A Third Recessive Allele, *stp^{mic}*, for Seedcoat Pattern at the *Stp* Locus in Common Bean

Mark J. Bassett²

Horticultural Sciences Department, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611

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ABSTRACT. The inheritance of a seedcoat pattern having white micropyle stripe (WMS) on a colored background was studied in two common bean (*Phaseolus vulgaris* L.) accessions from Centro Internacional de Agricultura Tropical—G12606 and G07262. The WMS character from G12606 was backcrossed into the recurrent parent 5-593, which has black seedcoats. Test crosses of the derived WMS stocks (BC₁ and BC₂) with genetic tester stocks *stp* (stippled seedcoat) BC₂ 5-593 and *stp^{hbw}* (flowers with half banner petal white) BC₃ 5-593, respectively, demonstrated in F₁ and F₂ progenies that WMS is controlled by an allele at the *Stp* locus. The gene symbol *stp^{mic}* is proposed for the allele expressing the WMS character. The dominance order at the *Stp* locus is *Stp* > *stp^{mic}* > *stp^{hbw}* > *stp*. Although *stp* and *stp^{hbw}* each produce a different color pattern on flowers, *stp^{mic}* does not produce patterned flowers. A selection from accession G07262 with a long, white micropyle stripe was crossed with 5-593 to derive a stock named F₃ *stp^{mic}* long micropyle stripe, which was then crossed to the genetic tester stock *t z virgatus* BC₂ 5-593 to produce F₁ and F₂. Expression of the long micropyle stripe was controlled by the interaction of *t* and *stp^{mic}* in the genotype *t z stp^{mic}*. The triple recessive interaction from genotype *t z stp^{mic}* was also observed.

Prakken (1970) wrote a comprehensive, critical review on the genetics of seedcoat colors of common bean (*Phaseolus vulgaris*), except for the red colors. Partly colored seedcoat pattern is one of the classes of patterned seedcoats in common bean. The partly colored seedcoat types are expressed only with the *t* gene (Lamprecht, 1934). Good illustrations of the many partly colored seedcoat patterns were provided by Lamprecht (1934), Leakey (1988), and Schreiber (1940). The various partly colored patterns are mostly controlled by modifying genes at the Z (for zonal) and L (for limiter) loci, and the complex interactions between Z and L alleles with *t* are described by Schreiber (1940). The work of Bassett (1996a, 1997) has confirmed the genetic model of Schreiber (1940) and extended the knowledge of interactions at the Z and L loci with *t/t*. Recently, Bassett (1996b) described a new locus for patterned seedcoats, viz., the stippled locus (*Stp*), which has two recessive alleles that each produce a different pattern in the seedcoat and, pleiotropically, a different pattern in the flowers.

This paper presents evidence for the existence of a third recessive allele at the *Stp* locus and its interactions with *t* and *z*.

Materials and Methods

In 1985 a program was initiated to develop genetic tester stocks, each with one or more recessive marker genes for seedcoat color or pattern backcrossed into the recurrent parent, Florida dry bean breeding line 5-593. Details of the backcross procedure are described elsewhere (Bassett, 1994a). Line 5-593 has a determinate habit, purple flowers, and shiny, black

seedcoats. The seedcoat genotype of 5-593 is *TP [C r] D J G B V Rk* (Bassett, 1994b; Prakken, 1970, 1972). Genetic stocks that were developed by backcrossing a marker gene to the 5-593 recurrent parent are designated *gene symbol* BC_n 5-593. For example, *v* BC₃ 5-593 indicates that the *v* allele is in backcross three to 5-593. Before the first backcross, the stock is designated F_n *gene symbol* 5-593.

Development of the genetic stocks F₄ *stp V* 5-593, *stp* BC₂ 5-593 and *stp^{hbw}* BC₃ 5-593 was described previously by Bassett (1996b). The *stp* allele not only produces a stippled seedcoat, but pleiotropically produces a patterned corolla, which was previously illustrated (Bassett, 1996b). The *stp^{hbw}* allele produces a stippled pattern on the seedcoat that is different from the *stp* pattern, and pleiotropically produces banner petals with the lower half being white (Bassett, 1996b).

Two seedcoat patterns that have not been reported were observed in two accessions from the germplasm bank of the Centro Internacional de Agricultura Tropical, viz., G12606 and G07262. G12606 has a vine habit, pink flowers (due to *v^{lae}*), and dark purple (nearly black) seedcoats with a short, white (or near white) micropyle stripe (Fig. 1). The hypothesis for the nearly black seedcoat color is [*? R*] *J G B v^{lae}* (Bassett, 1996c; Prakken, 1972). G07262 has a vine habit and is heterogeneous for two phenotypes for flower color and seedcoat pattern. The first type has pink flowers (*v^{lae}*) and dark purple (nearly black) seedcoats that have a short, white (or near white) micropyle stripe (Fig. 1). The second type has white flowers with blue (or bluish purple) veins on a white background and dark purple seedcoats with a long, white (or near white) micropyle stripe that extends around the micropyle end of the seed (Fig. 2). A plant with the second phenotype was used in the

experiments described below. To make the presentation easier to follow, the hypothesis that a new

Fig. 1. Ventral view of the seedcoat pattern (white micropyle stripe) produced by *stp^{mic}*.

