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ORIGINAL PAPER

An updated 'Essex' by 'Forrest' linkage map and first composite interval map of QTL underlying six soybean traits

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Abstract DNA marker maps based on single populations are the basis for gene, loci and genomic analyses. Individual maps can be integrated to produce composite maps with higher marker densities if shared marker orders are consistent. However, estimates of marker order in composite maps must include sets of markers that were not polymorphic in multiple populations. Often some of the pooled markers were not codominant, or were not correctly scored. The soybean composite map was composed of data from five separate populations based on northern US germplasm but does not yet include 'Essex' by 'Forrest' recombinant inbred line (RIL) population (E × F) or any southern US soybean cultivars. The objectives were, to update the E × F map with codominant markers, to compare marker orders among this map, the Forrest physical map and the composite soybean map and to compare QTL identified by composite interval maps to the earlier interval maps. Two hundred and thirty seven markers were used to construct the core of the E × F map. The majority of marker orders were consistent between the maps. However, 19 putative marker inversions were detected on 12 of 20 linkage groups (LG). Eleven marker distance compressions were also found. The number of inverted markers ranged from 1 to 2 per LG. Thus, marker order inversions may be common in southern compared to northern US germplasm. A total of 61 QTL among 37 measures of six traits were detected by composite interval maps, interval maps and single point analysis. Seventeen of the QTL found in composite intervals had previously been detected among the 29 QTL found in simple interval maps. The genomic locations of the known QTL were more closely delimited. A genome sequencing project to compare Southern and Northern US soybean cultivars would catalog and delimit inverted regions and the associated QTL. Gene introgression in cultivar development programs would be accelerated.

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Introduction

The *Glycine max* L. [Merr.] soybean genome had a diploid ancestor (n = 11), that underwent aneuploid loss or chromosome fusion (n = 10), allo- and auto-polyploidization (n = 20) about 4–16 million years ago and diploidization in the intervening period (Singh and Hymowitz 1988, Blanc and Wolfe 2004). Some DNA markers, genes, loci and QTL in soybean segregate in simple Mendelian fashion, facilitating genetic analysis. However, about 65% of loci have highly similar paralogs in the genome (Shultz et al. 2006a). The soybean composite genetic map has been developed by focusing on five US populations derived from northern US germplasm (Song et al. 2004) selected from the several dozen developed to date worldwide (Iqbal et al. 2001; Njiti et al. 2002; Yamanaka et al. 2001; Zhang et al. 2004). In contrast to the molecular map, the classical loci map contains 63 loci on 19 linkage groups (LG) and is a composite of 120 populations from worldwide germplasm (see references in Song et al. 2004). The consequence of a greater number of mapping populations is greater accuracy of the classical composite map.

The composite molecular map encompasses 20 LGs and 2,512 cM based on 600 of the 1,019 available microsatellites (BARC-SSRs; Song et al. 2004). However, three large populations (each $n > 200$) based on crosses among 'Minsoy', 'Noir' and 'Archer' form the core of the map. Small populations derived from 'A81-356022' × '*G. soja*' ('PI468916'; $n = 57$) and 'Harosoy' × 'Clarke' ($n = 76$) add many markers to the composite map but do not greatly alter marker orders. The soybean composite map does not include sufficient germplasm from southern US cultivars or Asian cultivars, two distinct gene pools with potential for genome rearrangements (Gizlice et al. 1996; Yamanaka et al. 2001; Dong et al. 2004). The soybean composite map does not include the data from the 'Essex' by 'Forrest' (E × F) mapping population (Lightfoot et al. 2005) the most advanced mapping population based on southern US adapted germplasm. The composite map does not include the microsatellite markers derived from BAC end sequences (McCouch et al. 2002) shown at SoyGD based on the soybean physical maps (Wu et al. 2004a, b; Shultz et al. 2006a) or mapped by segregation analysis (Kazi et al. 2005, 2006; Shultz 2006b).

The core composite map shown on SoyBase contains several types of markers in addition to microsatellites. There are 707 paralogous loci (sequences that diverged after one of the genome duplication events) mapped by about 300 RFLP markers. In addition, some dominant markers have been selected for map placement. They include 6 of about 600 mapped AFLP markers (Keim et al. 1997), and 72 of about 300 mapped RAPDs (Ferreira et al. 2000). Additional dominant markers were placed into the map shown at SoyBase (based on the composite map) if they are the marker closest to a QTL with positions estimated in relation to the microsatellite or RFLP marker map (not direct mapping in any of the core populations). The marker framework has been used to anchor the soybean physical map contigs shown in the AceDB at Soybase, the GBrowse at SoyGD and the C-map at LIS (Stein et al. 2002; Gonzales et al. 2005; Shultz et al. 2006a). Unfortunately, the single locus amplicon specificity of markers, marker orders, and the distance between markers have been shown to be imprecise estimates during physical mapping (Shultz et al. 2003; Shultz et al. 2006a). The composite genetic map remains an estimate that changes periodically as new markers are added (Chang et al. 1997; Cregan et al. 1999; Iqbal et al. 2001; Song et al. 2004; Shultz et al. 2006b; this paper).

A properly constructed, complete integrated genetic (Cregan et al. 1999; Song et al. 2004) and physical map (Wu et al. 2004a, b; Shultz et al. 2006a) of the soybean genome will be useful to determine whether newly detected QTL overlap with any of the more than a thousand QTL that condition more than 80 traits previously reported. In spite of the clustering of QTL, to date, about 85% of the QTL reported have not been confirmed in a second study. In addition, most soybean maps contain errors in their linkage maps. 'Errors' may be seen as different marker orders, different marker positions on LGs, markers on different LGs. However, genomic phenomena like inversion, insertion, deletion and transition of genomic regions will have some of the same effects on marker orders in maps. Gene conversion, meitoic drive and gametic or zygotic selection within mapping populations will also distort marker orders and segregation ratios.

