Positioning 3 Qualitative Trait Loci on Soybean Molecular Linkage Group E

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In soybean (Glycine max [L.] Merr.), 3 qualitative trait loci (Pb, Y9, and Y17) are located on classical linkage group 14, which corresponds to soybean molecular linkage group (MLG) E. The Pb locus conditions sharp/blunt pubescence tip; the y9 and y17 loci condition green/chlorotic foliage. The gene order is not known. Our objective was to determine the gene order on soybean MLG E of the Pb, Y9, and Y17 loci using previously mapped simple sequence repeat (SSR) markers. Allelism tests between y9 and y17 gave normal green foliage F1 plants, indicating nonallelism. Our F2 data from the allelism test could not distinguish between a 1:1 or a 9:7 ratio. The F2:3 family segregation indicated a very close genetic linkage between the y9 and the y17 loci. Two molecular mapping populations were developed. Population-1 segregated for Pb and y9, and population-2 segregated for Pb and y17. The gene order on soybean MLG E, using SSR markers, was Pb, Y9, and Y17.

Key words: chlorophyll mutants, Glycine max, molecular mapping, qualitative traits, soybean, SSR markers

Chlorophyll deficiencies are expressed during the seedling stage in some soybean mutant lines, late in plant development in other mutants, and throughout the growth period in other mutants. Starner and Hadley (1965) reported that T135 mutant plants contained significantly less of both light-harvesting chlorophyll a and b proteins than the other 5 chlorophyll-deficient mutant lines tested. Eskins et al. (1981) reported similar results with T135 when compared with 3 other chlorophyll-deficient mutant lines. T162 was not included in these 2 reports. The Pb locus is linked to chlorophyll-deficient mutant y9 with 27.3 ± 1.1% recombination (Thorson et al. 1989) and to chlorophyll-deficient mutant y17 with 27.0 ± 4.0% recombination (Devine 1998). The 2 chlorophyll-deficient mutants are nonallelic and are closely linked (Devine 1998), but a recombination value was not determined.

In soybean, about 25% of the mutants in the Soybean Genetic Type Collection are duplicate loci (Palmer et al. 2004). At the molecular level, the soybean genome underwent at least 2 large-scale genome duplication events, and perhaps more (Shoemaker et al. 1996), and might have gene duplication as high as 90% (Schlueter et al. 2004). Thus, y9 and y17 might have resulted from large-scale duplication events or a tandem duplication of a locus conditioning chlorophyll proteins. Our study was not to address this issue but to determine the gene order on soybean MLG E of the Pb, Y9, and Y17 loci, using simple sequence repeat (SSR) markers.

Materials and Methods

Allelism and Recombination of T135 and T162

Genetic Type T135 (female, y9y9 Y17Y17) was crossed with T162 (Y9Y9 y17y17). The F1 plants were advanced to the F2 in the USDA greenhouse at Ames, IA. The F2 seed were placed on germination paper in a growth chamber. The F2 seedlings were transplanted to peat pots in the USDA greenhouse. The green foliage F2 seedlings were transplanted to the Bruner Farm near Ames in summer 2004.
The chlorophyll-deficient F2 seedlings were grown in the USDA greenhouse and produced adequate seed for progeny testing. All the F2:3 families were grown at the Bruner Farm near Ames, IA, in summer 2005. Plant color was recorded for individual F1 and F2 plants. The F2:3 families were classified for plant color on a family basis.

Remnant seed of the only field-grown F2:3 family that was suspected to be double mutants (y9 y9 y17 y17; very weak yellow plants) were planted in the USDA greenhouse in fall 2005. These yellow plants (male parent) were crossed to T135 and T162 plants to confirm their genotype and were screened using SSR markers for MLG E. The F1 plants were recorded for plant color at the Bruner Farm in summer 2006.

Mapping Populations and DNA Extraction

Two segregating F2 populations, Pop-1 and Pop-2, were developed from crosses of Minsoy (PI 27890, Y9Y9 PbPb) × T135 (y9y9 pbpb) and Minsoy (Y17Y17 PbPb) × T162 (y17y17 pbpb), respectively (Table 1). The F1 plants were grown in the USDA greenhouse. The F2 plants were grown at the Bruner Farm in summer 2004. The F2 plants were individually numbered. A young trifoliolate leaf was collected and examined with a compound microscope at x80 magnification to classify pubescence tip as either sharp (Pb) or blunt (pb). The F2:3 families were grown at the Bruner Farm in summer 2005. Plant color was recorded for individual F1 and F2 plants. The F2:3 families were classified for plant color on a family basis. Sixteen plants were sampled from each F2:3 family and examined for pubescence tip morphology and leaf color to determine genotype of each F2 plant. Pop-1 had 300 F2 plants, and Pop-2 had 107 F2 plants (Table 1).