Fig. 2. (left) Ventral view of the long micropyle stripe seedcoat pattern (long white micropyle stripe) produced by *t stp^{mic}*. (right) Side view of the long micropyle stripe of *t stp^{mic}*.

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¹Professor.

Table 1. Segregation for color and patterns in the flowers and seedcoats of the F₂ from the crosses 1) *stp*^{mic} BC₁ 5-593 × *stp* BC₂ 5-593 and 2) *stp*^{mic} BC₂ 5-593 × *stp*^{hbw} BC₃ 5-593.

Cross	Purple flower, seedcoat with <i>stp</i> ^{mic} pattern	<i>stp</i> Flower, seedcoat with <i>stp</i> pattern ^z	<i>stp</i> ^{hbw} Flower, seedcoat with <i>stp</i> ^{hbw} pattern ^y	χ^2 3:1	P
1	325	114		0.219	0.64
2	219		67	0.378	0.54

^zThe gene *stp* produces patterned flowers; the periphery of the wing petals and upper edge of the banner petal are violet, whereas the nonviolet areas are white.

^yFor the gene symbol *stp*^{hbw}, the superscript letters hbw are an acronym for half banner white, where the lower half of the banner petal is white.

Table 2. Segregation for seedcoat patterns (with genetic hypothesis) in the F₂ from the cross F₃ *stp*^{mic} long micropyle stripe (*t* Z *stp*^{mic}) × *t* virgarcus BC₂ 5-593 (*t* z *Stp*)^z.

Self-c/exp/ambigua ^y <i>t</i> Z/- <i>Stp</i> /-	Virgarcus <i>t</i> z <i>Stp</i> /-	Long micropyle stripe <i>t</i> Z/- <i>stp</i> ^{mic}	Double recessive ^x <i>t</i> z <i>stp</i> ^{mic}	χ^2 9:3:3:1 ^w	P
97	27	44	8	5.616	0.13

^zAll F₂ plants had white flowers due to *t/t* and black seedcoats (or partial seedcoat color) due to *P B V*.

^ySelf-c/exp/ambigua = self-colored, expansa, and ambigua classes combined.

^xThe double recessive pattern of partly colored seedcoat is illustrated in Fig. 3.

^wThe orthogonal contrasts of Mather (1957) are χ^2_z (3:1) = 2.455, *P* = 0.12; χ^2_{stp} (3:1) = 1.939, *P* = 0.16; and χ^2_L = 1.222, *P* = 0.27.

allele (*stp*^{mic}) exists at the *Stp* locus, which produces a short, white micropyle stripe, will be indicated with the tentative gene symbol *stp*^{mic} rather than the usual acronym for the character, such as WMS. For G12606, the genetic hypothesis is *T stp*^{mic} *v*^{lne}. For the selection from G07262, the genetic hypothesis is *t* Z *stp*^{mic} *B V rk*^d, where the interaction of *t* Z with *stp*^{mic} produces the long micropyle stripe and the interaction of *t* V with *rk*^d produces blue veins on the otherwise white flowers. The blue vein hypothesis will not be pursued in this paper, but the complete description of flower color and pattern is necessary to support the hypothesis that *t* is present. The possibility of having *t* Z produce self-colored (totally colored) seedcoats was previously reported by Bassett and Blom (1991).

In Fall 1992, the cross F₄ *stp* V 5-593 × G12606 was made in the greenhouse, and the F₁ plants produced black seeds with a white micropyle stripe (data not shown). The F₂ was planted in the field in Spring 1993 and a selection was made for a plant with determinate habit and seed with a white micropyle stripe. In Fall 1993, a true breeding progeny designated F₃ *stp*^{mic} 5-593 was crossed with 5-593 in the greenhouse. The F₂ was planted in the field in Spring 1994 and selection was made for the *stp*^{mic} character with the 5-593 plant type. In Fall 1994, a true breeding progeny designated *stp*^{mic} BC₁ 5-593 was crossed with *stp* BC₂ 5-593 and backcrossed to 5-593 in the greenhouse. The F₂ from both crosses was planted in the field in Spring 1995. Data were taken in F₁ and F₂ on the patterns and colors of flowers and seedcoats. In the F₂ from the backcross to 5-593, selection was made for the *stp*^{mic} character with the 5-593 plant type. In Fall 1995, a true breeding progeny designated *stp*^{mic} BC₂ 5-593 was crossed with *stp*^{hbw} BC₃ 5-593 in the greenhouse. The F₂ was planted in the field in Spring 1996, and data were taken in F₁ and F₂ on the patterns and colors of flowers and seedcoats.