Some trivial marker order errors may be due to the use of RAPD, AFLP, RFLP, and even microsatellite markers without sufficient replication to be error free or to detect all residual heterogeneity. Small numbers of such map errors disproportionately expand map distances (Kearsey and Farquhar 1997; Njiti et al. 1998; DeWan et al. 2002). Expanded map distances cause type 1 and type 2 errors during gene, loci and QTL mapping by Mapmaker (Lander et al. 1987). This property allows some genetic causes of marker order changes to be distinguished from some marker scoring problems. Map expanding markers can be eliminated, but only in marker dense regions (Ekstrom 2003). Markers linked to loci that distort normal segregation loci can be eliminated by a simple chi square test (Vogl and Xu 2000). In addition, composite interval mapping (CIM) can reduce the frequency of type 1 errors by considering the

likelihood of the QTL position among several marker pairs (Zeng 1993, 1994; Jansen and Stam 1994; Basten et al. 2001). Finally, the emergent physical map and sequence resources for soybean have shown several instances where physical map orders were different from genetic map orders (Ruben 2000; Ruben et al. 2006; Hague et al. 2001; Ashfield et al. 2003; Searle et al. 2003; Shultz et al. 2003, 2006a; Triwitayakorn et al. 2005).

The soybean physical map (Wu et al. 2004a, b; Shultz et al. 2006a) was based on the cultivar Forrest's genome, gene map (Shopinski et al. 2003, 2006), and DNA sequence derived from BAC ends (Shultz et al. 2006a, b). The physical map was the first to be constructed from a partly polyploidy genome. A whole genome sequence based on shotgun reads is in progress and the physical map will be an important finishing tool. Therefore, the marker orders and distances in the map are important estimates. The E × F population was the second soybean map population to be released (Lightfoot et al. 2005); and was grown in six states (NJ, OH, IL, TN, VA, NC), Argentina and Thailand in 2005. Collaborators in these locations require regular map updates to compare with their trait data. NIL populations were developed from each recombinant inbred line (RIL) (Njiti et al. 1998; Meksem et al. 1999, 2001a; Lightfoot et al. 2005) that effectively expands the population size to 4,000 lines. The NILs allow fine maps of QTL to be developed simultaneously with QTL identification. Maps of related southern US germplasm have been developed, some by crossing to northern germplasm. A 'Williams' by Essex map was reported (Hyten et al. 2004). A map of 'Flyer' by 'Hartwig' was developed (Yuan et al. 2002; Kazi et al. 2006). A map of 'Pyramid' by 'Douglas' exists (Njiti et al. 2002).

Therefore, it was important to update the map of the Essex and Forrest genome provided to physical map users, E × F population users, to prepare for a joined map, to more closely delimit previously known QTL and to discover new QTL using a comprehensive set of technical and statistical error detection methods. Described herein are an updated genetic and QTL linkage map based on replicated, codominant markers and the E × F RIL population. QTL for six traits were analyzed by CIM. The updated map was compared to the composite genetic and physical maps.

Materials and methods

Plant material

In this study, the RIL population derived from a cross of Essex by Forest (E × F, $n = 100$; Lightfoot et al. 2005) was used. Both individual lines (E × F 78; Schmidt et al. 1999) and the majority of lines in the population were registered and released (Lightfoot et al. 2005). Some lines may be licensed separately and constitute the SDX™ gene pyramid (E × F 23; Iqbal et al. 2001; 2005) and both high and low isoflavone lines (E × F 52 and E × F 82; Kassem et al. 2004a). All lines were used in this study for trait analysis, but 94 were used for microsatellite marker maps because only 94 RILs, Essex, and Forrest DNA samples fit into the 96 well plates used. Excluded were RILs 95–100.

DNA isolation

Leaves were collected from seedlings grown in the greenhouse (2 weeks old), ground in liquid nitrogen, and DNA was purified as described earlier (Iqbal et al. 2001; Yuan et al. 2002). Using a fluorometer, DNA samples were quantified and diluted to 15 ng μl^{-1} to be used in PCR amplifications.

Microsatellite amplifications

Microsatellites markers used were obtained from Research Genetics, Inc., (Huntsville, AL). Amplifications of parent and RIL's DNA samples were carried out in 96 well plates format using a PE (Foster City, CA) 9600 thermal cycler. The electrophoresis of the PCR products was carried out using 5% (w/v) PAGE gels as described earlier (Yuan et al. 2002). The dried gels were used to expose Kodak (Ithaca, NY) X-ray films for 5–6 h and developed. The RILs were classified as 'A' for Essex and 'B' for Forrest. Each marker score was determined by two operators, equivocal scores were repeated in triplicate. Heterogeneous lines were recorded as not scored for that marker and the heterogenous RIL was used to extract a NIL population for future studies. Segregation distortion was detected by Chi-squared tests ($P < 0.05$) of 1:1 allele segregation after excluding heterogeneous lines.

Construction of the genetic linkage map

To analyze the linkage between markers, the program Mapmaker/EXP v. 3.0b was used (Lander et al. 1987). Microsatellite markers were initially grouped and anchored based on known LG (Soybase). All non-anchored markers were then tested against the groups at LOD 5.0, with any markers linking unambiguously to a group added to that group. All groups were then analyzed for linkage using the Kosambi centimorgan function, a maximum genetic distance of 30 cM, and a LOD score greater than 3.0. Marker order was

determined using the compare command, and taking the lowest log-likelihood score. The type I error was set at 5%. Markers were grouped to construct core LGs and anchored on the basis of their known locations (Cregan et al. 1999; Song et al. 2004).

