Which seed color gene of *r1* responds to inhibitors (*Inr*) and enhancers (*Enr*) of aleurone color?

--Stinard, PS

Stinard and Sachs (2002. J Hered 93:421-428) and Stinard *et al.* (2009. J Hered 100:217-228) recently reported on *r1* haplotype-specific inhibitors and enhancers of aleurone color. Genetic analysis of the *R1-r(sd2)* (*spotted dilute*) haplotype led Stinard *et al.* (2009) to conclude that one of the seed color genes (*S1* or *S2*) of the seed color (*S*) subcomplex of *R1-r(sd2)* contains a *dSpm* insert, and the other seed color gene responds to dominant alleles at inhibitory (*Inr*) or enhancing (*Enr*) loci (see Figure 4 of Stinard *et al.* 2009 for a proposed model). However, it was unclear which seed color gene carries the *dSpm* insert and which gene responds to *Inr* and *Enr*. Intragenic recombination experiments were designed in order to answer this question.

Intragenic recombination has been used a means of dissecting the structure and function of the genic components of the compound *r1* locus (see Kermicle, JL. 1988. pp. 81-89 in Nelson, OE Jr (ed). Plant transposable elements. Plenum Press, NY for a review). The *S* subcomplex of *R1-r(Std)*, the presumed progenitor of *R1-r(sd2)*, carries duplicate genes, *S1* and *S2* arranged as inverted repeats flanking a promoter region (Walker *et al.* 1995. EMBO J 14:2350-2363). Both *S1* and *S2* are expressed in the aleurone, so a mutation in one of the *S* genes of *R1-r(Std)* would not be expected to give a visible phenotype. However, intragenic recombination knocking out the *S2* gene followed by genetic analysis of the derivatives can help answer the question of which gene in *R1-r(sd2)* carries a *dSpm* insert and which one responds to inhibitors and enhancers. For these experiments, the *r1-sc:m3* allele was chosen to recombine with *S2*. *r1-sc:m3* carries a *Ds* insertion in the simplex *Sc* (aleurone *Self-color*) gene, which is homologous to and in direct orientation with respect to *S2* on chromosome 10 (Kermicle, JL. 1980. Science 208:1457-1459; in combination with conclusions of Walker et al. 1995). Recombination between *S2* and *r1-sc:m3* proximal to the *Ds* insertion point would result in the transfer of the *Ds* element to *S2*, knocking it out and at the same time placing it under control of *Ac* should *Ac* be present in the genome. Experiments were devised to discriminate between the following two possibilities:

(1) **Possibility 1: *S1* responds to *Inr* and *Enr* alleles.** *r1-sc:m3* was crossed to *R1-r(Std)* (genotype *S1 S2*). The F1 was crossed by *r1-g Enr1-Fcu Inr1*. If *Ds* is transferred to *S2* by intragenic recombination, and if *S1* responds to the mutable enhancer allele *Enr1-Fcu* and the inhibitory *Inr1* allele, then the derivatives carrying *s2::Ds* with *S1* would be expected to show aleurone color sectoring on a pale background in the presence of *Enr1-Fcu* and *Inr1*. Nonrecombinants would have full aleurone color due to the presence of the uninhibited, nonmutant *S2* gene. This cross was performed in 2008, and out of a total of 10,288 scorable kernels (with aleurone color), 11 mottled exceptions were obtained. Subsequent testing of the exceptions revealed that the mottling was not heritable in 10 of the exceptions—they were actually parental *R1-r(Std)* types. One exception did not germinate. Therefore, from an effective population of 9,353 kernels, no visible recombinants were

obtained. These negative results could mean that either the population size was not sufficient to isolate the desired recombinants, or that *S1* does not respond to *Enr* and *Inr* alleles.

(2) **Possibility 2: *S2* responds to *Inr* and *Enr* alleles.** *r1-sc:m3* was crossed to *R1-r(sd2)*. The F1 was crossed by *r1 inr1 Spm*. If *Ds* is transferred to *S2* by intragenic recombination, and if *S1* of *R1-r(sd2)* carries a *dSpm* insert, then the derivatives carrying *s2::Ds* with *s1::dSpm* would be expected to show typical small revertant sectors (*S1 s2::Ds*) on a colorless (*s1::dSpm s2::Ds*) background (no *Ac* present). Nonrecombinants (*s1::dSpm S2*) would have full aleurone color since an inhibitory *Inr1* allele was absent from this cross. This cross was performed in 2009, and out of a total of 8,740 scorable kernels (with aleurone color), 3 mutable exceptions were obtained, for a frequency of 3.4 X 10^{-4}. Subsequent testing of the exceptions revealed that all three were heritable. Two of the exceptions also responded to *Ac*, which is what would be expected if *Ds* was transferred to *S2* by intragenic recombination (*s1::dSpm s2::Ds)*; one exception did not respond to *Ac*. The non-responder to *Ac* could represent a spontaneous mutation in *S2* (*s1::dSpm s2*) rather than a recombination event. The frequency of *Spm Ac* double responders was 2.3 X 10^{-4}.

The recovery of the expected and heritable events from the latter intragenic recombination experiment demonstrates that it is the *S2* gene that responds to *Inr* and *Enr* alleles, and the *S1* gene of *R1-r(sd2)* carries a *dSpm* insert. The frequency of recombination between *Sc* and *S2* (2.3 X 10^{-4}) is comparable with that observed between other components of the *r1* gene complex (e. g. 4.49 X 10^{-4} between the *P* (*Plant color*) gene and *r1-sc:m3* restoring kernel color, Dooner, HK and Kermicle, JL. 1986. Genetics 113:135-143; and 3.93 X 10^{-4} and 3.68 X 10^{-4} for recombination between *P* and *S2* in *R1-r(Std)* resulting in deletion of sequences between *P* and *S2*, Dooner, HK and Kermicle, JL. 1971. Genetics 67:427-436.).