

“Effects of RNAi suppression of MLH1 gene expression on crossing over in tomato”

MLH1 protein is a DNA mismatch repair protein that has a crucial role in completing meiotic crossovers in budding yeast, plants, and animals. Here, we evaluate crossing over (CO) in tomato plants in which MLH1 gene expression is reduced to about 20% of wild-type levels by using an RNAi approach called Direct-Repeat-Induced-Gene-Silencing (DRIGS). In MLH1-DRIGS tomato plants, synapsis followed normal patterns, and we observed early recombination nodules (ENs, that are thought to mark DNA double-strand breaks) in frequencies and sizes similar to those of wild-type. However, pachytene SC spreads showed almost no MLH1 immunolabeling, and late recombination nodules (LNs, that mark COs) were smaller and more numerous than in wild-type. Notably, the number and distribution of these unlabeled LNs in MLH1-DRIGS plants showed evidence of interference. By diplotene, distinct MLH1 foci were observed on SCs in frequencies and locations similar to wild-type, and the MLH1 foci corresponded to LNs. However, the LNs were significantly smaller than diplotene LNs from wild-type. Chiasma frequencies and patterns in wild-type and MLH1-DRIGS plants were not significantly different, but seed set was lower in MLH1-DRIGS plants than in wild-type. Based on this data, we conclude that suppression of MLH1 expression has little effect on the distribution, frequency, or interference characteristics of class I COs, consistent with a late role for MLH1 protein in CO resolution, but reduced levels of MLH1 are likely to lead to DNA repair defects that particularly affect embryonic growth and cause reduced seed set. Our data also indicate that MLH1 protein is an important contributor to the morphology of late recombination nodules.