Traits and components used

No new trait data were included and trait sets were not altered in scope so that the CIM QTL could be more easily compared to earlier publications describing interval maps of the same loci. Table 1 shows the 38 components of six traits mapped in this population. The traits were: sudden death syndrome (SDS; Chang et al. 1996; Hnetkovsky et al. 1996; Njiti et al. 1998; 2002; Iqbal et al. 2001), soybean cyst nematode (SCN; Meksem et al. 1999, 2001a), manganese toxicity (Kilo and Lightfoot 1996; Kassem et al. 2004a), seed yield (Yuan et al. 2002), seed isoflavones (phytoestrogens) contents (Njiti et al. 1999; Meksem et al. 2001b; Kassem et al. 2004b;) and foliar trigonelline (TRG) content (Cho et al. 2002).

Agronomic traits were measured as components 1–6 and 29–38. Components 1–3 (resistance to the foliar symptoms of sudden death syndrome) were measured as disease incidence (DI), disease severity (DS) and used to calculate disease index (DX; Chang et al. 1996; Hnetkovsky et al. 1996; Iqbal et al. 2001; Njiti et al. 1998; 2002). Trait 4 (resistance to soybean cyst nematode) was based on the female index with Hg type 0 (Meksem et al. 1999, 2001a). Trait 5 was mean seed yield in six locations with SDS (Hnetkovsky et al. 1996). Traits 29–32 were seed yield in locations with no visible SDS (Yuan et al. 2002). Trait 36 was mean lodging in the seed yield locations. Trait 37 was the mean maturity date and trait 38 the mean height in those locations. Traits 33–34 (foliar trigonelline—TRG—content) were TRG-IP, the foliar TRG content in irrigated plots; and TRG-NIP the foliar TRG content in non-irrigated plots (Cho et al. 2002).

Seed composition; traits 6–15 were deglycosylated seed isoflavone contents estimated by HPLC in $\mu g\ g^{-1}$ of seed dry weight. Daid40, Gen40, and Gly40 were daidzein, genistein, and glycitein contents in the seed in the subpopulation of 40 RILs. Daid60, Gen60, and Gly60 were daidzein, genistein, and glycitein contents in the seed non-overlapping subpopulation of 60 RILs. Daid, Gen, Gly and TPHY were composite trait of daidzein, genistein, glycitein and total isoflavone content in the seed in the full population of 100 lines (Meksem et al. 2001b; Kassem et al. 2004b).

Mineral nutrition; traits 16–27 (resistance to manganese toxicity) was measured by the extent of leaf curl (L_CURL1 to 4), leaf chlorosis (CHLO1–4), and root necrosis (NECRO1–4) at 7, 14, 21, and 28 days (1–4 weeks) after treatment of RIL plants in hydroponics by 125 μM of manganese sulfate (Kilo and Lightfoot 1996; Kassem et al. 2004a).

QTL maps

The CIM method of WINQTL Cartographer v. 2.0 was used to map QTL and estimate their effects (Zeng 1993, 1994; Basten et al. 2001). A walk speed of 2 cM, with LOD values (LR 11.5) of at least 2.5 indicating the existence of QTL for the 34 trait components in this study (Table 1). All markers reported as unlinked or with ambiguous linkages were placed in a single linkage group and analyzed for QTL associations using the single marker analysis tool.

Results

Polymorphism and linkage

Six hundred soybean SSR markers were first tested for polymorphism between the parents Essex and Forrest. All polymorphic markers were mapped in the RIL population. A total of 368 markers were scored and initially mapped. First eliminated were markers that clustered very closely on the composite map (< 0.1 cM), that showed segregation distortion ($P < 0.05$) or that had been scored differently among replicated amplifications. After elimination, only 237 markers remained, including 204 microsatellites. They represented 34% of the 600 markers tested (Kassem et al. 2004a, b; Lightfoot et al. 2005). In comparison, RFLPs [5–6% per probe/enzyme combination; (Keim et al. 1992), RAPDs (8–9% per primer; Chang et al. 1997), AFLPs (10–15% per primer; Meksem et al. 2000, 2001a), and SNPs (5–10% per site; Meksem et al. 2000; Zhu et al. 2003)] were not as polymorphic between Essex and Forrest as microsatellites.

Among previously reported non-microsatellite markers just 6 of 27 RFLPs, 4 of 90 RAPDs, and 6 of 20 AFLPs appeared to be useful (non-distorted) markers. This set of markers was added to the map based on microsatellites after LG formation.

Construction of the genetic linkage map and QTL mapping

The core map covered approximately 1,879 cM and consisted of 171 linked markers on 20 LGs (Fig. 1). There were 66 markers unlinked at LOD3.0 but

Table 1 Ranges and means of the 35 traits in the RIL population and the 'Essex' and 'Forrest' parents

