Fr1 (root fluorescence) locus is located in a segregation distortion region on linkage group K of soybean genetic map

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Fr1 (root fluorescence) locus is located in a segregation distortion region on linkage group K of soybean genetic map

Abstract
We report the use of bulked segregant SSR analysis for rapid identification of DNA markers linked to the Fr1 locus in soybean. Pooled DNA extracts from 10 homozygous Fr1 Fr1 and 10 fr1 fr1 F2 plants, derived from a msMOS x Minsoy cross, were analyzed using 65 SSR markers. Five SSRs produced repeatable polymorphisms between paired bulks. Linkage with the Fr1 locus was tested using these five SSR primers and DNA from individual plants of each bulk. DNA polymorphisms generated by these five primers were linked to the Fr1 locus. Linkage of SSR loci with the Fr1 locus was verified by using an F2 population segregating for Fr1. The five SSR markers and Fr1 are on linkage group K of the USDA ARS/ISU molecular genetic map. The markers flanking Fr1 are Satt337 (11.0 cM) and Satt044 (0.6 cM). Fr1 previously was mapped on linkage group 12 of the classical genetic map. Thus classical genetic linkage group 12 has been correlated to linkage group K of the molecular genetic map. Six SSR markers were chosen on linkage group K to test the segregation ratio. All six SSRs tested were skewed toward the Minsoy genotype, one chi-square value was statistically significant. This suggested that a gametophyte factor may lie in the region close to Fr1 and most likely close to Satt046.

Disciplines
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Comments
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rameters \( m \), \( d \), \( h \), and \( i \) gave the best ex-
planation of stem thickness gene func-
tions in this cross (Table 4). Since the \( h \)
value is very similar to \( d \), but with nega-
tive sign, the function of the genes con-
trolling for thickness of stem seems to be
partially dominant to stem thinness. In
the second cross, the simple additive-
dominance model failed to explain gene
function, and both the simple and the
joint scaling tests indicated the existence
of at least one kind of nonallelic interac-
tion effects. The removal of the least im-
portant component of the model, \( l \), pro-
duced a good fit with the rest of the
parameters, that is, \( m \), \( d \), \( h \), \( i \), and \( j \). Also,
in this cross the results indicated that the
genes controlling thickness are partially
dominant to those for thinness of stem.
Although in this set \( j \) was significant at
the 5% level, the other parameters in the
best-fit model were very similar to those
of cross 1. This confirms that in such a situa-
tion—extreme phenotypic classes in
two diverse populations—generation
mean analysis could be applied in order
to investigate the dominance and epista-
sis relationships of the genes involved.

Minimum Number of Genes

The number of effective factors or the
minimum number of genes estimated in
set 1 and 2 indicated that these attributes
are oligogenic (controlled by a few genes;
Table 2). The corresponding standard er-
rors for the estimated number of genes
were very small. This indicates that when
the assumptions for this procedure are
approximately satisfied, the estimates of
the number of genes would be reasonably
accurate.

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Fr1 (Root Fluorescence)
Locus Is Located in a
Segregation Distortion
Region on Linkage Group K
of Soybean Genetic Map

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and R. C. Shoemaker

We report the use of bulked segregant
SSR analysis for rapid identification of
data markers linked to the Fr1 locus in soybean. Pooled DNA extracts from 10 ho-
mozygous Fr1 Fr1 and 10 fr1 fr1 F2 plants,
derived from a msMOS × Minsoy cross,
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SSRs produced repeatable polymor-
phisms between paired bulks. Linkage
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with the Fr1 locus was verified by using an
F2 population segregating for Fr1. The five
SSR markers and Fr1 are on linkage group
K of the USDA ARS/ISU molecular genetic
map. The markers flanking Fr1 are Satt337
(11.0 cM) and Sal044 (0.6 cM). Fr1 pre-
viously was mapped on linkage group 12
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cal genetic linkage group 12 has been
correlated to linkage group K of the mo-
lecular genetic map. Six SSR markers
were chosen on linkage group K to test the
regression ratio. All six SSRs tested were
skewed toward the Minsoy genotype, one
chi-square value was statistically signifi-
cant. This suggested that a gametophyte
factor may lie in the region close to Fr1
and most likely close to Satt046.

