A New Allele, t^c , at the T Locus for Partly Colored Seedcoats in Common Bean

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ADDITIONAL INDEX WORDS. *Phaseolus vulgaris*, inheritance, pleiotropy, seedcoat genetics, RAPD markers

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^c BC₁ 5-593' and '2-points t^c BC₂ 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^c BC₁ 5-593' x 5-593 and '2-points t^c BC₂ 5-593' x 5-593 produced F₂ 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 $t Fcr Fcr-2$ produce partly colored seedcoats and flower color restoration with t . The crosses '2-points t^c BC₁ 5-593' x 'self-colored t BC₂ 5-593' and '2-points t^c BC₂ 5-593' x 'minimus t BC₃ 5-593' produced F₂ populations that segregated 3:1 for colored:white flowers, respectively. Those results are consistent with the revised hypothesis that t^c can produce partly colored seedcoats without affecting flower color. The RAPD marker OM19₄₀₀, which is linked in repulsion to T , was used with the F₂ populations from the crosses '2-points t^c BC₂ 5-593' x 5-593 and '2-points t^c BC₂ 5-593' x 'minimus t BC₃ 5-593' and established that the t^c gene from PI 507984 is either an allele at T or tightly linked to T . F₃ data from the cross '2-points t^c BC₂ 5-593' x 5-593 also support the t^c hypothesis. On the basis of the above experiments, the gene symbol t^c is proposed for an allele at T that pleiotropically produces partly colored seeds and colored flowers.

It is well established in common bean (*Phaseolus vulgaris*) that the t allele of the T locus expresses two pleiotropic effects, viz., partly colored seedcoats and white flowers (Leakey, 1988). Bassett (1993) presented data supporting the hypothesis that two complementary genes, Fcr and $Fcr-2$, interact with t to produce partly colored seedcoats and colored flowers in common bean. The data also supported the hypothesis that one of the complementary genes was carried by the Plant Introduction (PI) accession 507984, and the other complementary gene was carried by Florida dry bean breeding line 5-593. The objective of this research was to reassess that hypothesis and report further on inheritance studies of plants with the partly colored seedcoat and colored flower derived from PI 507984.

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 BC₂ 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 I (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 BC₃ 5-593' was developed from the cross 'self-colored t BC₂ 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^c BC₁ 5-593', where t^c 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 BC₂ 5-593' produced a true breeding F₅ selection designated 'F₅ 2-points bishops violet (B.V.) flowers' in 1992. The partly colored 2-points seedcoat pattern is illustrated (Fig. 2). The subsequent cross 'F₅ 2-points B.V. flowers' x 5-593 produced the true breeding selection '2-points t^c BC₁ 5-593' in 1993. An additional backcross to 5-593, viz., '2-points t^c BC₁ 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.

Received for publication 13 July 1998. Accepted for publication 7 June 1999. Florida Agricultural Experiment Station journal series no. R-06415. We thank Hallie Smith, formerly with the Graphics Dept., Office of Instructional Resources, Univ. of Florida, Gainesville, for preparing the illustrations used in this paper. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

Fig. 2. The left seed (ventral view); 2-points pattern, i.e., two black dots at the micropyle end of the hilum with white seedcoat. The right seed; side view of the 2-points pattern.

the true breeding selection '2-points t^{cf} BC₂ 5-593' in 1994.

CROSSES USED IN THE INHERITANCE STUDY. The crosses '2-points t^{cf} BC₁ 5-593' x 5-593 and '2-points t^{cf} BC₁ 5-593' x 'self-colored t BC₂ 5-593' were made, and the F₂ populations were planted in the field in 1994. The crosses '2-points t^{cf} BC₂ 5-593' x 5-593 and '2-points t^{cf} BC₂ 5-593' x 'minus t BC₃ 5-593' 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.

TESTING PUTATIVE SEGREGATION AT t WITH A RAPD MARKER. A randomly amplified polymorphic DNA (RAPD) marker, OM19₄₀₀, which is linked in repulsion phase to the T gene (Brady et al., 1998), was employed to test for linkage between T and t^{cf} . The linkage distance between T and OM19₄₀₀ is 1.4 cM although no recombination has been detected to date. Seventy-eight plants were grown in the greenhouse from remnant F₂ seeds from the cross '2-points BC₂ 5-593' x 5-593. For each F₂ plant, DNA was isolated and scored for the presence of the marker (Brady et al., 1998). The plants were also classified for flower color and seedcoat pattern. Because F₂ seed production was poor, F₃ progeny testing was limited to the few seeds available. Eighty plants were grown in the greenhouse from remnant F₂ seeds of the cross '2-points t^{cf} BC₂ 5-593' x 'minus t BC₃ 5-593', and the same molecular genetic procedures were applied to this second cross.