About 3 g young leaves were sampled from each F2 plant and their parental lines for each of the 2 mapping populations. The young leaves were freeze-dried and ground into powders. DNA was extracted from the leaf powders using the CTAB method (Keim et al. 1988).

Bulked Segregant Analysis

Pop-1 and Pop-2 were used in bulked segregant analysis (Michelmore et al. 1991) to identify markers linked to the y9 and y17 regions. Two bulks were made for each population. Bulk 1 (B1) contained DNA aliquots from 10 F2 plants in Pop-1 homozygous for the wild-type phenotype, that is, green foliage. Bulk 2 (B2) contained DNA aliquots from 10 F2 plants in Pop-1 homozygous for y9. Similarly, Bulk 3 (B3) contained DNA aliquots from 10 F2 plants in Pop-2 homozygous for the wild-type phenotype, that is, green foliage. Bulk 4 (B4) contained DNA aliquots from 10 F2 plants in Pop-2 homozygous for y17. SSR analyses were performed with B1, B2, and the 2 parental lines (Minsoy and T135) and B3, B4, and the 2 parental lines (Minsoy and T162), respectively.

SSR Analyses

The F2 plants were screened using SSR markers (Integrated DNA Technologies, Skokie, IL). The Pb and Y9 loci were mapped to MLG E (Song et al. 2004). Therefore, only 20 SSR markers from MLG E were selected for mapping the Pb, Y9, and Y17 loci. SSRs were amplified by polymerase chain reaction (PCR) in 15 μl mixture containing the following: 50 ng genomic DNA, 1 × PCR buffer, 2.00 mM MgCl2, 170 μM of each deoxynucleoside triphosphate, 0.15 μM of each primer (adapted from Song et al. 2004), and 0.25 U Tag DNA polymerase (Bioline, Boston, MA). The PCR was performed in a MJR PTC-100 thermal cycler (MJ Research, Inc., Waltham, MA) for 32 cycles of 45 s at 94 °C,

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>F2 data</th>
<th>F2:3 data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. plants</td>
<td>χ² (9:7)</td>
</tr>
<tr>
<td>Green</td>
<td>109</td>
<td>0.48</td>
</tr>
<tr>
<td>Segregating (Green and chlorotic)</td>
<td>98</td>
<td>0.61</td>
</tr>
<tr>
<td>Chlorotic</td>
<td>1.09</td>
<td>0.30</td>
</tr>
<tr>
<td>Total</td>
<td>207</td>
<td>1.09</td>
</tr>
</tbody>
</table>
Amplified products were resolved and evaluated by electrophoresis on 5% agarose 3:1 gels (AMRESCO, Solon, OH), containing 0.50 μg/ml ethidium bromide, in 1× Tris/borate/ethylenediaminetetraacetic acid (EDTA) buffer or on 8% (w/v) denaturing gels (29:1 acrylamide–bisaacrylamide, 5.6 M urea, and 30% [v/v] formamide) in 1× Tris/acetate/EDTA buffer. The band patterns were checked and photographed under ultraviolet light.

**Linkage Analyses**

The molecular linkage maps for Pop-1 and Pop-2 first were calculated individually with JOINMAP® 3.0 (Van Ooijen **Figure 1.** Soybean MLG E constructed using JOINMAP. (A) y9 Locus from Pop-1, cross Minsoy (PI 27890, Y9 Y9 Pb Pb) × T135 (y9y9 pb pb); dotted line indicates a distance greater than 50 cM; (B) y17 Locus from Pop-2, cross Minsoy (PI 27890, Y17 Y17 Pb Pb) × T162 (y17y17 pb pb); (C) Integrated map of the 2 populations for the y9 and the y17 loci; (D) MLG E from the USDA-ARS-ISU map (Song et al., 2004). Distances shown in centiMorgans (cM).
The thresholds for linking 2 markers together were log of odds 4.0 and recombination value 0.4 (Lander et al. 1987). The genetic distances were converted from recombination rates using the Kosambi map function (Kosambi 1944). Then, the 2 maps were integrated using JOINMAP® 3.0 (Van Ooijen and Voorrips 2001).

**Results and Discussion**

**Allelism Test**

The allelism test between T135 and T162 gave all green foliage F1 plants, suggesting that \( y_9 \) and \( y_{17} \) were nonallelic. This is in agreement with Devine (1998). It was not possible to distinguish between the \( y_9 \) and the \( y_{17} \) phenotypes of these F2 greenhouse–grown plants. Thus, individual F2 plants were classified as either chlorotic (greenhouse grown) or green (field grown). The F2 data were in agreement with a 9:7 or a 1:1 ratio of green:chlorotic plants (Table 2).

