleff (1987) identified several soybean mutants from a mutation breeding program with increased tolerance to sulphonylurea herbicides. Genetic analysis of these mutants revealed that the tolerance was conditioned by a different single recessive gene in each mutant. Sebastian et al. (1989) reported that the resistance of a newly derived mutant soybean to sulphonylurea was monogenic, semidominant, and not allelic to any of the previously identified recessive genes *hs*1, *hs*2, or *hs*3. The resistant allele found in this mutant was designated as *Als*1 and the corresponding sensitive wild-type allele

as *als*1. From these studies, however, it was not known whether there was any minor gene(s) involved with CS in soybean. Using RFLP techniques, we (Mian et al. 1997) have mapped two CS loci in a soybean population derived from a cross of PI97100 × Coker 237. Based on the visual scoring of seedling damage from chlorimuron ethyl, we found one major QTL on USDA linkage group E (Shoemaker and Specht 1995) and one minor QTL on an unknown linkage group.

Most of the published reports on CS in soybean are based on damage rating on young seedlings. From a practical standpoint, it is important to know the effect of chlorimuron ethyl on soybean seed yield and other agronomic traits such as seed number m^{-2}, seed weight, plant height, lodging, seed protein, and seed oil. The conventional method of evaluating the effect of chlorimuron ethyl on soybean seed yield requires the maintenance of a weed-free experimental area, usually by hand weeding (Larry and Shaw 1992).

Using conventional breeding methods it is difficult to identify and dissect the effects of the two CS loci on seed yield. Inherent differences (such as growth habit, maturity, etc.) among soybean genotypes also add complexity to such studies. Often, it is necessary to use near-isogeneic lines to quantify the allelic effect of a gene on a trait. With the aid of molecular mapping it is now possible to overcome most of these difficulties. Using such techniques, one can simultaneously determine the individual effect of several QTLs conditioning a trait in a segregating population. Multiple traits can be evaluated in a single experiment.

In field experiments to evaluate F_2-derived lines from the cross of PI97100 × Coker 237 for tolerance to the soybean cyst nematode, *Heterodera glycines* Ichinohe, the experimental areas were treated with chlorimuron ethyl for weed control. PI97100 was found to be highly sensitive to the labeled rate of chlorimuron ethyl under field conditions (Mian et al. 1997). The plots were scored for CS at the seedling stage, the same plants were grown to maturity, and seed yield and other agronomic data were collected. The objective of this research was to quantify the association of the two CS marker loci with soybean seed yield, seed number m^{-2}, seed weight, plant height, lodging, seed protein, and seed oil.

Materials and methods

One hundred and eleven F_2-derived lines from a cross of PI97100 × Coker 237 were used to create a genetic linkage map and to evaluate the agronomic traits. The indeterminate PI97100 was classified as sensitive to chlorimuron ethyl and the determinate Coker 237 was classified as tolerant (Mian et al. 1997).