In Fall 1993, the cross 5-593 × G07262 (grown from seed with the long micropyle stripe) was made in the greenhouse, and the F₂ was planted in the field in Spring 1994. Selection in F₂ was made for plants with determinate habit and seeds with the long micropyle stripe. In Fall 1994, a true breeding progeny designated F₃ *stp*^{mic} long micropyle stripe was crossed to the genetic stock *t* z virgarcus BC₃ 5-593 in the greenhouse. The development of *t* z virgarcus BC₃

5-593 has been previously described (Bassett, 1996a). The F₂ was planted in the field in Spring 1995, and data were taken in F₁ and F₂ on flower color and seedcoat patterns.

Results and Discussion

For the cross *stp*^{mic} BC₁ 5-593 × *stp* BC₂ 5-593, the F₁ plants had purple flowers and seedcoats with the pattern of the *stp*^{mic} BC₁ 5-593 parent (data not shown). Those results do not show complementation for seedcoat pattern (which would produce an unpatterned seedcoat), but suggest that the two parental stocks are allelic for the same seedcoat pattern locus, with the *stp*^{mic} parent being dominant to the *stp* parent. The F₂ segregated only for the two parental phenotypic classes in the ratio of 3 *stp*^{mic}:1 *stp* (Table 1). Also, the F₂ segregants with *stp*^{mic} seedcoat pattern had unpatterned flowers. Those results support the hypothesis of allelism for seedcoat pattern and dominance of the micropyle stripe over no micropyle stripe.

The hypothesis that the micropyle stripe pattern locus is allelic with *stp* was tested another way with the cross *stp*^{mic} BC₂ 5-593 × *stp*^{hbw} BC₃ 5-593. The F₁ produced plants with purple flowers and seedcoats with the micropyle stripe (with fine dots) pattern (data not shown). Those results do not show any evidence of complementation, where the seedcoat pattern of neither parent is expressed because of the influence of the dominant allele from the opposite parent. Instead, one of the parental patterns, white micropyle stripe (with fine dots) is expressed and indicates the dominance of that pattern over the *stp* pattern. The F₂ segregated for only

the two parental classes in a ratio of 3 *stp*^{mic}:1 *stp*^{hbw} (Table 1). Also, the F₂ seg-

Fig. 3. (left) Ventral view of the seedcoat pattern produced by *t* z *stp*^{mic}. (right) Side view of the same genotype effect, primarily the elimination of the colored micropyle stripe that would appear with *t* z *Stp* (virgarcus).

regants with *stp^{mic}* seedcoat pattern had unpatterned flowers; hence, *stp^{mic}* differs from *stp* and *stp^{hbw}*, each of which produces a unique flower color pattern (Bassett, 1996b). Those results support the hypothesis of allelism for the seedcoat patterns of the two parents and dominance of the *stp^{mic}* allele over the *stp^{hbw}* allele. Combining the results of the previous work with the *stp* locus (Bassett, 1996b) with the present work, the dominance order of alleles at the *stp* locus is: *Stp* > *stp^{mic}* > *stp^{hbw}* > *stp*.

The selection from G07262 used in the experiments described above had a long micropyle stripe (Fig. 2). The genetic hypothesis given for the longer stripe was *t Z stp^{mic}*, where the interaction of *t* and *stp^{mic}* produced the elongation of the micropyle stripe of *stp^{mic}* alone (with *T*). The genetic hypothesis was tested with the cross F₃ *stp^{mic}* long micropyle stripe (*t Z stp^{mic}*) × *t z virgarcus* BC₂ 5-593 (*t z Stp*). The F₁ plants had white flowers with blue veins and seedcoats with the ambigua pattern, which is produced by *t/t Z/z* (Schreiber, 1940; data not shown). If the F₃ *stp^{mic}* long micropyle stripe parent did not carry *t/t*, then the *t/t* in the virgarcus parent would have been masked by *T* in the female parent. Consequently, the F₁ flowers would have been purple. The F₂ of the test cross segregated for the four classes expected from dihybrid segregation at *Z/z* and *Stp/stp^{mic}* and fit a 9:3:3:1 ratio for those classes (Table 2). The seedcoat pattern produced by the homozygous recessive class *t z stp^{mic}* is illustrated in Fig. 3. The *t Z/- Stp/-* class (Table 2) combines the plants with *t/t Z/Z* (either self-colored or expansa) and *t/t Z/z* (ambigua) because these two genotypic classes cannot be classified unambiguously. The other three phenotypic classes are highly distinctive and easily classified, viz., virgarcus, long micropyle stripe, and the double recessive class (Table 2). All the F₂ plants had white flowers, which is consistent with the hypothesis that the F₁ was homozygous (*t/t*). No alternative hypothesis, based on established genetics of common bean, could be found that fit the observed data. Thus, the three-factor interaction of *t z stp^{mic}* produces a virgarcus pattern *without* a colored micropyle stripe, having instead a white micropyle stripe (Fig. 3).

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