Soybean root fluorescent mutants are
important in characterizing germplasm di-
versity (Delannay and Palmer 1982), in tis-
sue culture (Roth et al. 1982), and in ge-
netic linkage studies (Devine et al. 1993;
Griffin et al. 1989; Palmer and Chen 1998).
Five loci controlling root fluorescence
have been reported (Delannay and Palmer
1982; Sawada and Palmer 1987) and the
genomic locations of several of these loci
have been defined. Fr2 was placed on a
molecular genetic map approximately 6.5
cM from RFLP markers pBLT 73 and 6 cM
from pBLT 42 (Devine et al. 1993). Fr1 and
Fr3 have been located on the classical ge-
netic map. Fr1 is 41 cM distant from the
Ep (seed coat peroxidase level) locus on classical
linkage group 12 (Griffin et al. 1989). Fr1 is
located on Ulc linkage group (Mansur et al. 1996); however, no detailed data were described in the article. Fr3 is
mapped on classical linkage group 9
(Palmer and Chen 1998). The linkage re-
lationships of the Fr2, Fr4, and Fr5 loci on
molecular genetic maps are unknown.

A classical genetic map of soybean has
been developed with great effort over
many years (Palmer and Hedges 1993). In-
tegration of this map with the soybean
molecular map would be useful for design-
ing breeding strategies for soybean im-
provement and using the molecular map
as a bridge to obtain a comprehensive map
of important genes (Shoemaker and Specht
1995). In soybean, diversity at the
DNA sequence level is low (Keim et al.
1989). This has made the construction of
an RFLP-based genetic map difficult and
tedious.

Recently, simple sequence repeat (SSR)
markers have been developed (Akkaya
et al. 1993; Weber and May 1989). Because
SSRs are highly polymorphic, stable, and
simple, they have great potential for mo-
lecular mapping. The combination of SSR
polymorphism and bulked segregant anal-
ysis (Michelmore et al. 1991) will facilitate
the identification of markers that are tight-
ly linked to the gene of interest.

Segregation distortion has been report-
ed in wide crosses of rice (Xu et al. 1997),
maize (Pereira and Lee 1995), barley (De-
vaux et al. 1995), common bean (Paredes
and Gepts 1995), Aegilops tauschii (Faris
et al. 1998), and soybean (Honeycutt et al.
1990). Many instances of segregation dis-
tortion have been reported through stud-
ies of isozymes (Ishikawa et al. 1987a,b;
Wu et al. 1988) and RFLP alleles (McCouch
et al. 1998; Saito et al. 1991). The mecha-
nisms of segregation distortion are not
well understood, but they may result from
action of gametophytic factors, competi-
tion among gametes, or from the abortion
of male or female gametes. Studies by Nak-
agehara (1972, 1986) localized gametophy-
tic gene loci on chromosome 3 based on a
locus responsible for the partial or total
elimination of gametes carrying one of the
alleles at that locus. Faris et al. (1998) re-
ported that there are segregation distor-
tion loci on chromosomes 1D, 3D, 4D, 5D,
and 7D of Ae. tauschii.
In the present study we describe the application of bulked segregant analysis as a method for rapidly identifying SSR markers linked with the Fr1 locus. Our goals were to place Fr1 on the molecular genetic map and thus to associate classical linkage group 12 with a molecular linkage group.