The procedures for constructing the genetic map have been described in Lee et al. (1996). The experimental area at the University of Georgia Plant Sciences Farm near Athens, G.A., in 1995 (Athens-1995) was treated with trifluralin (α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine, 0.84 kg a.i./ha) and alachlor [2-chloro-2′, 6′-diethyl-N-(methoxymethyl)acetanilide, 2.24 kg a.i./ha] on 19 April 1995, and post emergence chlorimuron ethyl (0.009 kg a.i./ha) on 15 June 1995. The experimental area at Clemson University Edisto Res. and Educ. Ct. near Blackville, S.C., in 1995 (Blackville-1995) was treated with pre-plant trifluralin (0.84 kg a.i./ha), chlorimuron ethyl (0.047 kg a.i./ha), and metribuzin [4-amino-6-(1, 1-dimethylethyl)-3-(methylthio)-1, 2, 4-triazin-4(4H)-one, 0.36 kg a.i./ha] on 10 May 1995. The planting dates for Athens-1995 and Blackville-1995 were 22 May and 24 May 1995, respectively. The herbicide, Canopy, was the formulation of chlorimuron ethyl and metribuzin applied at Blackville-1995, while the herbicide, Classic, was the formulation of chlorimuron ethyl applied at Athens-1995. A fumigant nematicide (ethylene dibromide, 53.8 kg a.i./ha at Athens and 1,3-dichloropropene, 74.4 kg a.i./ha at Blackville) was applied prior to planting for control of the soybean cyst nematode *H. glycines* Ichinohe. In addition, a liquid nematicide, phenamiphos [ethyl 3-methyl-4-(methylthio) phenyl (1-methylethyl) phosphoramidates, 5.7 to 7.7 kg a.i./ha] was applied on the soil surface at both locations at the R_2 stage of soybean development (Fehr and Caviness 1977). Additional information on the field experiments for the Athens-1995 and Blackville-1995 environments can be found in Mian et al. (1997). Information on the experiment conducted at Athens, G.A., in 1994 (Athens-1994), environment is the same as Athens-1995 except for the followings: (1) the herbicide application dates — alachlor and trifluralin were applied on 2 May 1994, and chlorimuron ethyl was applied on 13 July 1994; (2) in addition to the above herbicides, sethoxydim {2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cychlohexen-1-one, 0.32 kg a.i./ha} was applied as a post-emergence herbicide on 15 July 1994; and (3) the seeds were planted on 31 May 1994.

The plants in each plot for the Athens-1995 and Blackville-1995 experiments were visually scored for herbicide injury. The scoring procedure is outlined in Mian et al. (1997). There was no noticeable seedling damage from chlorimuron ethyl at Athens in 1994 and the plots were not scored for CS.

The plants were grown to maturity and data were collected for plant height, lodging, maturity, seed yield, seed weight, seed number, seed oil, and seed protein. Plant height was measured as the average length of plants from the ground to the terminal bud of the plant at maturity. Lodging rating was recorded at maturity on a scale of 1 (all plants erect) to 5 (all plants prostrate). Maturity was recorded as the number of days after August 31 when 95% of the pods had reached a mature pod color (Fehr and Caviness 1977). For seed yield the plots were first end-trimmed and then machine-harvested. Seed yield was expressed as mg ha^{-1} on a 13% moisture basis. Seed weight was expressed in mg $seed^{-1}$ based on a 100-seed sample per plot. Seed number m^{-2} was calculated as seed yield m^{-2}/weight $seed^{-1}$. Protein and oil contents were determined on a plot basis. Seeds were sent to USDA-ARS, National Center for Agricultural Utilization Research at Peoria, Ill., for analysis. Two samples of 18 – 20 g of seeds/plot were analyzed for protein and oil composition with a Infratec NIR Food and Feed Grain Analyzer (Model 1255). Analysis of the seed was conducted on an as-is basis and then mathematically converted to a moisture-free basis.

Phenotypic data were analyzed by the GLM procedure of SAS (SAS Institute, Cary, N.C.) to determine significant differences among the 111 F_2-derived progeny. For the two CS QTLs the F_2-derived lines were divided into three genotypic classes according to their allelic configuration (i.e., the lines homozygous for the PI97100 allele, the heterozygotes, and the lines homozygous for the Coker 237 allele). Single-factor analysis of variance (ANOVA) was then performed to determine the significance of each trait among genotypic class means using the *F*-test from the type-III mean squares obtained from the GLM procedures of SAS. A two-way ANOVA was also used to detect epistatic interactions between the two CS QTLs.

Results and discussion

There were significant differences among the progeny lines for all of the phenotypic traits measured in all three environments. The CS marker loci (cr168-1 and Blt015-2) classes showed significant differences in seed yield and seed number m^{-2} (Table 1), but had no differences in plant height, lodging, maturity, seed weight, seed protein, and seed oil (data not shown).