All 207 F2 plants were progeny tested as F2:3 families. There were no progeny rows that were all green plants. Because it was not possible to distinguish accurately the number of green and chlorotic plants within each F2:3 progeny row, they were classified only as segregating. In some progeny rows, a clear distinction between all \( y_9 \) plant phenotype and all \( y_{17} \) plant phenotype was possible, but not for all F2:3 progeny rows. Thus, these progenies were classified as all chlorotic. There was one F2:3 progeny row (A05-1531) that consisted of uniform, very weak chlorotic plants. Remnant seed of this putative double-recessive mutant (\( y_9 y_9 y_{17} y_{17} \)) were planted in the USDA greenhouse and crossed as male parent to T135 and T162 plants. The results were 16 F1 green plants with T135 and segregation of green and chlorotic plants in the F2

**Table 3.** Segregation of the \( y_9 \) locus and SSR markers on soybean MLG E in Pop-1 from an F2 population of the cross Minsoy (\( Y_9 Y_9 P_b P_b \)) × T135 (\( y_9 y_9 p_b p_b \))

<table>
<thead>
<tr>
<th>Marker</th>
<th>Composite distance(^a) (cM)</th>
<th>Distance(^b) (cM)</th>
<th>Segregation(^c)</th>
<th>( \chi^2 ) (1:2:1)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Satt575</td>
<td>3.30</td>
<td>2.40</td>
<td>88</td>
<td>134</td>
<td>78</td>
</tr>
<tr>
<td>P_b</td>
<td>13.60</td>
<td>0</td>
<td>86</td>
<td>139</td>
<td>75</td>
</tr>
<tr>
<td>Satt384</td>
<td>19.30</td>
<td>14.96</td>
<td>85</td>
<td>146</td>
<td>69</td>
</tr>
<tr>
<td>Satt691</td>
<td>19.70</td>
<td>15.43</td>
<td>86</td>
<td>144</td>
<td>70</td>
</tr>
<tr>
<td>Satt720</td>
<td>20.80</td>
<td>15.56</td>
<td>85</td>
<td>145</td>
<td>70</td>
</tr>
<tr>
<td>( y_9 )</td>
<td>22.50</td>
<td>27.68</td>
<td>65</td>
<td>166</td>
<td>69</td>
</tr>
<tr>
<td>Satt606</td>
<td>39.77</td>
<td>*</td>
<td>83</td>
<td>139</td>
<td>78</td>
</tr>
<tr>
<td>Satt573</td>
<td>35.79</td>
<td>*</td>
<td>72</td>
<td>158</td>
<td>70</td>
</tr>
<tr>
<td>Satt598</td>
<td>34.20</td>
<td>*</td>
<td>81</td>
<td>151</td>
<td>68</td>
</tr>
<tr>
<td>Satt268</td>
<td>44.27</td>
<td>*</td>
<td>74</td>
<td>137</td>
<td>89</td>
</tr>
</tbody>
</table>

\(^{a}\) SSR markers did not show linkage to \( P_b \) with our data but have been shown to be located on MLG E (Song et al. 2004).

\(^{b}\) Composite distance on MLG E from composite 2003 map at http://soybeanbreederstoollbox.org/.

\(^{c}\) Distance from the \( P_b \) locus.

\(^{d}\) A, homozygous for the allele from Minsoy at this locus; B, homozygous for the allele from T135 at this locus; H, heterozygous for the allele at this locus.

**Table 4.** Segregation of the \( y_{17} \) locus and SSR markers on soybean MLG E in Pop-2 from an F2 population of the cross Minsoy (\( Y_{17} Y_{17} P_b P_b \)) × T162 (\( y_{17} y_{17} p_b p_b \))