**Material and Methods**

**Plant Material**

msMOS (a male-sterile line obtained from Midwest Oilsseed, Adel, Iowa; Fr1 Fr1) as a female parent, was crossed with Minsoy (fr1 fr1) in Ames, Iowa, in the summer of 1995. The resulting F1 seeds were planted at the University of Puerto Rico, Iowa State University Soybean Nursery, Isabela Substation, Isabela, Puerto Rico. An F2 population of 111 individuals was used to test linkage between SSR markers and the Fr1 locus. F2 seeds were planted on germination paper for scoring root fluorescence. Fluorescence was observed by irradiating roots with ultraviolet light (Delannay and Palmer 1982). After scoring, F2 seedlings were transferred to a growth chamber, leaf material was harvested, and DNA was isolated. F2 plants were single-plant threshed, and F2 seeds were planted in Puerto Rico to generate the F3 generation. F3 seeds were planted on germination paper for scoring root fluorescence to determine F2 genotypes (Fr1 Fr1, Fr1 fr1, fr1 fr1).

**DNA Extraction and Bulk DNA Preparation**

DNA isolation was conducted as described by Keim et al. (1988). Equal quantities of DNA were combined from each of 10 homozygous F2 Fr1 Fr1 plants and from each of 10 homozygous F2 fr1 fr1 plants to obtain two DNA bulks.

**SSR/BSA (Bulked Segregation Analyses)**

SSRs were developed and initially placed on linkage groups as reported by Cregan et al. (in press). Primer sequences specific to each SSR can be obtained through Soybase, the soybean genome database at http://129.186.26.94.

For SSR/BSA analyses, PCR amplifications were carried out in a total volume of 20 μl containing 60 ng of soybean genomic DNA, 1.5 mM MgCl2, 0.3 μM of sense and antisense primers, 200 μM of dNTPs, 1× PCR buffer, and 2.5 units of Taq DNA polymerase. Cycling consisted of 30 s at 94°C, 30 s at 47°C, and 30 s at 68°C for 40 cycles on a Perkin-Elmer 960 thermal cycler. PCR products were run on 2.5% Metaphor gel in TBE (0.089 M Trisborate, 0.089 M boric acid, 0.002 M EDTA) buffer with ethidium bromide incorporated into the gel. Initial screening was performed on samples of F1 Fr1 (msMOS), fr1 fr1 (Minsoy), pooled DNA from 10 Fr1 Fr1 (pool Fr1) genotypes, and pooled DNA from 10 fr1 fr1 (pool fr1) genotypes.

**Linkage Analysis**

The segregation ratios of Fr1 and each molecular marker in the F2 population were tested for fitness to a 1:2:1 genotypic ratio by chi-square test. After finding that three SSRs (Sat055, Sat044, Satt337) showed segregating excess BB class and less AA class, six more SSR markers (Satt137, Satt247, Satt046, Sat116, Satt326, Satt001) were chosen to investigate segregation ratios. Three (Satt137, Satt046, Satt326) out of these six markers showed polymorphisms and were analyzed further.

The MapMaker program (Lander et al. 1987) was used to construct a linkage map by using Fr1 segregation data. A logarithm of odds ratio (LOD) score of 3 was used as the lower limit for accepting linkage between two markers. Distances between markers were calculated in centiMorgans (cm) derived with the Kosambi function (Kosambi 1944).

**Results**

The root fluorescence test on the F2 progenies, derived from 80 F2 plants, yielded 14 homozygous Fr1 Fr1, 42 segregating, and 24 homozygous fr1 fr1 families (Table 1). This segregation fit the expected ratio of 1:2:1 ($\chi^2 = 2.7$). Ten homozygous Fr1 Fr1 and 10 homozygous fr1 fr1 F2 plants were selected for preparation of the two DNA bulks.

<p>| Table 1. Segregation of the Fr1 (root fluorescence) locus and six simple sequence repeat markers in an F2 soybean population from msMOS × Minsoy |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Trait or markers</th>
<th>No. of F2 plants</th>
<th>Observed no.</th>
<th>$\chi^2$ (1:2:1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Satt137</td>
<td>78</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Satt055</td>
<td>80</td>
<td>12</td>
<td>42</td>
</tr>
<tr>
<td>Satt337</td>
<td>80</td>
<td>12</td>
<td>41</td>
</tr>
<tr>
<td>Satt046</td>
<td>79</td>
<td>10</td>
<td>41</td>
</tr>
<tr>
<td>fr1 fr1</td>
<td>80</td>
<td>14</td>
<td>42</td>
</tr>
<tr>
<td>Sat044</td>
<td>80</td>
<td>13</td>
<td>43</td>
</tr>
<tr>
<td>Satt326</td>
<td>78</td>
<td>14</td>
<td>43</td>
</tr>
</tbody>
</table>

* Genotypes: AA = msMOS; HH = heterozygous; BB = Minsoy.
* Significant deviation from 1:2:1 ratio at 5% level.