Number	Trait and symbol	Unit	RIL population	
			Average	Range
1	Mean disease index (MNDI)	Score	48.5	4.4–94
2	Mean disease severity (MNDS)	Score	1.5	1.1–2.3
3	Mean disease index (MNDX)	Score	9.3	1.1–23.9
4	Soybean cyst nematode (SCN)	Score	53	0–101.3
5	Yield during SDS (YLD-SDS)	kg ha^{-1}	3.3	2.9–3.76
6	Daidzein (DAID)	μg g^{-1}	1,314	874.5–2,181
7	Genistein (GEN)	μg g^{-1}	996.8	695.5–1,329
8	Glycitein (GLY)	μg g^{-1}	206.1	116–309
9	Daidzein60 (DAID60)	μg g^{-1}	1,336.9	882–2,181
10	Genistein60 (GEN60)	μg g^{-1}	993.9	771–1,329
11	Glycitein60 (GLY60)	μg g^{-1}	211.7	145–309
12	Total daidzein40 (TDEIN40)	μg g^{-1}	1,279.6	874.5–2,033.5
13	Total genistein40 (TGEIN40)	μg g^{-1}	1,001.1	695.5–1,280.7
14	Total glycitein40 (TGLY40)	μg g^{-1}	197.6	116–295.5
15	Total isoflavones (TPHY)	μg g^{-1}	2,516.8	1,774.2–3,759
16	Leaf curl (L_CURL1)	Scale 0–5	1.05	1–1.5
17	Leaf chlorosis (CHLO1)	Scale 0–5	1.05	1–1.35
18	Root necrosis (NECRO1)	Scale 0–5	1.08	1–1.35
19	Leaf curl (L_CURL2)	Scale 0–5	1.37	1.1–2.35
20	Leaf chlorosis (CHLO2)	Scale 0–5	1.23	1.1–2.25
21	Root necrosis (NECRO2)	Scale 0–5	2.02	1.1–4.5
22	Leaf curl (L_CURL3)	Scale 0–5	1.64	1.1–3.25
23	Leaf chlorosis (CHLO3)	Scale 0–5	1.5	1.1–2.6
24	Root necrosis (NECRO3)	Scale 0–5	2.86	1.1–4.5
25	Leaf curl (L_CURL4)	Scale 0–5	2.18	1–3.65
26	Leaf chlorosis (CHLO4)	Scale 0–5	1.58	1.1–3.55
27	Root necrosis (NECRO4)	Scale 0–5	2.48	1.15–4.1
28	YLD-Ridgway-96	kg ha^{-1}	4.43	3.64–4.95
29	YLD-Carbondale-96	kg ha^{-1}	2.77	1.65–3.78
30	YLD-Desoto-96	kg ha^{-1}	3.62	2.78–4.29
31	YLD-Desoto-97	kg ha^{-1}	2.94	2.48–3.51
32	MEAN-YLD-96–97	kg ha^{-1}	3.44	2.64–4.13
33	Trigonelline content (TRG-IP)	μg g^{-1}	98.85	59.87–126.96
34	Trigonelline content (TRG-NIP)	μg g^{-1}	417.94	245.95–618.18
35	Flower color (FLC)	W, P	43 W, 47 P	–
36	Mean lodging (LODG)	Score	2.3	1–5
37	Mean maturity date (MNDAP)	days	108	100–114
38	Plant height (PlntHT)	cm	111	87–129

Traits 1–3 (resistance to sudden death syndrome); see Chang et al. (1996), Hnetkovsky et al. (1996), Kassem (2003), and Njiti et al. (1998, 2002). Trait 4 (resistance to soybean cyst nematode); see Kassem (2003) and Meksem et al. (1999, 2001a). Traits 5 and 28–31 (seed yield); see Yuan et al. (2002) and this work. Traits 6–15 (seed isflavone content): the total seed isoflavone content was estimated by HPLC in μg g^{-1} of seed dry weight. Daid40, Gen40, and Gly40: Daidzein, Genistein, and Glycitein seed contents in the subpopulation of 40 RILs. Daid60, Gen60, and Gly60: Daidzein, Genistein, and Glycitein seed contents in the subpopulation of 60 RILs and TPHY: total isoflavones, see Kassem et al. (2004b) and Meksem et al. (2001b). Traits 16–27 (resistance to manganese toxicity): Leaf Curl (L_CURL1–4), Leaf Chlorosis (CHLO1–4), and Root Necrosis (NECRO1–4) at 7, 14, 21, and 28 days after treatment of RIL plants in hydroponics by 125 μM of manganese sulfate (Kassem et al. 2004a; Kilo and Lightfoot 1996). Traits 32–33 (foliar trigonelline—TRG—content); see Cho et al. (2002). *TRG-IP* foliar TRG content in irrigated plots, *TRG-NIP* foliar TRG content in non-irrigated plots. Trait 35 (Flower color)

anchored by comparison to the composite map. Most of the marker orders among the linked markers were consistent with the consensus genetic linkage map (Song et al. 2004); however, 19 marker order inversions were detected on 12 LGs (Fig. 1; Supplementary Fig. 1) and 11 marker compressions were detected on 5 LGs.

QTL mapping of the 38 trait components was performed using the QTL Cartographer v. 2.0 (Basten et al. 2001). A total of 61 QTL for 29 of the trait components were detected and located in genomic regions on 15 LGs of the soybean genome (Table 2 and Fig. 1). The LOD scores of these QTL ranged from 2.50 (*pht2*) to 27.96 (*sdsyld3*).

Soybean sudden death syndrome (SDS) resistance was previously evaluated using three parameters; mean disease incidence (MNDI), mean disease severity