<table>
<thead>
<tr>
<th>Marker</th>
<th>Composite distance(^a) (cM)</th>
<th>Distance(^b) (cM)</th>
<th>Segregation(^c)</th>
<th>( \chi^2 ) (1:2:1)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Satt575</td>
<td>3.30</td>
<td>6.95</td>
<td>33</td>
<td>52</td>
<td>22</td>
</tr>
<tr>
<td>P_b</td>
<td>13.60</td>
<td>0</td>
<td>32</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>Satt691</td>
<td>19.70</td>
<td>16.98</td>
<td>27</td>
<td>56</td>
<td>24</td>
</tr>
<tr>
<td>Satt720</td>
<td>20.80</td>
<td>17.44</td>
<td>27</td>
<td>55</td>
<td>25</td>
</tr>
<tr>
<td>Satt384</td>
<td>19.30</td>
<td>18.21</td>
<td>28</td>
<td>55</td>
<td>24</td>
</tr>
<tr>
<td>Satt651</td>
<td>32.10</td>
<td>26.85</td>
<td>25</td>
<td>61</td>
<td>20</td>
</tr>
<tr>
<td>( y_{17} )</td>
<td>27.00</td>
<td>30.31</td>
<td>25</td>
<td>61</td>
<td>21</td>
</tr>
<tr>
<td>Satt573</td>
<td>35.79</td>
<td>59.91</td>
<td>31</td>
<td>53</td>
<td>23</td>
</tr>
<tr>
<td>Satt598</td>
<td>34.20</td>
<td>63.06</td>
<td>32</td>
<td>46</td>
<td>29</td>
</tr>
<tr>
<td>Satt606</td>
<td>39.77</td>
<td>67.91</td>
<td>28</td>
<td>53</td>
<td>26</td>
</tr>
<tr>
<td>Satt268</td>
<td>44.27</td>
<td>76.20</td>
<td>27</td>
<td>55</td>
<td>25</td>
</tr>
</tbody>
</table>

\(^{a}\) Composite distance on MLG E from composite 2003 map at http://soybeanbreederstoollbox.org/.

\(^{b}\) Distance from the \( P_b \) locus.

\(^{c}\) A, homozygous for the allele from Minsoy at this locus; B, homozygous for the allele from T162 at this locus; H, heterozygous for the allele at this locus.
generation. The results were 13 F1 chlorotic plants with T162 and all chlorotic F2 plants. Furthermore, all F2 plants had SSR patterns in agreement with T162. These results do not support our view that F2,3 family A05-1531 was the double-recessive genotype. The very weak chlorotic F2,3 plants were Y9Y9 y17y17. Other genes, genetic interactions, environment, and genotype × environment interactions contributed to this extreme phenotype. Thus, y9 and y17 are closely linked to each other, which is in agreement with Devine (1998) (Table 2).

Genetic Distances between the \( Pb \) and \( Y9 \) (Pop-1) and the \( Pb \) and \( Y17 \) (Pop-2) Loci

In Pop-1, the \( Pb \) and \( Y9 \) loci were 27.68 cm apart (Figure 1A), and in Pop-2, the \( Pb \) and \( Y17 \) loci were 30.31 cm apart (Figure 1B). These values are in agreement with Thorson et al. (1989) of 27.3 ± 1.1% for \( Pb-Y9 \) and with Devine (1998) of 27.0 ± 4.0% for \( Pb-Y17 \).

Molecular Mapping of the \( y9 \) (Pop-1) and the \( y17 \) (Pop-2) Loci

The molecular screening of the 2 parents and the contrasted bulks of Pop-1 for each SSR marker of MLG E indicated tight linkage between the \( y9 \) locus and the SSR marker Satt720. In Pop-2, tight linkage of \( y17 \) and SSR marker Satt651 was shown for MLG E.

To construct a detailed map for T135 (\( y9 \)) and T162 (\( y17 \)), additional polymorphic markers on MLG E were used. These SSR markers for Pop-1 were Satt575, Satt691, Satt384, Satt606, Satt573, Satt598, and Satt268 (Table 3). For Pop-2, the SSR markers were Satt575, Satt691, Satt720, Satt384, Satt573, Satt598, Satt606, and Satt268 (Table 4). For Pop-1, the \( y9 \) locus was located at 12.25 cm from Satt691 and Satt720 (Figure 1A). For Pop-2, the \( y17 \) locus was located at 3.46 cm from Satt651 and 29.60 cm from Satt573 (Figure 1B).

A map constructed to integrate Pop-1 and Pop-2, using JOINMAP® 3.0 (Van Ooijen and Voorrips 2001), showed that \( y9 \) and \( y17 \) were positioned between markers Satt575 and Satt573 (Figure 1C). The gene order was \( Pb, y9, y17 \), and \( Pb \).

Pop-1 (Figure 1A) and Pop-2 (Figure 1B) and the integrated map of Pop-1 and Pop-2 (Figure 1C) showed similar molecular marker order when compared with the USDA-ARS-ISU molecular map of Song et al. (2004) for MLG E (Figure 1D). However, large map distances between \( y9 \) and Satt606 and between \( y17 \) and Satt573 were noticed. There was no segregation distortion of markers in this chromosome region (Tables 3 and 4). An explanation awaits fine-structure mapping in this region.

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