In total, 65 SSR markers distributed on the 23 linkage groups of the soybean molecular genetic map (Cregan et al., in press) were tested with DNA from Fr1 Fr1 parent, fr1 fr1 parent, a pool of 10 Fr1 Fr1 genotypes, and a pool of 10 fr1 fr1 genotypes (Figure 1). Of these, 4 (6%) did not produce any amplification product and were not analyzed further. The pool size of 10 genotypes for BSA analyses was determined on the basis of detection limit, desired interval of the genome to be covered (Giovannoni et al. 1991; Michelmore et al. 1991), and our population size. Six of the remaining 65 SSRs detected polymorphisms between pooled Fr1 and fr1 DNAs. These polymorphisms were repeated to verify reproducibility. Primers for these six SSRs were used to amplify DNA from each of the 80 F2 progeny. Again, all six markers tested were skewed toward the Minsoy genotype, and one chi-square value was statistically significant (Table 1, Figure 2). In all cases, fewer msMOS gametes were transmitted than expected. Segregation analysis with 80 F2 progeny determined that these two SSRs (Sat044 and Satt337) were linked to the Fr1 locus (Figure 3) with LOD scores of 30 and 16, respectively. The two markers flanking Fr1 locus were Sat044 and Satt337. The map locations of the two flanking loci agreed with the soybean molecular genetic map (Cregan et al., in press). The fragment amplified by Sat044 in the Fr1 Fr1 bulk and Fr1 Fr1 parent was present in all 14 Fr1 plants and was not present in any fr1 individuals. The fragment amplified in the
fr1 bulk and fr1 parent was present in all 24 homozygous fr1 fr1 plants. For Satt337, the fragment amplified in the Fr1 Fr1 bulk and the Fr1 Fr1 parent was present in 13 of 14 plants scored as Fr1 Fr1 and was present in three of 24 plants scored as fr1 fr1. The fragment amplified in the fr1 fr1 bulk and fr1 fr1 parent was present in 23 of 24 plants scored as fr1 fr1. The closest linkage (0.6 cM) was found with SSR marker SatL044. The SSR profiles of some of the individuals amplified with SatL044 primers (Figure 4). The two SSR markers flanking the Fr1 locus are on linkage group K in USDA ARS/ISU map (Cregan et al., in press).

Discussion

Mapping genes with molecular markers can be laborious and costly. One of the most time-consuming aspects of mapping, the screening of the entire mapping population with every probe or primer to be tested, can be eliminated by applying bulked segregant analysis (Michelmore et al. 1991). Bulked segregant analysis simultaneously provides information on polymorphisms between the parents and possible linkage between a marker and a targeted gene. This process can reduce cost by several-fold, particularly when used with PCR-based techniques such as SSR and RAPD analyses. Bulked segregant analysis combined with RAPD also has been used to detect markers linked to many traits including disease resistance genes (Mouzeyar et al. 1995; Yaghoobi et al. 1995), a male-sterile gene in rice (Zhang et al. 1994), and a fertility restoration gene in rapeseed (Delourme et al. 1994). To our knowledge this is the first report using bulked segregant analysis with SSR for molecular mapping in plants. SSR/BSA efficiently identified markers linked to the gene of interest and allowed their rapid placement on a genetic map.

In this study the combination of SSR markers and bulked segregant analysis was faster and less expensive than using RFLPs to find markers flanking the Fr1 locus. We tested 61 polymorphic SSR markers with pools of DNA from 10 Fr1 Fr1 and 10 fr1 fr1 genotypes and found that the Fr1 locus mapped to linkage group K of the USDA ARS/ISU map and was flanked by two SSR markers, Satt337 and SatL044.