Results and Discussion

GENETIC ANALYSIS OF t^{cf} . The F₁ plants from the crosses '2-points t^{cf} BC₁ 5-593' x 5-593 and '2-points t^{cf} BC₂ 5-593' x 5-593 had self-colored seeds and colored flowers (data not shown), and the genotype of the F₁ plants is interpreted to be T/t^{cf} . Most F₂ plants had self-colored seeds (only ≈11% or 8%, respectively, had partly colored patterns, data not shown), but all the F₂ plants had colored flowers (Table 1). The absence of at least some F₂ plants with white flowers is inconsistent with the hypothesis made by Bassett (1993), while also working with materials derived from PI 507984. If the colored flowers in '2-points t^{cf} BC₁ 5-593' were really due to genotype t Fcr Fcr -2, then the F₂ would segregate for white flowers, and the expected ratio would depend on the genetic hypothesis for 5-593. For example, if 5-593 has T Fcr Fcr -2, then the expected ratio would be 57 colored:7 white. If 5-593 has either T Fcr Fcr -2 or T Fcr Fcr -2⁺, then the expected ratio would be 15 colored:1 white. If the F₁ plants had the genotype T/t^{cf} , then no F₂ segregants with white flowers would be expected.

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, t^{cf} 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 t^{cf} (data not shown).

In earlier work, the t 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^{ers} , and F₃ 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 t^{cf} BC₁ 5-593' x 'self-colored t BC₂ 5-593', the F₁ plants had partly colored seeds and colored flowers due to t^{cf}/t (data not shown). Most of the F₂ plants had partly colored seeds (data not shown), and the flower color segregated for a good fit to a 3:1 ratio of colored to white, respectively (Table 1).

For the cross '2-points t^{cf} BC₂ 5-593' x 'minus t BC₃ 5-593', the F₁ plants had partly colored seeds and colored flowers (data not shown). Most of the F₂ plants had partly colored seeds (data not shown), and the segregation for flower color fit a 3:1 ratio for colored to white, respectively (Table 1). The data from these two crosses are consistent with a new hypothesis that plants with colored flowers and partly colored seedcoats are due to t^{cf} (where t^{cf} is dominant to t).

MOLECULAR TEST OF LINKAGE OF t^{cf} AND T . Although the data from all four F₂ populations are consistent with the new hypothesis that t^{cf} (a new allele at T) controls the expression of partly colored seeds on plants with colored flowers, they are also consistent with an alternative hypothesis that a newly discovered locus, independent of

Table 1. Segregation for flower color in the F₂ from four crosses: 1) 2-points t^{cf} BC₁ 5-593 x 5-593, 2) 2-points t^{cf} BC₁ 5-593 x self-colored t BC₂ 5-593, 3) 2-points t^{cf} BC₂ 5-593 x 5-593, and 4) 2-points t^{cf} BC₂ 5-593 x minus t BC₃ 5-593.

Cross no.	Flower color		Ratio tested	χ^2	P
	Colored	White			
1	162				
2	70	30	3:1 ^z	1.33	0.25
			57:7 ^y	37.30	<0.001
			15:1 ^x	96.30	<0.001
3	420				
4	161	48	3:1 ^z	0.46	0.50
			57:7 ^y	31.05	<0.001
			15:1 ^x	99.70	<0.001

^zSegregation ratio expected from selfing an F₁ parent with genotype t^{cf}/t . For the combined data from crosses 2 and 4, viz., 231 colored:78 white, the χ^2 (3:1) = 0.01, P = 0.92.

^ySegregation ratio expected from selfing an F₁ parent with genotype T/t $+Fcr$ $+Fcr$ -2.

^xSegregation ratio expected from selfing an F₁ parent with either of two genotypes, T/t Fcr Fcr $+Fcr$ -2 or T/t $+Fcr$ Fcr -2 Fcr -2.

Table 2. Segregation for seedcoat pattern and the RAPD marker OM19₄₀₀ in the F₂ and F₃ progeny of the cross '2-points t^{cf} BC₂ 5-593' x 5-593.

Segregation data of F ₂				Segregation data of F ₃	
No. of observations ^z	Phenotypic classes			Frequency of progeny types	Progeny description
	Seedcoat pattern	OM19 ₄₀₀ marker	Genetic hypothesis		
19	Self-colored	Absent	<i>T/T</i>	19	True breeding self-colored
42	Self-colored	Present	<i>T/t^{cf}</i>	30	Segregated 3:1 for self-colored to partly colored ^y
				12	True breeding self-colored
17	Partly colored	Present	<i>t^{cf}/t^{cf}</i>	16	Expressed partly colored ^x
				1	True breeding self-colored ^x

^zFor the data 19, 42, 17 for genotypes *T/T*, *T/t^{cf}*, and *t^{cf}/t^{cf}*, respectively, the χ^2 (1:2:1) = 0.564, *P* = 0.75.

