Interspecific Reproductive Barriers in Tomato (IRBT) Meeting Abstracts

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Abs # P56012

Temporal and spatial mapping of reproductive barriers in the developing pistils of *Solanum pennellii*, a tomato wild species

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Many wild species in the tomato clade show both sympatric and allopatric distributions and possess both self-incompatibility (SI) and unilateral incongruity (UI) barriers. Thus, this group offers a useful model system to analyze the molecular mechanisms and evolution of mating barriers in plants. Using a semi-*in vitro* system to track pollen tube growth in styles, we have successfully determined the developmental timing and stylar location of reproductive barriers (both SI & UI) in three accessions of *S. pennellii*. Two of the accessions, LA2560 & LA1340 are self-incompatible, whereas the accession LA0716 is self-compatible. However, all the three accessions reject pollen unilaterally from *S. lycopersicum*, the domesticated tomato. Our studies demonstrate that UI and SI are developmentally regulated. Further, our analysis shows that early stages of developing pistils (i.e., 5 days before the bud break) in all three accessions of *S. pennellii* lack these barriers and allow the growth of pollen tubes that are normally rejected by fully-developed pistils, thus indicating that both SI as well as UI machinery is fully activated only in the later stages of development. Current analysis indicates that SI barriers appear on day -5 while UI seems to be operative not until a day later. These results are being confirmed by *in vivo* pollination studies. We have also found that the UI barriers seem to operate in or near the stigma (i.e., within the top 20% of the style), where as SI barriers cause pollen tube inhibition deeper in the style (~below 40% of the style), as found in our *in vivo* analysis. We are also tracking the appearance of proteins known to be involved in SI, particularly the S-RNases and HT-proteins. Early results indicate that the appearance of HT proteins coincides temporally with the activation of mating barriers in the developing pistil. We are further leveraging the information by proteome and transcriptome profiling to identify the novel components of molecular machinery associated with SI and UI.

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Unilateral Incongruity in Tomato: Role of Self-Incompatibility Factors

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Self-Incompatibility (SI), wherein self pollen is rejected by styles, is widespread in plants and functions to prevent inbreeding. In gametophytic SI, RNases encoded at the *S*-locus (S-RNases), are the female SI determinants. Apart from specificity determinants, additional pistil factors are required for SI, including the asparagine-rich HT-family proteins. Interspecific pollen rejection is less well understood than intraspecific SI. Often interspecific pollinations are only successful in one direction; this phenomenon is known as unilateral incongruity or incompatibility (UI). In tomato, genetic studies of *Solanum pennellii* X *S. lycopersicum* and *S. habrochaites* X *S. lycopersicum* crosses have directly implicated the *S*-locus in UI, but the role of SI proteins in UI is complex. We examined the mode of pollen tube rejection and assessed the potential role of SI genes in crosses between wild tomato species. We find that there are at least two modes of interspecific pollen rejection – rapid (in the upper 15% of the style) and slow (in the lower half of the style). Neither mode necessarily requires high levels of S-RNase expression. Two asparagine-rich HT-family genes, *HT-A* and *HT-B*, are tightly linked and map to a UI QTL on Chromosome 12. While *HT-A* is functional in all wild tomato accessions tested, the *HT-B* gene contains a point mutation that should eliminate expression in all tested accessions of *S. habrochaites*, regardless of whether plants were self-compatible or self-incompatible. Proteomic analysis supports this conclusion. Therefore, neither S-RNases nor HT-B protein appear to be essential for UI pollen rejection in wild tomato relatives, but HT-A protein and *S*-locus encoded factors other than *S*-RNases may play important roles in UI.