A New Allele, $t^{cf}$, at the $T$ Locus for Partly Colored Seedcoats in Common Bean

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Abstract. Common bean (Phaseolus vulgaris L.) plants with partly colored seeds and colored flowers were derived from PI 507984 in two genetic tester stocks, '2-points $t^{cf}$ BC$_1$ 5-593' and '2-points $t^{cf}$ BC$_2$ 5-593'. These stocks were produced by backcrossing to the recurrent parent, Florida dry bean breeding line 5-593, which has black self-colored seeds and purple flowers due to the genotype $T^P V$. The crosses '2-points $t^{cf}$ BC$_1$ 5-593' x 5-593 and '2-points $t^{cf}$ BC$_2$ 5-593' x 5-593 produced $F_2$ populations in which all plants had colored flowers. Those results, when considered with previously published work, do not support the previously reported hypothesis that the genes $T$ and $F_c$ interact with $t$ to produce partly colored seedcoats and flower color restoration with $t$. The crosses '2-points $t^{cf}$ BC$_1$ 5-593' x self-colored $t$ BC$_1$ 5-593' and '2-points $t^{cf}$ BC$_2$ 5-593' x 'minimus' BC$_2$ 5-593' produced $F_2$ populations that segregated 3:1 for colored: white flowers, respectively. Those results are consistent with the revised hypothesis that $t^{cf}$ can produce partly colored seedcoats without affecting flower color. The RAPD marker OM19, which is linked in repulsion to $T$, was used with the $F_2$ populations from the crosses '2-points $t^{cf}$ BC$_1$ 5-593' x 5-593 and '2-points $t^{cf}$ BC$_2$ 5-593' x 'minimus' BC$_2$ 5-593' and established that the $t^{cf}$ gene from PI 507984 is either an allele at $T$ or tightly linked to $T$. $F_3$ data from the cross '2-points $t^{cf}$ BC$_1$ 5-593' x 5-593 also support the $t^{cf}$ hypothesis. On the basis of the above experiments, the gene symbol $t^{cf}$ is proposed for an allele at $T$ that pleiotropically produces partly colored seeds and colored flowers.

Materials and Methods

Development of genetic tester stocks. Florida dry bean breeding line 5-593 has the black seedcoat genotype $T^P [C r] D J G B V Rk$ (Bassett, 1994a) and has been used for many years as a recurrent parent in a program to produce genetic tester stocks. These stocks contain one or more recessive marker genes backcrossed into 5-593 with selection against all other marker genes (Bassett, 1994b). Line 5-593 has colored (purple) flowers due to the genotype $T^P V$ (Leakey, 1988).

Genetic tester stock 'self-colored $t$ BC$_1$ 5-593' has the standard recessive $t$ described by Leakey (1988). This stock was developed during the study of the unusual genotype for white seedcoats found in 'Early Wax' (Bassett and Blom, 1991) and has self-colored black seeds and white flowers due to genotype $t P V$. The self-colored seedcoat is due to selection for $Z$ and $l$ (Schreiber, 1940) and an unidentified recessive gene that converts minimus (same as ‘expansa’ of Schreiber, 1940) into self-colored (Bassett, unpublished data).

Genetic tester stock 'minimus $t$ BC$_2$ 5-593’ was developed from the cross 'self-colored $t$ BC$_1$ 5-593' x 5-593. The partly colored seedcoat pattern is not only minimus but has fibula arcs (Lamprecht, 1934, see his Fig. 23), which are white bows on either side of the hilum just outside the hilum ring (Fig. 1).