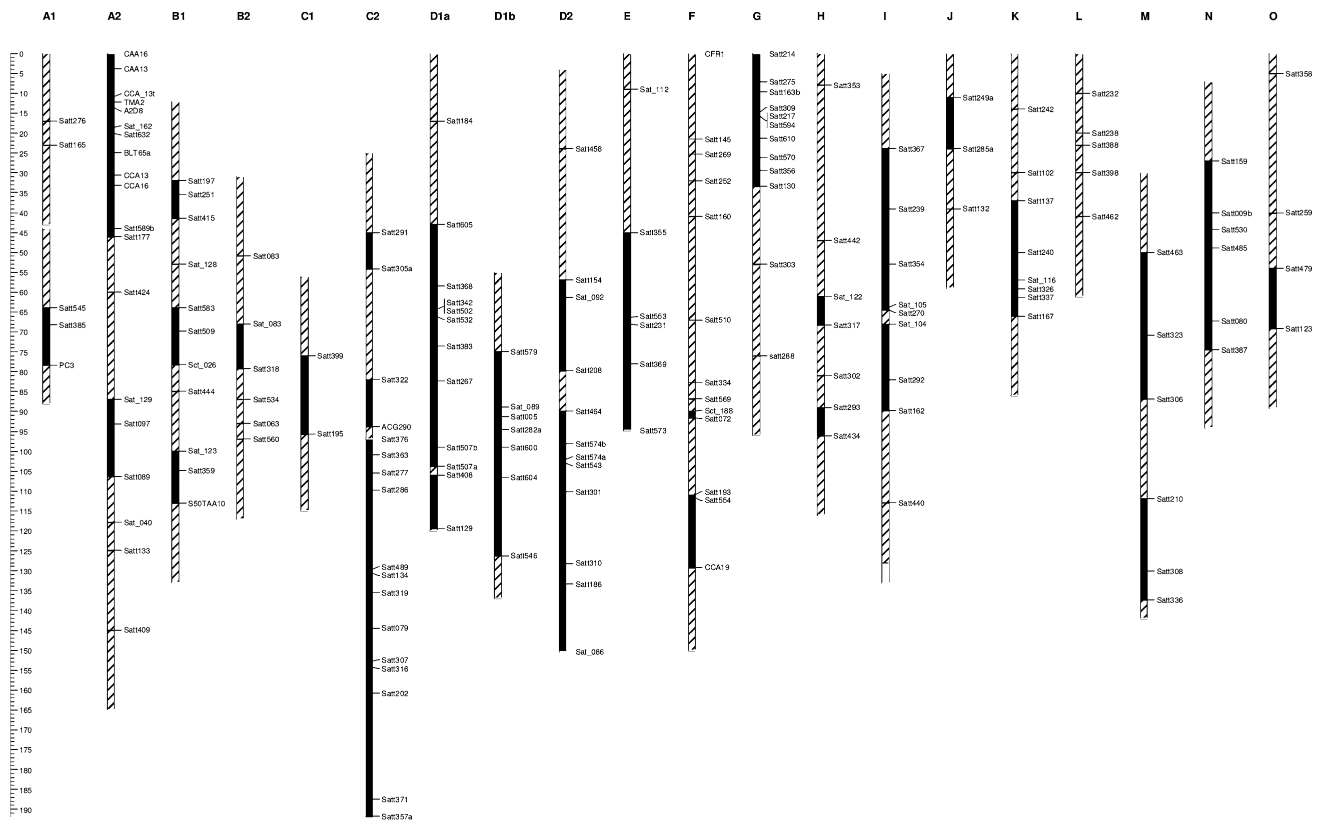

Fig. 1 The new Essex by Forrest genetic linkage map used to identify and locate QTL. Microsatellite markers were designated. Colinear regions of the E × F map and the consensus map are shown as *hatched bars*. Strongly significant inversions (19) and distance compression/expansion (11) between the composite and the E × F map are shown by the *black bars* under the marker names. SattXXX, Sat_XXX, or Sct_XXX identify BARC-microsatellite markers. RFLP markers were designated by a 1–3 letter prefix and a 1–3 number suffix. RAPD markers were named with the prefix O followed by another letter and a number 1–20. AFLP markers were named with a three or six letter prefix (from A, T, G or C) and a 0–3 number suffix. SCARs derived from AFLPs and RAPDs were named with the same notation as the band they were derived from. The linkage groups were named using the consensus map (Cregan et al. 1999). The *cursor* shows approximate map distance between markers in cM. E × F groups that start after 0 cM were missing markers found at the subtelomeric regions in the composite map

(MNDS), and mean disease index (MNDX; Chang et al. 1996, 1997; Hnetkovsky et al. 1996; Iqbal et al. 2001; Njiti et al. 1998, 2002). Six QTL underlying resistance to SDS were reported from MAPMAKER/QTL. Using QTL Cartographer v. 2.0, a QTL underlying resistance to SDS (MNDI, MNDX; LG G) was verified (Table 2). Previously, one QTL for MNDI (*di1*) found on LG N, was identified in the interval between markers Satt080–Satt387. The same QTL underlies MNDS (ds2) and MNDX (*dx1*) (Table 2). Five QTL for MNDS (*ds1–ds5*) were previously mapped on LGs. C2 (interval Satt489–Satt286), F-1 (interval Satt160–Satt252), G (1G, $\mathrm{OPOI03}_{512}$ to ATGACC_{230}; 2G, ATGACC_{230}–Satt214), and N (Satt080–Satt387) (Table 2). Loci on LG C2 and F were associated with seed yield in fields showing SDS symptoms.

For resistance to soybean cyst nematode (SCN), two QTL (*scn1* and *scn2*) that corresponded to the genes *Rhg4* on LG A2 and *rhg1* on LG G identified previously (Meksem et al. 2001a), were confirmed in this study.

Seed yield in the absence of disease was previously reported controlled by six loci (Yuan et al. 2002). In this study, an additional QTL for mean seed yield was identified. The new QTL for mean yield (*yld1*) was linked to the marker Satt079 located on LG C2 (Table 2). The major QTL for seed yield linked to Satt137 on LG K detected by Yuan et al. (2002) by interval mapping was significant in only one location Desoto 1997 (*yldD3*), on the composite interval map.

Foliar trigonelline (TRG) content was also studied previously (Cho et al. 2002). Four QTL underlying foliar TRG content were identified on different LGs. Two QTL for foliar TRG content in irrigated plots (TRG-IP) were found LG A2-1 (linked to the marker TMA2) and F-1 (in the marker interval Satt252-Satt269). Two QTL were found in non-irrigated plots

