New Genes, \textit{stp} and \textit{stp}^{hbw}, for Flower and Seedcoat Pattern in Common Bean

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Abstract. The inheritance of novel flower and seedcoat patterns was studied in three parental materials: PI 390775 and ‘Springwater Half Runner’ (SHR), which have patterned flower and seedcoat colors, and 5-593, a Florida dry bean breeding line with unpatterned purple flowers and seeds. Using crosses between 5-593 and the other two parents, an analysis of \textit{F}_1, \textit{F}_2, backcross \textit{F}_1, and backcross \textit{F}_2 data demonstrated that a single recessive allele in each of the patterned parents controlled flower and seedcoat pattern. Genetic tester stocks were used to demonstrate that the recessive gene for patterning in PI 390775 was nonallelic with \textit{C}, \textit{T}, and \textit{Mar}, the three genes previously known to control seedcoat pattern in common bean. An allelism test between the recessive pattern genes from PI 390775 and SHR demonstrated that they were allelic and that the gene from SHR was dominant. The gene symbols \textit{stp} (for the gene from PI 390775) and \textit{stp}^{hbw} (for the dominant gene from SHR) are proposed, where \textit{stp} stands for stippled seedcoat pattern and the superscript letters \textit{hbw} stand for half banner white.

Prakken (1970) wrote a comprehensive, critical review of all previous work on the genetics of seedcoat colors of common bean (\textit{Phaseolus vulgaris} \textit{L.}), except for the red colors. He described two types of patterned seedcoats: the partly colored patterns that express only with the \textit{t} gene and a very large allelic series of patterns that are controlled at the complex \textit{C} locus. Prakken (1970) omitted reference to seedcoat patterns of the corona and margo types, but these have been described well recently by Bassett (1994a, 1995) and Leakey (1988). Bassett (1995) reported that the corona character of Lamprecht (1933) is controlled as a pleiotropic effect of the \textit{v}^{lae} allele and is not controlled by an independent \textit{Cor} locus.

During a visit to the plant introduction (PI) station at Pullman, Wash., in 1989, I saw a seed sample of PI 390775 on display. My intuitive judgment was that the seedcoat pattern of PI 390775 might be controlled by a gene other than the previously reported effects at \textit{T} and \textit{C} (Leakey, 1988; Prakken, 1970). Similarly, having obtained seed of ‘Springwater Half Runner’ I suspected that the inheritance of its unusual seedcoat pattern was not controlled by either \textit{T} or \textit{C}. The research reported herein describes inheritance studies done with two common bean materials: PI 390775 and ‘Springwater Half Runner’.

Materials and Methods

The accession PI 390775 was selected for study based on its seedcoat appearance. It had an unusual seedcoat pattern that was not in the published literature, viz., it is covered with small colored dots (stippling) on a cartridge buff (beige) background. In some genetic backgrounds, the dotting coalesces to produce a fine marbling pattern (Fig. 1). The range of variation, observed in materials that have the stippled character in \textit{BC}_1 to 5-593, is illustrated by the extremes of the range (Fig. 1). PI 390775 also has
Florida dry bean breeding line 5-593 was used as the recurrent parent in the study. The line has a determinate habit, purple banner petals are white or nearly white (Fig. 2). The lower half of the seedcoat genotype of 5-593 is P(Cr)D J G B V Rk (Bassett, 1994b; Prakken, 1970, 1972).

A program to develop genetic tester stocks, each with one or more recessive marker genes for seedcoat color or pattern backcrossed into the recurrent parent 5-593, was initiated in 1985. Details of the backcross procedure are described by Bassett (1994c). The tester stock, r BC2 5-593, has white flowers due to r (derived from ‘Early Wax’), but self-colored black seeds due to having the genes in 5-593 that fully extend dark pattern color in the presence of r (Bassett and Blom, 1991). The tester stock, c* BC2 5-593, was derived from ‘Prakken 75’ an all-recessive tester stock obtained from R. Prakken of the Netherlands (Bassett, 1994d). The tester stock, mar BC 5-593, was derived from Lamprecht line V0553 (now PI 527753) and has dull, dark purple seeds (if grown in greenhouse) with a black hilum ring and nearly white corona (Bassett, 1994a). If the stock is grown in the field, the margo region is dark purple, but the remainder of the seedcoat is brown.

