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Deciphering molecular mechanism underlying 10-stamen phenotype in *Capsella bursa-pastoris*

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Motivation and aim: Flower development is a complex morphogenetic process dependent on the precise regulation of genes that determine the identity and number of floral organs. The study of *Arabidopsis* mutants with various floral abnormalities led to the rise of the ABC-model of flower development, in which homeotic genes play a key role, defining the formation of sepals (A-class genes), petals (A- and B-class genes), stamens (B- and C-class genes), and carpels (C-class genes) in the particular quantity in the correct location. Investigating these genes is vital for understanding the evolution of flowering plants, as alterations in their function and regulation can lead to significant morphological transformations, promoting plant adaptation.
We are investigating a natural homeotic *Capsella bursa-pastoris* mutant with a unique floral morphology: 10 stamens instead of 6, accompanied by the absence of petals (replaced by stamens). This "decandric" phenotype is absent in *Arabidopsis*, making it a valuable system for uncovering new mechanisms of homeotic gene function and regulation. Our goal is to elucidate the genetic basis of this unusual phenotype.
Materials and methods: Mutant plants were crossed with the wild type (*C. bursa-pastoris* with 6 stamens and 4 petals). F2 plants were phenotyped to determine the inheritance pattern of the aberrant trait. Bulked Segregant Analysis (BSA) using next-generation sequencing (NGS) was employed to map loci controlling atypical flower morphology. Differential gene expression was analyzed using RNA-seq.
Results: Segregation ratio of the mutant phenotype in the F2 generation of crosses between mutant and wild-type plants comprises 1:2:1 between stamenoid vs. intermediate vs. wild-type second whorl organs. This suggests a monogenic mode of inheritance for the trait, with incomplete dominance of mutant allele. BSA identified the location of a locus associated with the 10-stamen trait on chromosome R4 of *C. bursa-pastoris*. Differential expression analysis revealed 80 genes with reduced expression in the "decandric" mutant compared to the wild type, of which 57 genes are located on chromosome R4. Notably, the key regulator of stamen and carpel development, the *AGAMOUS (AG)* gene, resides in this region. However, its expression was not altered in the mutant ecotype. GO-annotation of Up-regulated genes is enriched in genes controlling pollen formation. Down-regulated genes are enriched in stress-related processes.
Comparative sequence analysis of the *AG* gene in 66 *C. bursa-pastoris* ecotypes, including the "decandric" mutant and wild type, showed that the only substitution unique to the "decandric" mutant is located in the fourth intron at R4:8,853,384 T -> A. This substitution may be significant, as regulatory elements are known to reside in the second intron of the *AG* ortholog in *Arabidopsis*. Alternatively, epigenetic changes might be involved.
Conclusions: The results indicate that decandrous phenotype in *C. bursa-pastoris* is defined by a change in AG or closely linked region. In the former case this is the most likely change in the regulatory element conferring the possibility of ectopic expression in the second whorl. Since *AG* expression is not altered, it is possible that *AG* regulation occurs at the post-transcriptional level, for example, via the action of non-coding RNAs. The concerted downregulation of genes of the same chromosome where the causative gene is located suggests the involvement of epigenetic mechanisms.
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