Table 2 QTL underlying 29 agronomic traits in soybean

Number	Trait	QTL (q−)	LG	Marker/interval	cM position[a]	LOD	Additive	R^2	Reference or new
(a) Agronomic trait QTL									
1	MNDI	*Di1*	N	Satt080–Satt387	51.61	2.56	−9.50	0.159	Hnetkovsky et al. (1996), Iqbal et al. (2001) and Kassem (2003)
		di2	G	Satt214–Satt275	0.5	2.62	7.805	0.0745	New
2	MNDS	*ds1*	C2	Satt489–Satt286	99.21	2.52	0.12	0.1543	Hnetkovsky et al. (1996), Iqbal et al. (2001) and Kassem (2003)
		ds2	N	Satt080–Satt387	51.61	2.62	−1.15	0.1424	Hnetkovsky et al. (1996), Iqbal et al. (2001) and Kassem (2003)
		ds3	F	Satt160–Satt252	0.0	2.81	0.81	0.063	Kassem (2003)
		ds4	G	OIO3–ACC230	95.1–103.71	3.90	1.04	0.1186	Hnetkovsky et al. (1996), Iqbal et al. (2001) and Kassem (2003)
		ds5	G	ACC230–Satt214	103.7–110.5	5.96	1.31	0.1730	Hnetkovsky et al. (1996), Iqbal et al. (2001) and Kassem (2003)
3	MNDX	*dx1*	N	Satt080–Satt387	49.61	2.67	−2.45	0.1733	Hnetkovsky et al. (1996), Iqbal et al. (2001) and Kassem (2003)
		dx2	G	Satt214–Satt275	0.5	2.56	1.8719	0.0692	New
4	SCN	*Scn1*	A2	CAA13–CCA13	2.01–24.1	4.71	13.06	0.1440	Meksem et al. (1999)
		Scn2	G	Satt217–ATGCGA190	81.3–89.5	5.54	13.09	0.1518	Meksem et al. (1999)
5	YLD-SDS	*sdsyld1*	C2	Satt307–Satt319	117.87	3.81	4.279	0.1786	New
		sdsyld2	F	Satt510–Satt569	71.41	20.76	15.968	0.618	New
		sdsyld3	F	Satt334	78.05	27.96	15.968	0.618	New
29	Cdale96	*yldC*	H	Sat_122–Satt317	61.33	5.05	−0.277	0.2584	New
30	Desoto96	*yldD1*	C2	Satt371–307	145.47	4.78	−0.173	0.1933	New
31	Desoto97	*yldD2*	C2	Satt286–Satt363	98.07	4.34	−0.085	0.1517	New
		yldD3	K	Satt137	36.99	2.51	0.066	0.0934	Yuan et al. (2002)
32	MYLD	*yld1*	C2	Satt079	66.01	4.0	3.77	0.1323	This work
		yld2	C2	Satt307–Satt079	121.26	3.02	0.286	0.1625	New
34	TRG-NIP	*trgnip1*	C2	Satt357–Satt371	2.01	2.90	47.50	0.2573	Cho et al. (2002)
		trgnip2	G	Satt217	81.41	2.70	35.62	0.1486	Cho et al. (2002)
36	LODG	*ldg1*	E	Satt355–Satt553	45.15	3.55	−0.264	0.1543	New
		ldg2	I	Satt354	46.22	3.02	−0.232	0.1138	New
37	MNDAP	*Dap1*	G	Satt214–Satt275	0.5	2.84	−0.414	0.0858	New
		Dap2	I	Satt270–Sat_105	50.11	3.49	−0.471	0.1137	New
38	PlntHT	*pht1*	C2	Satt291–Satt305a	45.75	4.00	1.958	0.1545	New
		pht2	E	Satt231–Satt369	70.23	2.50	−1.614	0.0945	New
(b) Isoflavone QTL									
8	Gly	*gly1*	B1	Satt251–Satt197	49.9–52.8	7.74	26.04	0.2567	Meksem et al. (2001a, b) and Kassem et al. (2004b)
		gly2	A2	Satt097–Sat_129	–	3.36	17.040	0.1023	New
		gly3	B1	Satt251–Satt415	46.38	15.02	32.610	0.4871	New
		gly4	D1a	Satt368–Satt605	43.84	3.47	−13.835	0.0892	New
9	Daid60	*daid60–1*	N	Satt485–Satt080	38.01	2.63	−132.21	0.5591	Meksem et al. (2001a, b) and Kassem et al. (2004b)
		daid60–2	A2	CAA13–CAA16	–	4.69	285.378	0.1626	New
		daid60–3	M	Satt323–Satt463	60.04	4.02	−338.603	0.2401	New
10	Gen60	*gen60-1*	A2	CAA13	–	2.86	160.461	0.0993	New
		gen60-2	M	Satt323–Satt463	60.04	3.75	−241.005	0.2274	New
11	Gly60	*gly60-1*	A2	CAA16–CCA_13t	–	3.66	40.131	0.1271	New
		gly60-2	G	Satt356–Satt130	12.18	2.89	41.047	0.1071	New
		gly60-3	M	Satt323–Satt463	60.04	2.51	−42.929	0.1491	New
13	TGein40	*tgein40-1*	A2	CAA13	–	3.74	−190.576	0.135	New
		tgein40-2	M	Satt323–Satt463	60.04	2.62	210.712	0.1742	New

Table 2 continued

Number	Trait	QTL (q−)	LG	Marker/interval	cM position[a]	LOD	Additive	R^2	Reference or new
14	Tgly40	*tgly40-1*	A2	CAA13	–	2.85	−34.664	0.1094	New
15	Tphy	*Tphy1*	G	Satt356	12.18	2.78	122.999	0.1024	New
(c) Mn toxicity QTL									
16	LCURL1	*lcurl1-1*	F	Satt145–CFR1	10.65	2.61	−0.055	0.283	New
		lcurl1-2	I	Sat_104–Satt162	65.62	4.61	−0.062	0.3308	New
17	CHLO1	*Chlo1-1*	F	Sat_133	0.01	3.88	−0.13	34.10	Kassem et al. (2004a)
18	NECRO1	*necro1-1*	C2	Satt286	102.11	4.32	0.11	0.2779	Kassem et al. (2004a)
		necro1-2	G	Satt317	83.51	3.37	0.09	0.1833	Kassem et al. (2004a)
		necro1-3	C2	Satt291–Satt305a	45.75	5.83	−0.066	0.2452	New
		necro1-4	F	Satt510–Satt334	71.41	7.92	0.090	0.3884	New
19	LCURL2	*lcurl2-2*	B2	Sat_083–Satt318	51.49	4.43	0.209	0.293	New
20	CHLO2	*Chlo2-1*	C2	Satt322–ACG290	82.23	3.78	0.1202	0.2375	New
21	NECRO2	*necro2-1*	M	Satt210–Satt336	130.75	4.50	−0.468	0.27	New
		necro2-2	N	Satt080–Satt159	38.07	3.41	−0.377	0.1922	New
		necro2-3	N	Satt009b	28.52	4.38	−0.419	0.2195	New
22	LCURL3	*lcurl3-1*	H	Satt293–Satt434	89.08	4.37	0.322	0.3045	New
24	NECRO4	*necro4-1*	D1a	Satt129–Satt408	109.66	2.53	−0.415	0.2089	New
		necro4-2	E	Satt355	45.15	2.84	0.311	0.1311	New
26	CHLO4	*chlo4-1*	D1b	Sat_089–Satt282a	76.27	3.41	0.244	0.1839	New

