Using Synthetic Gene Clusters to Model Resistance Gene Evolution by Meiotic Recombination in *Arabidopsis thaliana*

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ABSTRACT

Plants have evolved multiple surveillance mechanisms to detect the presence of disease-causing organisms. One mode of surveillance is based on dozens of constitutively expressed resistance (*R*) genes. *R* genes recognize pathogen gene products as signals of invasion. We are interested in how plants evolve *R* genes to keep pace with rapidly evolving pathogen populations. The mechanisms that drive the evolution of new *R* genes are poorly understood. There is data that supports the relevance of recombination in the evolution of resistance gene clusters in plants. However, a more comprehensive understanding of the molecular biology of recombination and the impact recombination has on *R* gene evolution is necessary. The objectives of this dissertation were to develop a genetic screen that models meiotic unequal crossing over at a synthetic *RPP8* (*synthRPP8*) resistance gene cluster and to assess the effect of abiotic stress on recombination with the synthetic *RBCSB* gene cluster (*synthRBCSB*) in *Arabidopsis*. The genetic screen utilized in these studies specifically identifies a novel recombinant gene and a concomitant gene duplication that results from meiotic unequal crossing-over by coupling chimeric gene formation to the activation of the firefly luciferase gene. Two *synthRPP8* clusters were constructed and extensive optimization of screening conditions were performed. An initial screen of ~1 million *synthRPP8* transgenic plants was performed and plants that expressed the luc^+ phenotype were isolated and analyzed. Unexpectedly, background bioluminescence was found to interfere with the identification of *bona fide* luc^+ *synthRPP8* recombinants. An abiotic stress response assay was performed and the data suggests activation of a putative stress response element in the promoter of *RPP8* is responsible for background levels of *in vivo* luciferase activity. The background bioluminescence could not be sufficiently reduced. Therefore, two additional *synthRPP8* constructs, *synthRPP8-3* and *synthRPP8-4*, were constructed and are currently being examined for their utility to model meiotic unequal crossing-over. UV-C treatment was shown to stimulate somatic unequal crossing over, as well as upregulate defense/stress response genes and transcription factors. Meiotic recombination may also be affected by stress. Therefore, the effect of UV-C irradiation on the frequency of unequal meiotic recombination between paralogous *RBCSB* genes and on the expression of genes associated with the defense/stress response was examined. We observed a ~2-fold increase in the frequency of meiotic recombination after UV-C irradiation but this increase was not statistically significant. We did not detect a significant alteration in the steady-state *MYB10, PR-1* and *HSF-3* mRNA levels by semi-quantitative RT-PCR. The expression data we gathered provided minimal support for whether the UV-C treatment was an effective DNA damaging agent.

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