PI 507984 was used to develop the genetic tester stock '2-points $t^{cf}$ BC$_1$ 5-593', where $t^{cf}$ is a proposed symbol for a new allele at $T$ that controls the partly colored seedcoat function of $t$ but does not convert colored flowers to white. The cross PI 507984 x 'self-colored $t$ BC$_2$ 5-593' produced a true breeding $F_3$ selection designated $F_3$, '2-points bishops violet (B.V.) flowers’ in 1992. The partly colored 2-points seedcoat pattern is illustrated (Fig. 2). The subsequent cross $F_3$, '2-points B.V. flowers’ x 5-593 produced the true breeding selection '2-points $t^{cf}$ BC$_2$ 5-593’ in 1993. An additional backcross to 5-593, viz., '2-points $t^{cf}$ BC$_2$ 5-593' x 5-593 produced

![Fig. 1. The left seed (ventral view): fibula arcs on either side of the hilum (just outside the hilum ring). The middle seed; minimus pattern limited to only white irregular shaped blotches on the bottom right side of the seed. The right seed; classic minimus pattern.](image-url)
The crosses ‘2-points’ were made, and the F2 populations were planted in the field in 1994. The crosses ‘2-points’ were for flower color and seedcoat pattern. Because F2 seed
the presence of the marker (Brady et al., 1998). The plants were also

Discussion

Results and Discussion

Fig. 2. The left seed (ventral view); 2-points pattern, i.e., two black dots at the

Molecular test of linkage of r4 and t. Although the data from all four F2 populations are consistent with the new hypothesis that r4 (a new allele at T) controls the expression of partly colored seeds on plants with colored flowers, they are also consistent with an

Electrophoregrams were scored for each F2 plants from the crosses ‘2-points’ x ‘self-colored’ and ‘self-colored’ x ‘minimus’ in 1994. The crosses ‘2-points’ x ‘self-colored’ and ‘self-colored’ x ‘minimus’ were made, and the F2 populations were planted in the field in 1994. The crosses ‘2-points’ x ‘self-colored’ x ‘minimus’ x ‘minimus’ were made, and the F2 populations were planted in the field in 1995. Flower color and seedcoat phenotype (self- vs. partly colored) data were collected for each cross.

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The data on seedcoat pattern (especially for crosses 2 and 4 in Table 1) were not presented because the flower color data show perfectly the segregation for the two recessive alleles at T. Furthermore, presentation of the seedcoat pattern data would open up an entirely new requirement for demonstration, viz., the difference in pattern expression given by the two genes, r4 and t (data not shown). The present data are not sufficient to support a complete genetic hypothesis for the 2-points pattern, which only expresses with r4 (data not shown).

In earlier work, the r allele from ‘Early Wax’ was crossed to 5-593 and later repeatedly backcrossed into 5-593. During each cycle of the conversion, a large number of F2 progenies were analyzed (Bassett and Blom, 1991). In each F2, selection was made for plants with the genotype t z l, and F2 plants were used to make the next backcross to 5-593 (Bassett, 1997). Plants with partly colored seeds and colored flowers were never observed among the F2 progeny, although ample opportunity existed for such recombinants. Therefore, 5-593 cannot have genotype Fcr Fcr-2, and the former hypothesis presented by Bassett (1993) is incorrect. When Bassett (1993) published the hypothesis, it was thought that 5-593 probably carried only one of the two dominant genes (Fcr and Fcr-2) needed to convert flower color. This hypothesis is also not supported by the data.

For the cross ‘2-points’ x ‘self-colored’ and ‘self-colored’ x ‘minimus’ in 1994. The crosses ‘2-points’ x ‘self-colored’ x ‘minimus’ x ‘minimus’ were made, and the F2 populations were planted in the field in 1995. Flower color and seedcoat phenotype (self- vs. partly colored) data were collected for each cross.

Table 1. Segregation for flower color in the F2 from four crosses: 1) 2-points r4 BC2, 5-593 x 5-593, 2) 2-points r4 BC2, 5-593 x self-colored t BC2, 5-593, 3) 2-points r4 BC2, 5-593 x 5-593, and 4) 2-points r4 BC2, 5-593 x minimus t BC2, 5-593.