The QTL are found using the CIM of WINQTL CART. All QTL were reported with LOD scores greater than 2.5 (WINQTL CART). New QTL are reported as 'New' and previously reported QTL are indicated with references. Traits with no QTL detected are not shown

[a] All 'new' linkage group positions are based on nearest marker position on the soybean composite map. All others are based on reported position in the literature

(TRG-NIP) on LG C2 (Satt357–Satt371) and G (Satt217), which is in agreement with Cho et al. (2002).

A total of 16 QTL for seed isoflavones (daidzein, genistein, and glycitein) contents were identified on different LGs (Table 2). Three QTL for daidzein were identified on LGs A2, N and M (Table 2), two QTL for genistein were identified on LGs A2 and M, and six QTL for glycitein were identified on LGs A2, B1, D1a, G and M (Table 2). Total phytoestrogen contents were associated with a locus on linkage group G. The two QTL that underlies glycitein content (*gly1*, *gly3* LG B1, Satt251–Satt197) was previously reported to be a single locus (Meksem et al. 2001b; Kassem et al. 2004b). The genomic region was delimited further in this study (by new markers) from 12 to 2.9 and 9.2 cM, respectively (Table 2 and Fig. 1).

Resistance to manganese toxicity was also studied previously on the basis of leaf curl (l_curl1–4), leaf chlorosis (Chlo1–4), and root necrosis (Necro1–4) at 7, 14, 21, and 28 days after treatment with 125 μM manganese sulfate in hydroponics (Kilo and Lightfoot 1996; Kassem et al. 2004a). A total of 16 QTL for resistance to manganese toxicity were identified on 15 LGs (Table 2). One QTL was previously found on each of the LGs A2-1 (CAA, Necro1), A2-2 (Satt089, Necro4), B2 (Satt163, Chlo4), D1b + W (Satt282–Satt005, Necro4), D1a + Q (Satt502–Satt368, Necro4), E (Satt305–Satt553, Necro2), F-1 (Satt160, L_curl2), F-3 (Satt193, Necro4), F-4 (Sat_133, Chlo1), G (Satt317, OI03-ACGCAT90, and Satt214; Necro1, L-curl3, and Necro3), H (Satt434–Satt293, Chlo3), I (Satt440–Satt294, L-curl4), and O-2 (Satt420–Satt479, Satt479; Chlo2, L-curl2, and Chlo4), two QTL were found on LG B1-2 (Satt415–Satt251, Necro4 and L-curl2; Satt358, Chlo3), four QTL were found on LG C2 (Satt307–Satt079, L-curl2; Satt489Satt286, Necro1 and 2; Satt363Satt520, Chlo2, L-curl3, and Necro3), and two QTL found on LG K (Satt306, Necro3; Satt337, Necro1). The four QTL previously reported on LGs B2, C2, G, and I were confirmed in this study (Kassem et al. 2004b).

Discussion

A genetic linkage map of the soybean genome using 204 core microsatellite markers along with 33 other markers is reported. The map covers about 1,879 cM and consists of 32 fragments of 20 LGs. In reported single population maps, the linkage distance ranged from 2,800 cM (Mansur et al. 1996; Cregan et al. 1999) to 3,600 cM (Song et al. 2004; Zhang et al. 2004). Although the markers are substantially evenly distributed among the LGs, gaps still remain that would cause QTL detection to fail. Gaps of distances greater than 20 cM were observed on all LGs and need to be filled with more markers. In prospect, the full Essex × Forrest genetic linkage map marker set could contain all the 417 previously mapped

markers (June 2006). Additional polymorphic non-satellite markers included 27 RFLPs, 20 AFLPs, 90 RAPDs, and 50 BAC-End-Sequencing (BES) derived SSR markers. That set is linked to an available 1,053 BES derived microsatellites (Kazi et al. 2006; Shultz et al. 2006a, b) and 13,747 sequence tagged sites. The non-SSR additional markers may currently be used for single point analyses, not interval maps due to their tendency to expand map intervals. Extensive marker score replication, error detection and removal will be necessary for the additional markers.

In the core E × F map, the longest gap distances per linkage group ranged from 20 cM on LG G to 40 cM on LG D2. However, such gaps (> 20.0 cM) were reported in almost all soybean genetic linkage maps (Lark et al. 1993; Mansur et al. 1996; Cregan et al. 1999; Zhang et al. 2004; Song et al. 2004). Microsatellite clustering was observed in the ExF map as reported earlier in soybean (Cregan et al. 1999; Song et al. 2004), rice (McCouch et al. 2002), and tomato maps (Broum and Tanksley 1996; Areshchenkova and Ganal 1999). Marker clusters and marker distance expansions may reflect inversions between genomic regions between the population parents that reduce the rate of recombination in the inverted region (Fig. 1). In the physical map there are several marker pairs and triplets on single contigs (Shultz et al. 2003, 2006a) showing that regions of low recombination frequency do exist that are at different regions in different populations.