There were consistently skewed segregation ratios within an interval on linkage group K (Table 1). Based on chi-square analysis of SSR segregation data, chi-square values peaked in the interval between Satt046 and Satt337 (Figure 2). This suggests the presence of a gametophytic factor (Redei 1965; Li 1967; Gonella and Peterson 1975; Rashid and Peterson 1992) in the interval affecting transmission of the msMOS gametes. Segregation distortion is expected to be more extreme if the distance between a marker locus and the gametophytic factor gene is very close.

Development of the classical genetic map has proceeded slowly in soybean because it is difficult to make crosses and generate large numbers of hybrid seeds, and there are few cytogenetic markers and low genetic variation in the germplasm (Keim et al. 1989). To exploit fully the potential of a molecular genetic map, molecular and conventional markers must be integrated into one linkage map. Integration of the maps can also be pursued by screening near-isogenic lines (NIL) (Young et al. 1988). According to Shoemaker and Specht (1995), about half of the 19 soybean classic linkage groups have been associated with their corresponding molecular genetic linkage groups. But classical linkage group 12 has not been asso-

![Figure 2. Effect of map distance (cM) from Satt046 locus on segregation ratios (chi square). * indicates chi-square value of Fr1.](image2)

![Figure 3. Position of the Fr1 locus on molecular genetic linkage group of soybeans. (A) A part of the soybean genetic group K showing the linkage map around the Fr1 locus obtained from analysis of 80 F2 plants derived from the cross between msMOS (Fr1 Fr1) and Minsoy (fr1 fr1). (B) A linkage map of linkage group K constructed from the Glycine max × Glycine soja population (Shoemaker et al. 1996). Linkage group K extends beyond the hash marks.](image3)

![Figure 4. SSR profiles of some of the F2 individual genotypes of the pools with SatL044. * indicates recombinants. P1 = parent 1 (msMOS), P2 = parent 2 (Minsoy).](image4)
asiated with any of the molecular genetic linkage groups. Fr1 is known to reside on classical linkage group 12 (Griffin et al. 1989). Our results have shown that the Fr1 locus is located on molecular genetic linkage group K. Therefore classical linkage group 12 is now integrated into molecular genetic linkage group K.

From the Interdepartmental Plant Physiology Program and Department of Botany (Jin and Horner), and the Departments of Agronomy and Zoology/Genetics and the USDA Agricultural Research Service CICGR (Palmer and Shoemaker), Iowa State University, Ames, IA 50011. We gratefully acknowledge Drs. David Grant and Arla Bush for helpful discussions during the course of this project, and Drs. David Grant, John Imsande, and Joanne Labate for critically reviewing the manuscript. This is a joint contribution: journal paper J-17701 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa (projects 3256 and 3352) and the U.S. Department of Agriculture, Agricultural Research Service, Corn Insects and Crop Genetics Research Unit. The mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by Iowa State University or the USDA and does not imply its approval to the exclusion of other products that may also be suitable. Address correspondence to H. T. Horner at the address above.

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Quantitative Genetics of Leaf Morphology in Crepis tectorum ssp. pumila
(Asteraceae)

S. Andersson

The present study of Crepis tectorum ssp. pumila employed the factorial mating design to examine the genetic (co)variance structure of leaf morphology in a population that appears to be homozygous for a major gene causing deeply lobed leaves. One set of factorials was based on widely dispersed plants (different patches, mean crossing distance 24 m), while the second was based on crosses between plants within 0.25 m² plots (within-patch crosses). Variance component analyses revealed significant levels of additive variation in leaf size and four measures of leaf shape, indicating a potential for further adaptive change— even after the fixation of a major mutation. Offspring from short-range crosses had a significantly shorter leaf perimeter and were more likely to produce weakly lobed leaves (a recessive character) than progeny representing the longer crossing distances, suggesting that this patchily distributed population is subdivided into patches of related individuals.