Crosses with PI 390775. The cross PI 390775 x 5-593 was made in 1989 and the F2 progeny were grown in the field in 1991. All F2 plants with indeterminate habit were rogued from the plot at the time the determinate segregants began flowering. Data were taken on flower and seedcoat color in the F2 and for all determinate F2 progeny. Selection was made in the F2 for plants with the flower and seedcoat color and color pattern of PI 390775 but the determinate habit of 5-593. Plants with the stippled seeds and patterned flower color were labeled STP plants.

An F2 STP plant was progeny tested in the greenhouse and an F3 progeny plant was used in the backcross F2 STP x 5-593. The F2 progeny were planted in the field in 1991. Data were taken on flower and seedcoat color and color pattern in the F1 and F2. Selection was made in the BC1-F1 for STP plants. Remnant BC1-F2 seed were planted in the greenhouse in 1993 and the BC1-F2 progenies were planted in the field in separate plots in 1994. The same type of data were taken as in the BC1-F2.

A single BC1-F2 STP plant was progeny tested in the greenhouse and a BC1-F3 STP progeny plant was selected for use in a series of test crosses for allelism: BC1-F3 STP x c* BC2 5-593, r BC2 5-593 all black x BC1-F3 STP, and BC1-F3 STP x mar BC 5-593. The F2 of the first cross was planted in the field in 1992 and the F2 of the latter two crosses were planted in the field in 1993. Data were taken on flower and seedcoat color and color pattern in the F1 and F2 progenies.

Crosses with SHR. In 1990, the cross SHR x 5-593 was made, and the F2 was planted in the field in 1991. Data were taken on flower and seedcoat color and color pattern in the F1 and F2. Selection was made in the F2 for plants with the flower and seedcoat color patterns of SHR, but having the determinate habit of 5-593.

An SHR-type F2 selection was progeny tested in the greenhouse

![Fig. 2. The flower on the left illustrates the color pattern controlled by stp<sup>un</sup>; the flower on the right illustrates the color pattern controlled by stp.](image)

![Fig. 3. Drawings of seeds with the stippled pattern controlled by stp<sup>bstp</sup>](image)

Table 1. Segregation for flower and seedcoat pattern in the F2 from the following crosses: 1) PI 390775 x 5-593 and 2) F2 STP x 5-593.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Unpatterned flowers and seedcoats&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Patterned flowers and seedcoats&lt;sup&gt;b&lt;/sup&gt;</th>
<th>χ&lt;sup&gt;2&lt;/sup&gt;</th>
<th>P value</th>
<th>3:1 value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>57</td>
<td>9</td>
<td>4.546</td>
<td>0.033</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>105</td>
<td>27</td>
<td>1.455</td>
<td>0.22</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>For cross no. 1, the flowers were bishops violet and the seedcoats were either black or unpatterned dark purple (the latter probably due to P C J G b V); for cross no. 2, the flowers were bishops violet and the seedcoats were black.

<sup>b</sup>The flowers had white banner petals with violet tips and white wing petals with 2- to 3-mm violet borders; the seedcoats had a stippled pattern in black dots on cartridge buff.

In 1989, ‘Springwater Half Runner’ (SHR) seeds were obtained from the Ferry Morse Seed Co. The seedcoat has a pattern similar to that of PI 390775 in that most of the seedcoat is stippled, but the stippling patterns are less variable than that of the PI (Fig. 3). The dark pattern color is solid in the hilum ring and corona region. The immediate micropyle area is stippled, but a solid dark pattern color surrounds the micropyle and continues over most of the micropyle end of the seed. The pattern is similar to many seed types with the margo (mar) character, except that the remainder is stippled instead of solidly colored in light pattern color. The flower color pattern has not been reported in the literature. The lower half of the banner petal is white except for a 2- to 3-mm violet border (Fig. 2, flower on right).