A revision of QTL locations and size was presented based on 38 components of six agronomic traits. A total of 61 QTL were detected with LOD scores ranging from 2.52 to 9.80. Of the 29 QTL identified previously (Lightfoot et al. 2005) 17 were also significant by CIM and were delimited further by new markers and the new statistical analysis. However, for each trait component 1–4 new QTL were detected, 43 new QTL in total. The new QTL will be analyzed further in NIL populations (Njiti et al. 1998; Meksem et al. 1999, 2001a). Among the QTL identified by earlier studies but not by CIM, four had been confirmed in NIL populations (Njiti et al. 1998; Meksem et al. 1999, 2001a).

Previously, we reported 6 QTL for SDS resistance using MAPMAKER/QTL and one-way analysis of variance (ANOVA; Chang et al. 1996; Hnetkovsky et al. 1996; Iqbal et al. 2001; Njiti et al. 2002). Five of these QTL previously reported were confirmed here. The QTL located on LG F (Satt160) was new in this study and appeared to be a major seed yield determinate. The locus may be a useful selection target for a SDS resistance breeding programs.

For resistance to SCN, the two QTL corresponding to genes *Rhg4* on LG A2 and *rhg1* on LG G were also confirmed here using CIM. The complete resistance (FI < 10) was conditioned by the two genes *Rhg1* and *Rhg4*, as suggested previously (Meksem et al. 2001a). The variation among susceptible lines did not consistently associate with any loci.

For seed yield in fields showing SDS symptoms, two QTL were detected, one on each of the LGs C2 and F, whereas in fields free from disease C2 and G were significant. Interestingly, QTL for seed coat hardness (C2: Keim et al. 1990a, b) seed yield (F; Reyna and Sneller 2001) and resistance to SCN (G; Concibido et al. 2004) were identified about 3 cM from the QTL for seed yield (SoyBase 2005). For foliar TRG content, a potential component of water deficit tolerance, two QTL for non-irrigated plots were found on LG C2 and G, were confirmed here (Cho et al. 2002). However, neither QTL previously detected in irrigated plots was detected. It was interesting that both QTL overlapped with seed yield loci suggesting they may have a common basis or represent the action of clustered genes.

Sixteen QTL at nine separate locations were detected for seed isoflavones content. Eight loci were reported earlier (Meksem et al. 2001b; Kassem et al. 2004b). Only two of the 8 QTL were confirmed by CIM. These two QTL underlie genistein and daidzein contents and were located on LGs B1 (Satt415-Satt197) and N (Satt485-Satt080), respectively. Among the QTL detected by CIM were four intervals that appeared to overlap with loci underlying QTL reported in Canada (

Primomo et al. 2005a, b). Of particular note, the new analysis by CIM in E × F allowed the detection of the loci on LG M that were very significant in Canada.

For resistance to manganese toxicity, 16 QTL are reported here including 3 QTL close to those previously reported (Kassem et al. 2004a). Thirteen QTL were newly discovered and reported here. Resistance to manganese toxicity is a complex trait related to manganese uptake, partitioning within the cell, transport, leaf abscission and pH homeostasis. Leaf chlorosis appears to result from the inhibition of ribulose bisphosphate carboxylase (EC 4.1.1.39) by manganese. Therefore, large number of genes are expected to underlie resistance to manganese toxicity. The large number of QTL detected for this trait probably was also partly caused by the number of trait components (12) used to measure the trait. Certainly some of the QTL may be type 2 errors or QTL related to the single location used in the study. No other studies, to date, have reported QTL for resistance to manganese (Mn) toxicity to corroborate the QTL. Correspondence of the QTL among trait measurements indicates some of the QTL do underlie resistance to manganese toxicity.

The QTL on LG B2 was reported to underlie both leaf chlorosis at 21 and 28 days after treatment (Chlo3 and Chlo4; Kassem et al. 2004a); however, in this study it underlies only leaf curl2 (lcurl2-1, Sat_083). The other three previously known QTL on LGs C2, F and G (Kassem et al. 2004a) were confirmed in this study.

Interestingly, QTL for SCN resistance (Concibido et al. 2004), seed sucrose (Maughan et al. 2000), sclerotinia stem rot (Arahana et al. 2001), and seed weight (Hoeck et al. 2003), were mapped within 8–20 cM from the QTL, on LG A2, that underlies root necrosis at 28 days after treatment (necro4) reported here. The same QTL (necro4) is approximately 30 cM from the seed yield QTL noted on LG A2 suggesting that resistance to manganese toxicity and increased yield maybe combined in breeding programs. Similarly, resistances to SDS, SCN, and manganese toxicity may be combined with increased isoflavones contents and increased seed yield.

In conclusion, the majority of marker orders were consistent between the composite map and the E × F map. Nineteen putative map or marker inversions were detected on 12 of 20 LGs. Each inferred an inversion had occurred. A total of 61 QTL among 37 measures of six traits were detected and the positions of genes, loci and QTL were confirmed in relation to the anchor markers previously reported. Therefore, comparing the E × F population map with the composite map suggests marker order inversions are common in soybean mapping. The marker orders that underlie the physical map should be analyzed critically as a framework for whole genome shotgun sequence. The list of potential inversions emphasizes the need for a critical analysis of marker orders against the physical map because contradictions are expected as marker density increases (Kazi et al. 2006; Shultz et al. 2006a, b). Inversions between genomic regions in different germplasm pools will complicate gene transfer between pools. A genome sequencing project based on Forrest, to compare a Southern to a Northern US soybean cultivar (Williams 82) would catalog and delimit inverted regions and the associated QTL. Gene introgression in cultivar development programs would be accelerated by identifying inverted regions and cataloging the affected QTL. Directed breeding would be enabled.

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