<table>
<thead>
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<th>Cross no.</th>
<th>Flower color tested</th>
<th>Ratio</th>
<th>χ²</th>
<th>P</th>
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<tr>
<td>1</td>
<td>Colored</td>
<td>White</td>
<td>162</td>
<td>0.25</td>
</tr>
<tr>
<td>2</td>
<td>70</td>
<td>30</td>
<td>3:1</td>
<td>1.33</td>
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<td></td>
<td>15:1</td>
<td>96.30</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>420</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>4</td>
<td>161</td>
<td>48</td>
<td>3:1</td>
<td>0.46</td>
</tr>
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<td></td>
<td>15:1</td>
<td>99.70</td>
<td>&lt;0.001</td>
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Segregation ratio expected from selfing an F1 parent with genotype r4 t.

Segregation ratio expected from selfing an F1 parent with genotype T Fcr Fcr-2.

Segregation ratio expected from selfing an F1 parent with either of two
genotypes, T Fcr Fcr-2 or T Fcr Fcr-2.
Table 2. Segregation for seedcoat pattern and the RAPD marker OM19<sub>460</sub> in the F<sub>2</sub> and F<sub>3</sub> progeny of the cross ‘2-points<sup>r</sup> BC<sub>2</sub> 5-593’ × 5-593.

<table>
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<th>No. of observations&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Phenotypic classes</th>
<th>Segregation data of F&lt;sub&gt;2&lt;/sub&gt;</th>
<th>Segregation data of F&lt;sub&gt;3&lt;/sub&gt;</th>
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<tr>
<td></td>
<td>Seedcoat pattern</td>
<td>OM19&lt;sub&gt;460&lt;/sub&gt; marker</td>
<td>Genetic hypothesis</td>
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<tr>
<td>19</td>
<td>Self-colored</td>
<td>Absent</td>
<td>T/T</td>
</tr>
<tr>
<td>42</td>
<td>Self-colored</td>
<td>Present</td>
<td>T/T&lt;sup&gt;r&lt;/sup&gt;</td>
</tr>
<tr>
<td>17</td>
<td>Partly colored</td>
<td>Present</td>
<td>T/t&lt;sup&gt;r&lt;/sup&gt;</td>
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<td>z l&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>t z&lt;sup&gt;d&lt;/sup&gt;</td>
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</table>

<sup>a</sup>For the data 19, 42, 17 for genotypes T/T, T/t<sup>r</sup>, and T/t<sup>r</sup>/t<sup>r</sup>, respectively, the χ<sup>2</sup> (1:2:1) = 0.564, P = 0.75.

<sup>b</sup>For the data 232 self-colored and 75 partly colored totaled over 30 progenies, the χ<sup>2</sup> (3:1) = 0.053, P = 0.82. The mean F<sub>3</sub> progeny size was 10.23, with a range of 14 to 3 plants per progeny.

<sup>c</sup>For the data 16 and 1 the χ<sup>2</sup> (15:1) = 0.004, P = 0.95, based on the hypothesis that true breeding self-colored lines carry t<sup>r</sup> T fib.

T<sub>T</sub>, controls the phenotype. A molecular marker, OM19<sub>460</sub> that is linked in repulsion phase with T<sub>T</sub> (Brady et al., 1998), was employed to distinguish between these two possibilities.