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and an SHR-type F₁ progeny plant was used in the backcross 5-593 × F₁ SHR. The F₂ was planted in the field in 1992. Data were taken on flower and seedcoat color and color pattern in the F₁ and F₂. Selection was made in the BC₁-F₂ from 5-593 × F₁ SHR for plants with the flower and seedcoat traits of SHR.

Remnant BC₁-F₂ seed were planted in the greenhouse in 1993 and the BC₁-F₂ progenies were planted in the field in separate plots in 1994. The same type of data were taken as with the BC₁-F₂.

A single BC₁-F₂ SHR selection was progeny tested in the greenhouse in Fall 1992 and a selection for BC₁-F₂ SHR was made. A final allelism test was made by crossing BC₁-F₂ SHR × BC₂-F₁ STP, the latter parent being a self progeny from the BC₁-F₂ STP parent plant described above. The F₂ was planted in the field in 1993. Data were taken on flower and seedcoat color and color pattern in the F₁ and F₂.

### Results and Discussion

Crosses with PI 390775. The F₁ plants from the cross PI 390775 × 5-593 had bishops-violent flowers and unpatterned black seedcoats (data not shown). The F₂ segregated for indeterminate and determinate plant habit. Among the determinate segregants it was noted that the patterned flowers and patterned seedcoats always cosegregated, but there was a significant deviation from the expected 3:1 segregation for unpatterned to patterned plants, respectively, due to a shortage of the latter (Table 1). Because further investigation supported a single gene hypothesis, other genetic models were not tested. The F₂, selection with patterned flowers and seeds was demonstrated to be true breeding for those traits in an F₂ progeny test grown in the greenhouse (12 plants). The hypothesis formulated was that a single recessive gene controls the seedcoat stippling and the flower color patterning. The patterning trait was given the label STP.

The F₁ plants from the backcross F₁, STP × 5-593 had bishops-violent flowers and black seeds without any pattern in either organ. The BC₁-F₂ segregated for only two phenotypic classes, unpatterned flowers and seeds or patterned flowers and seeds, and a satisfactory goodness of fit to a 3:1 segregation ratio was observed for the classes, respectively (Table 1). Thus, there was further support that a single locus controls patterning in flowers and seedcoats.

To critically test the single-gene hypothesis, an F₁ progeny test was conducted. The F₁ progeny of all the stippled F₂ parents were true breeding. Segregating F₂ progenies from unpatterned F₂ parents fit a 3:1 ratio of unpatterned to stippled, respectively (Table 2). There was a 1:2:1 ratio for true-breeding stippled progenies, segregating progenies, and true-breeding unpatterned progenies, respectively (Table 2). Thus, I have conclusively demonstrated that a single gene (tentatively given the symbol stp) controls the STP trait, but it is possible that the STP locus is an unreported allele at the C, T, or Mar loci, which have been previously shown to control seedcoat pattern (Leakey, 1988; Prakken, 1970).