^yFor the data 232 self-colored and 75 partly colored totaled over 30 progenies, the χ^2 (3:1) = 0.053, *P* = 0.82. The mean F₃ progeny size was 10.23, with a range of 14 to 3 plants per progeny.

^xFor the data 16 and 1 the χ^2 (15:1) = 0.004, *P* = 0.95, based on the hypothesis that true breeding self-colored lines carry *t^{cf} Z fib*.

T, controls the phenotype. A molecular marker, OM19₄₀₀, that is linked in repulsion phase with *T* (Brady et al., 1998), was employed to distinguish between these two possibilities.

Seventy-eight F₂ plants from the cross '2-points t^{cf} BC₂ 5-593' x 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 F₂ and F₃ and the marker data observed in F₂ (Table 2), we hypothesize that all F₂ plants fall into one of three genotypic classes: *T/T*, *T/t^{cf}*, or *t^{cf}/t^{cf}*. Nineteen *T/T* F₂ plants were self-colored and lacked the OM19₄₀₀ marker (Table 2). Forty-two F₂ plants were self-colored and contained the OM19₄₀₀ marker (Table 2). The expected genotype of these plants was *T/t^{cf}*. As expected, the F₃ 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^{cf}*. All the F₃ progeny of the remaining 12 F₂ 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 seeds in individuals with a *t/t* genotype. Seventeen F₂ plants were partly colored and contained the OM19₄₀₀ marker (Table 2). The expected genotype of these plants was *t^{cf}/t^{cf}*. Thus, the expected segregation ratio of 1:2:1 for the F₂ genotypic classes *T/T*, *T/t^{cf}*, and *t^{cf}/t^{cf}*, respectively, gave a satisfactory fit to the observed data (Table 2).

One of the 17 F₃ progenies with *t^{cf}/t^{cf}* was true breeding (with six plants) for self-colored seedcoats (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^{cf} z l^{ers}* plus a fourth factor (locus unknown) to express 2-points (Bassett, unpublished data). Also, the parents differ at a hypothetical fibula arcs locus, where 5-593 carries *Fib* and 2-points carries *fib* (Bassett, unpublished data). The *Fib* gene strongly restricts the partly colored seedcoat patterns *expansa*, *virgatus*, and *Anasazi* (Bassett, unpublished data). The genotype *t z l^{ers}* produces pure white seed (Bassett, 1997), and the genotype *t^{cf} z l^{ers}* apparently does also (Bassett, unpublished data). Thus, the cross '2-points t^{cf} BC₂ 5-593 x 5-593' will produce F₁ plants with *T/t^{cf} Z/z l/l^{ers} Fib/fib*. With *t^{cf}/t^{cf}*, only the genotype *Z/Z fib/fib* will give true breeding self-colored seeds (*l^{ers}* has no effect with *t^{cf} Z*). Thus, ≈1/16 of the *t^{cf}/t^{cf}* progenies is expected to be true breeding for self-colored seeds (Table 2).

A second F₂ population of 80 plants from the cross '2-points t^{cf} BC₂ 5-593' x 'minus t BC₃ 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 F₂ plants had the OM19₄₀₀ band. The expected genotypes of the

progeny were *t^{cf}/t^{cf}*, *t^{cf}/t*, or *t/t*. Furthermore, the fact that the OM19₄₀₀ marker is present in both *t* and *t^{cf}* genetic backgrounds suggests an ancestral relationship between the two alleles. The most probable ancestral lineage is that the *t* allele must have been derived from *t^{cf}* (or vice versa) rather than *t* and *t^{cf}* being derived independently from *T*.

REFLECTIONS ON THE ORIGINAL HYPOTHESIS. The hypothesis of Bassett (1993) was established in accordance with the accepted standards of proof established by the Genetics Committee of the Bean Improvement Cooperative (Myers and Weeden, 1988). A genetic model based on F₂ data was tested with F₃ progeny tests and found to be consistent with the previous generation. It is a sobering realization that good experimental support does not always confirm the true nature of genetic variation. In this case we speculate that the *t^{cf}* locus is linked with another gene in PI 507984 that disturbs the segregation frequency. By a perverse coincidence the linkage distance was just right to produce a simulation of the segregation expected from two complementary dominant genes. The putative linkage to the gene producing disturbed segregation was apparently broken in later generations, making it possible to obtain the undisturbed 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).

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