Seventy-eight F<sub>2</sub> plants from the cross ‘2-points<sup>r</sup> BC<sub>2</sub> 5-593’ × 5-593 were grown, and the seedcoat pattern types were classified as self-colored or partly colored (Table 2). Based on seedcoat pattern classification in F<sub>2</sub> and F<sub>3</sub> and the marker data observed in F<sub>2</sub> (Table 2), we hypothesize that all F<sub>2</sub> plants fall into one of three genotypic classes: T/T, T/t<sup>r</sup>, or T/t<sup>r</sup>/t<sup>r</sup>. Nineteen T/T F<sub>2</sub> plants were self-colored and lacked the OM19<sub>460</sub> marker (Table 2). Forty-two F<sub>2</sub> plants were self-colored and contained the OM19<sub>460</sub> marker (Table 2). The expected genotype of these plants was T/T<sup>r</sup>. As expected, the F<sub>3</sub> progeny of 30 of these plants segregated 3:1 for self-colored to partly colored, respectively, and their genotypes were confirmed to be T/T<sup>r</sup>. All the F<sub>2</sub> progeny of the remaining 12 F<sub>2</sub> plants were self-colored rather than segregating for self- and partly colored seeds. This is not unexpected because, as Bassett and Blom (1991) observed, the epistatic effects of a gene(s) similar to that found in the background of this cross can prevent expression of partly colored waxed seeds in individuals with a t<sup>r</sup> T<sub>T</sub> genotype. Seventeen F<sub>2</sub> plants were partly colored and contained the OM19<sub>460</sub> marker (Table 2). The expected genotype of these plants was T/t<sup>r</sup><sup>r</sup>. Thus, the expected segregation ratio of 1:2:1 for the F<sub>2</sub> genotypic classes T/T, T/t<sup>r</sup><sup>r</sup>, and T/t<sup>r</sup>/t<sup>r</sup>, respectively, gave a satisfactory fit to the observed data (Table 2).

One of the 17 F<sub>2</sub> plants segregated partly colored (Table 2). A tentative genetic hypothesis to explain this result can be derived from the incomplete genetic hypothesis developed for the 2-points pattern, which is T<sup>r</sup> z<sup>f</sup> plus a fourth factor (locus unknown) to express 2-points (Bassett, unpublished data). Also, the parents differ at a hypothetical fibula locus, where 5-593 carries Fib and 2-points carries fib (Bassett, unpublished data). The Fib gene strongly restricts the partly colored seedcoat pattern expansa, virgarcus, and Anasazi (Bassett, unpublished data). The genotype t<sup>r</sup> z<sup>f</sup> produces pure white seed (Bassett, 1997), and the genotype t<sup>r</sup> z<sup>f</sup> T<sub>T</sub> apparently does also (Bassett, unpublished data). Thus, the cross ‘2-points<sup>r</sup> BC<sub>2</sub> 5-593 × 5-593 will produce F<sub>2</sub> plants with T/t<sup>r</sup> Z<sup>f</sup> z<sup>f</sup> Fib/fib. With t<sup>r</sup>/t<sup>r</sup>, only the genotype Z<sup>f</sup> Fib/fib will give true breeding self-colored seeds (p<sup>f</sup> has no effect with t<sup>r</sup> Z). Thus, 1/16 of the t<sup>r</sup>/t<sup>r</sup> progenies is expected to be true breeding for self-colored seeds (Table 2).

A second F<sub>2</sub> population of 80 plants from the cross ‘2-points<sup>r</sup> BC<sub>2</sub> 5-593’ × ‘minimum<sup>a</sup> BC<sub>2</sub> 5-593’ was also analyzed. These plants all produced partly colored seeds and segregated 3:1 for colored to white flowers, respectively (data not shown), and all the F<sub>2</sub> plants had the OM19<sub>460</sub> band. The expected genotypes of the progeny were t<sup>r</sup>/t<sup>r</sup>, t<sup>r</sup>/t<sup>r</sup>, or t<sup>r</sup>/t<sup>r</sup>. Furthermore, the fact that the OM19<sub>460</sub> marker is present in both t<sup>r</sup> and t<sup>r</sup> genetic backgrounds suggests an ancestral relationship between the two alleles. The most probable ancestral lineages are T<sub>T</sub> and T<sub>t</sub> being derived independently from T<sub>T</sub>. The segregation presented in Table 1. The seeds of PI 507984 are very large and suggest a possible ancestry related to the large-seeded domesticated varieties of Phaseolus coccineus L. (scarlet runner bean). It is well established that germplasm derived from crosses between P. vulgaris and P. coccineus have a high frequency of loci with disturbed segregation (Smartt, 1970).

**Literature Cited**


Bassett, M.J. 1994b. 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.