A single BC₁-F₂ STP selection was true breeding for the STP trait in a BC₁-F₁ progeny test (12 plants). Three allelism tests were conducted with a single BC₁-F₂ STP progeny plant. The F₁ plants from the cross BC₁-F₂, STP × c° BC₂, 5-593 had unpatterned bishops-violent flowers and black seeds with very tiny cartridge buff flecks typical of Cle°, i.e., the phenotype was nonallelic (data not shown). The gene symbol stp was tentatively adopted for the locus controlling the stippled seedcoat phenotype. The hypothesis of nonallelism was confirmed in the F₁, in which independent segregation at C and Stp was observed: 151 C Stp, 59 C stp, 45 c° Stp, 13 c° stp (χ² for 9:3:3:1 = 2.912, P = 0.41). The F₁ plants from the allelism test cross STP × 5-593 all black × BC₁-F₂ STP had unpatterned bishops-violent flowers and self-colored black seeds (data not shown), a nonallelic phenotype. The hypothesis of nonallelism was confirmed in the F₂, in which independent segregation at T and Stp was observed: 171 T Stp, 53 T stp, 38 t Stp, 13 t stp (χ² for 9:3:3:1 = 6.348, P = 0.10). The F₁ plants from the allelism test cross BC₁-F₂ STP × mar BC₂, 5-593 had unpatterned bishops-violent flowers and unpatterned black seeds, a nonallelic phenotype.

### Table 2. Segregation for flower and seedcoat pattern in the F₂ progenies from 27 randomly selected F₂ parents from the cross F₃ STP × 5-593.

<table>
<thead>
<tr>
<th>Genotype of F₂ parent</th>
<th>Unpatterned flowers and seedcoats</th>
<th>Patterned flowers and seedcoats</th>
<th>χ²</th>
<th>P value</th>
<th>3:1 value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stp/Stp</td>
<td>43</td>
<td>11</td>
<td>0.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Stp/Stp</td>
<td>48</td>
<td>12</td>
<td>0.029</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>Stp/Stp</td>
<td>40</td>
<td>10</td>
<td>0.121</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>Stp/Stp</td>
<td>38</td>
<td>8</td>
<td>1.252</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>Stp/Stp</td>
<td>44</td>
<td>15</td>
<td>1.939</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Stp/Stp</td>
<td>42</td>
<td>10</td>
<td>0.261</td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td>Stp/Stp</td>
<td>49</td>
<td>9</td>
<td>0.077</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>Stp/Stp</td>
<td>40</td>
<td>13</td>
<td>0.485</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Stp/Stp</td>
<td>41</td>
<td>16</td>
<td>2.348</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Stp/Stp</td>
<td>33</td>
<td>13</td>
<td>0.261</td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td>Stp/Stp</td>
<td>34</td>
<td>17</td>
<td>1.889</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>361</td>
<td>134</td>
<td>1.132</td>
<td>0.29</td>
<td></td>
</tr>
</tbody>
</table>

*For six true breeding STP parents, 11 segregating normal parents, and 10 true breeding normal parents, χ² (1:2:1) = 2.111, P = 0.35.

The flowers were bishops violet and the seedcoats were black.

The flowers had white banner petals with violet tips and white wing petals with 2- to 3-mm violet borders; the seedcoats had a stippled pattern in black dots on cartridge buff.

The heterogeneity of χ² at 10 df is 7.53; P = 0.75 – 0.50.

The flowers were bishops violet except that the lower half of the banner was white, and the seedcoats had the largely stippled pattern of the SHR parent.

### Table 3. Segregation for flower and seedcoat pattern in the F₂ from two crosses: 1) ‘Springwater Half Runner’ (SHR) × 5-593 and 2) F₁ SHR × 5-593.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Unpatterned flowers and seedcoats</th>
<th>Patterned flowers and seedcoats</th>
<th>χ²</th>
<th>P value</th>
<th>3:1 value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>90</td>
<td>29</td>
<td>0.025</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>105</td>
<td>27</td>
<td>1.455</td>
<td>0.23</td>
<td></td>
</tr>
</tbody>
</table>

*The flowers were bishops violet and the seedcoats were black.

The flowers were bishops violet except that the lower half of the banner petal was white, and the seedcoats had the largely stippled pattern of the SHR parent.
had unpatterned purple (bishops violet) flowers and unpatterned black seeds (data not shown). In the F3 progeny, patterned flowers and patterned seedcoats always cosegregated (Table 3). The data fit to a 3:1 segregation ratio for the parental phenotypes of 5-593 and SHR, respectively, with respect to flowers and seedcoats (Table 3), and it was hypothesized that a single recessive gene controls the stippled seedcoats and patterned flowers. The combination of flower and seedcoat traits was given the label SHR. The F3 selection for SHR flower and seedcoat type was true breeding in the greenhouse F1 progeny test (10 plants).