The regression of seed yield on the genotypic classes at the major CS marker locus cr168-1 explained 23% of the variation in seed yield in Athens-1995, 13% in Blackville-1995, and 15% in Athens-1994 (Table 1). This marker locus explained 83% of the variation in CS scores for Athens-1995 and 63% for the Blackville 1995 environment (Mian et al. 1997). The lines homozygous for the PI97100 allele at this locus were sensitive to chlorimuron ethyl. The lines homozygous for the PI97100 allele at the cr168-1 marker locus yielded 0.26, 0.21 and 0.26 mg ha^{-1} less compared to the lines homozygous for Coker 237 at this locus in Athens-1995, Blackville-1995, and Athens-1994, respectively. The heritability of seed yield of this population was 79% in Athens-1995, 76% in Blackville-1995, and 78% in the Athens-1994 environment (based on four replications/environment). Marker locus Cr168-1 thus explained a significant portion of the genetic variation in the seed yield of this population.

The minor CS locus (Blt015-2) explained 11% of the variation in seed yield in Athens-1995, and 6% of the variation in seed yield in Athens-1994 (Table 1). The association of this locus was not significant ($P = 0.17$) in Blackville-1995. However, combined over the three environments, Blt015-2 explained 11% of the variation in seed yield ($P < 0.01$). At this locus, however, the Coker 237 allele was sensitive to chlorimuron ethyl and accordingly the lines homozygous for the Coker 237 allele at this locus yielded less than the lines homozygous for the PI97100 allele. The two marker loci together explained as much as 34% of the variation in seed yield (Athens-1995), which is nearly half of the genetic variation in the trait. This is interesting considering the fact that the soybean plants in these experiments displayed apparent recovery from the chlorimuron ethyl damage and resumed normal growth and development later in the growing season. The effect of CS loci on seed yield at Athens in 1994 was even more surprising, because no visible damage to seedlings occurred from chlorimuron ethyl in that environment.

At the V14 growth stage (Fehr and Caviness 1977), the lines homozygous for the Coker 237 allele at the marker locus cr168-1 were 20% taller than the lines homozygous for the PI97100 allele at this locus in the Athens-1995 and Blackville-1995 environments (Mian et al. 1997). However, the plant height at maturity of these two groups of plants did not differ in any of the three environments (data not shown). Even though the final plant height did not differ, the sensitive lines probably closed the canopy later in the growing season than the tolerant lines.

Marker cr168-1 explained between 14 and 22% of the variation in seed number m^{-2} (Table 1). The

Table 1 Association of the two independent chlorimuron ethyl sensitivity (CS) marker loci (Cr168-1 and Blt015-2) with seed yield and seed number m^{-2}

Environment	Genetic class	Marker locus Cr168-1			Marker locus Blt015-2		
		CS score[a]	Seed yield mg ha^{-1}	Seed number number m^{-2}	CS score	Seed yield mg ha^{-1}	Seed number number m^{-2}
Athens-1995:		***[b]	***	***	**	**	**
	Coker 237/Coker 237	2.9 ± 0.2[c]	1.59 ± 0.04	1145 ± 30	6.5 ± 0.5	1.30 ± 0.04	906 ± 30
	Coker 237/PI97100	4.8 ± 0.1	1.53 ± 0.03	1056 ± 22	4.8 ± 0.3	1.51 ± 0.03	1092 ± 23
	PI97100/PI97100	7.5 ± 0.2	1.33 ± 0.04	927 ± 26	4.7 ± 0.4	1.48 ± 0.04	1061 ± 32
	R^2 (%)[d]	83	23	22	10	11	10
Blackville-1995:		***	***	***	*	NS	NS
	Coker 237/Coker 237	2.9 ± 0.1	1.39 ± 0.04	1135 ± 27	4.8 ± 0.5	1.24 ± 0.07	984 ± 51
	Coker 237/PI97100	3.4 ± 0.1	1.32 ± 0.03	1048 ± 24	3.8 ± 0.2	1.35 ± 0.03	1066 ± 23
	PI97100/PI97100	5.9 ± 2.2	1.18 ± 0.04	941 ± 27	3.6 ± 0.3	1.33 ± 0.04	1047 ± 31
	R^2 (%)	63	13	20	7	–	–
Athens-1994:		–	***	***		*	*
	Coker 237/Coker 237	–	2.32 ± 0.05	1670 ± 42	–	2.16 ± 0.06	1520 ± 42
	Coker 237/PI97100	–	2.25 ± 0.04	1618 ± 32	–	2.30 ± 0.04	1590 ± 29
	PI97100/PI97100	–	2.06 ± 0.04	1469 ± 30	–	2.36 ± 0.05	1706 ± 38
	R^2 (%)	–	15	14	–	6	7

