

Soybean Hilum Examination: *Morphology of Hilum Development*

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The hilum is the scar that forms as a result of detachment from the funiculus which is the point of attachment to the pod and maternal plant. The micropyle, which served as the point of entry for the pollen tube, is often visible at one end of the hilum (Bewley et al., 2013). In *Arabidopsis*, decreased pigmentation was associated with decreased dormancy (Bewley et al., 2013).



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FIGURE 1. Seven common soybean hilum colors (top to bottom): Yellow, Gray, Buff, Red-Brown, Brown, Imperfect Black and Black (Imperfect yellow hilum seeds are not represented).

FORMATION OF PIGMENT

Early in seed development, pigments are produced in the inner integuments in the endothelium layer as colorless compounds that are later oxidized during maturation to a darker color. These pigments include flavonoids, proanthocyanidins, and anthocyanins (Bewley et al., 2013). Barion et al. (2016) found darker hilum colors to be associated with higher levels of isoflavone, a target trait for nutraceutical uses of soy to treat cancer and heart disease symptoms.

REGULATION OF PIGMENTATION

Seed coat and hilum pigmentation in soybeans is controlled by 6 genetic loci, which have been determined via classical genetics and the specific genetic location and molecular mode of action for some has recently been elucidated through molecular techniques (Kovinich et al., 2011).

The I, or inhibitor locus, controls the spatial distribution of pigment within the seed. The dominant allele, I, inhibits all pigment. Other alleles, in order of decreasing dominance, ii, ik, and i, allow pigment in increasing regions of the seed coat: the hilum, a saddle-shaped region surrounding the hilum, and the entire seed respectively (See Figure 1. in Cho et al., 2017) (Carpentieri-Pipolo et al., 2015). As Cho et al. (2013) discuss, the dominant I locus alleles inhibit pigmentation through gene silencing via siRNAs. These siRNAs are coded by CHS 1-3-4 and target CHS7/8, which then serve as templates for secondary siRNA to downregulate chalcone synthase. The expression of these genes is tissue specific, being more concentrated in the less pigmented regions of the seed, and the recessive alleles are deletions of promoter regions found in the dominant alleles (Cho et al., 2013). Cho et al. (2013) also found that these siRNAs are produced early in seed development. Vu et al. (2018) demonstrated that when CHS7 is overexpressed (via genetic engineering), the hilum color is lightened from imperfect black to buff due to the downregulation of chalcone synthase. Kim et al. (2014) identified another locus they name HC in the I locus region (CHS3) that determines between yellow and black hila.

The dominant allele T at the T locus confers tawny pubescence while the recessive allele, t, confers gray pubescence (Toda et al., 2012). Toda et al. (2012) showed that the T locus produces a sf3'h1 protein associated with flavonoid pigment biosynthesis, that in the seed, accumulates in the hilum and funiculus tonoplasts. Additionally, a dominant allele at the T locus typically results in a darker hilum color (Carpentieri-Pipolo et al.,

REGULATION OF PIGMENTATION, CONT.

2015). Anthocyanin presence in the seed coat is controlled by the R locus, with the dominant R allele resulting in anthocyanin presence and a black seed coat and/or hilum (depending on I locus), and the recessive r allele resulting in the absence of anthocyanins, and a brown seed coat or hilum (Kovinich et al., 2011). Gillman et al. (2011) identified four distinct loss-of-function recessive mutations in the R2R3 MYB transcription factor that gave the brown phenotype. The W1, Wp, and O loci are believed to only affect seed color with an i genetic background. In an iRt background, W1 leads to an

imperfect black seed coat and w1 to a buff seed coat; the W1 loci primarily influences flower color. The recessive allele at the Wp loci leads to a lighter gray seed coat. The O loci impacts seed coat under an iRt genetic background, with the recessive allele, o, leading to a red-brown seed color (Kovinich et al., 2011).

The relationships between these loci and phenotypic outcomes are summarized by Palmer et al. (2004, pp. 184) in Table 1.

TABLE 1. Summary of genotype and phenotype relationships related to hilum colors in soybeans (Palmer et al., 2004).

Genes	Seed coat and hilum self-color	Saddle and hilum color	Hilum color	Hilum color
	<i>i</i>	<i>i-k</i>	<i>i-i</i>	<i>I</i>
<i>T R</i>	Black	Black	Black	Gray
<i>T r O</i>	Brown	Brown	Brown	Yellow†
<i>T r o</i>	Red brown	Red brown	Red brown	Yellow†
<i>t R W I</i>	Imperf. black	Imperf. black	Imperf. black	Gray
<i>t R w I</i>	Buff	Buff	Buff	Yellow
<i>t r</i>	Buff	Buff	Buff	Yellow

†Sometimes called imperfect yellow (Cober et al., 1998).

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