The F1 plants from the backcross 5-593 x F1 SHR had unpatterned bishops violet flowers and unpatterned black seeds (data not shown). The BC1-F1 segregated for only two phenotypic classes, unpatterned flowers and seeds or patterned flowers and seeds, and the data fit to a 3:1 segregation ratio was observed for the classes, respectively (Table 3). Those data further supported the hypothesis that a single locus controls flower and seedcoat pattern.

To test the single gene hypothesis critically, an F2 progeny test was conducted, and the gene symbol stp

\[ \chi^2 = 0.001. \]

Using the orthogonal contrasts of Mather and Pilger (1957),

\[ \chi^2 = 0.50 - 0.25. \]

The BC1-F2 segregated for only two phenotypic classes: either the flower and seedcoat pattern of PI 390775 (Table 5). The data fit to a 3:1 segregation ratio was observed for the classes, respectively (Table 5). Thus, I have conclusively demonstrated that a single gene controls the SHR trait, but it is not known whether there is an allelic relationship with a previously published gene. Because there was a certain similarity between the stp marker from PI 390775 and the marker gene in the SHR parent, an allelism test was conducted.

The BC1-F2 plant from the cross BC1-F1 SHR x BC1-F1 STP had the flower and seedcoat pattern of the SHR parent, which is an allelic interaction between the recessive marker gene in SHR and the stp gene from PI 390775 (data not shown). Thus, it appears that the allele in the SHR parent is dominant. The F1 from the allelism test segregated for only two phenotypic classes: either the flower and seedcoat pattern of the SHR parent or the flower and seedcoat pattern of PI 390775 (Table 5). The data fit to a 3:1 segregation ratio for the two classes, respectively (Table 5). Thus, the results presented above have confirmed that the marker genes for flower and seedcoat pattern in the SHR parent and PI 390775 are allelic and that the allele from SHR is dominant. For the dominant allele, the gene symbol stp

\[ \chi^2 = 0.001. \]

The BC1-F2 plant had the flower and seedcoat pattern that was observed, although a disturbed segregation occurred. The observed data were 187 Mar stp, 91 Mar stp, 79 Mar stp, 38 mar stp (\( \chi^2 \) for 9:3:3:1 = 16.954, \( P = 0.001 \)). Using the orthogonal contrasts of Mather (1957),

\[ \chi^2_{MR} = 4.497, P = 0.034; \]

\[ \chi^2_{MC} = 12.355, P < 0.001; \]

\[ \chi^2_{L} = 0.102, P = 0.75. \]

The disturbance was due to an excess of the margo and stippled segregants, but no explanation for the highly significant deviation from expected is apparent. On the basis of the above allelism tests, it is clear that the STP character is not controlled by a new allele at any of the three known genes for seedcoat patterning, viz., C, T, or Mar. Thus, it is proposed that the gene symbol stp be formally given to the recessive allele at a new locus for stippled seedcoat pattern in common bean, now identified in PI 390775.

TABLE 4. Segregation for flower and seedcoat pattern in the F3 progenies from 32 randomly selected F2 parents from the cross F3 SHR x 5-593.

<table>
<thead>
<tr>
<th>Genotype of F3 parent</th>
<th>Unpatterned flowers and seedcoats</th>
<th>Patterned flowers and seedcoats</th>
<th>( \chi^2 )</th>
<th>P value</th>
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</thead>
</table>
| Stp

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Bassett, M.J. 1994c. The griseoalbus (gray-white) seedcoat color is controlled by an allele (p<sup>gr</sup>) at the P locus in common bean. HortScience 29:1178–1179.