[a] Score = 1 (no damage) to 10 (plant death), data from Mian et al. (1997)
[b] ***, **, *, NS indicate significant differences at $P < 0.001$, 0.01, 0.05, and no significant differences at $P < 0.05$ based on single-factor analysis of variance, respectively
[c] Mean value ± the standard error
[d] R^2 (%) shows the percent of total variation in the trait explained by the marker locus

sensitive lines (homozygous for PI97100 allele) produced a lower seed number m^{-2} compared to the tolerant lines (homozygous for the Coker 237 allele). Marker Blt015-2 explained 10 and 7% of the variation in seed number m^{-2} in the Athens-1995 and Athens-1994 environments, respectively, and in these cases the lines homozygous for PI97100 allele at the Blt015-2 locus produced a higher seed number m^{-2} than the lines homozygous for the Coker 237 allele at this marker locus (Table 1). The effect of this locus was not significant in Blackville-1995. Combined over the environments, however, marker Blt015-2 explained significant ($P < 0.01$) variation ($R^2 = 11\%$) in seed number m^{-2}. The heritability of seed number m^{-2} in this population was 71% for Athens-1995, 70% for Blackville-1995, and 75% for the Athens-1994 environment (based on four replications/environment). The two CS marker loci together explained as much as 50% of the genetic variation in seed number m^{-2} in this population. Such a strong association of the CS loci with seed number m^{-2} and their lack of association with seed weight indicate that the effect of chlorimuron ethyl on seed yield was due mainly to its effect on the seed number m^{-2} rather than its effect on seed weight. This is substantiated by the strong positive correlation ($r = 0.74$ to 0.94) found between seed yield and seed number m^{-2}, and the weak correlation between seed yield and seed weight ($r = 0.13$ to 0.24), in these experiments.

The growth habit locus (*Dt1*) explained between 6 and 14% of the variation in seed yield in this population (data not shown). This soybean population was segregating for growth habit, and Lee et al. (1996) mapped the trait to the *Dt1* locus on USDA linkage group L. The effect of growth habit on seed yield was largely dependent on the environment. In the Blackville-1995 environment, the indeterminate lines had an 18% greater seed yield than the determinate lines. However, in the Athens-1994 environment the determinate lines yielded 9% more than the indeterminate lines.

Lee et al. (1996) identified two major loci for maturity in this population. In the Athens-1995 environment, both maturity loci (CpTI and R051 on USDA linkage group K) were significantly associated with seed yield (combined $R^2 = 25\%$). Marker locus CpTI also explained significant variation in seed number m^{-2} ($R^2 = 7.8\%$) in that environment (data not shown). The maturity marker loci, however, had no association with these traits in the remaining environments.

From these experiments, we were able to determine the effect of chlorimuron ethyl on seed yield and other agronomic traits of this soybean population as attributable to each of the two CS QTLs. The results demonstrate that the CS loci could have been easily mistaken as yield QTLs in this population, particularly in the Athens-1994 environment where no visible symptom of CS was present. It would also be possible to report a maturity QTL or the *Dt1* locus as a QTL for seed yield. Thus, we feel that studies designed for the identification of soybean seed-yield QTLs should be treated with caution to avoid any cultural practice that has the potential to influence seed yield differentially among the genotypes involved.

The environmentally dependent effects of growth habit and maturity on seed yield indicate that, in order to identify the seed-yield QTLs for soybean, simultaneous or *a priori* identification of QTLs for other traits that can possibly affect the seed yield of the population is necessary. This will facilitate the separation of the stable seed-yield QTL from some of the obvious environmentally sensitive QTLs for other traits that can differentially affect seed yield.

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