Naturally occurring flower mutation in offspring of a large fruited raspberry chance seedling

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A red raspberry population was established from an open pollination of a large fruited chance seedling. Subsequently, three different floral phenotypes were observed in this population. Type 1 is equivalent to the wild type raspberry flower phenotype with five sepals and petals, with stamens and carpels present. Type 2 has six sepals and petals, with stamens and carpels present. Type 3 has sepaloid and carpeloid structures, but no petals and stamens.

The population was evaluated for floral phenotype and for fruit weight, length and drupelet number. Type 1 and 3 fruits are smaller and have a smaller number of drupelets than type 2 fruits.

The cause of the mutation is thought to be a category B MADS-box gene, most likely PISTILLATA (PI), as APETALA3 has several homologs in other Rosaceae members, thus making it more robust against impairment. MADS- and K-box containing genes from Rubus occidentalis (black raspberry) were defined by Hidden Markov model search. A relationship tree was produced through amino acid sequence homology. Although no homolog for PI was found initially, a BLAST search found a non-annotated sequence with high homology to *Arabidopsis thaliana* PI.

An expression study was conducted on type 1 and type 3 whole flower and whorl cDNA with primers created based on Rubus occidentalis PI homolog sequence. Fragments amplified only for type 1 samples, in whole flower and in whorl 2 (petal) and 3 (stamen) samples, which indicates a defect category B MADS-box gene in type 3 phenotypes.

A further PCR with type 1 and 3 genomic DNA as template showed no visible fragment size difference, making a transposon insertion implausible and pointing to a possible SNP, or a disturbance in the promoter region as cause.

Currently, there are type 1 and type 3 mother plants crossed with two raspberry cultivars for fruit size evaluation on the resulting populations.

In the future, sequencing will be conducted on type 1 and type 3 genomic DNA for the PI region to find the possible sequence difference. Additionally, a silencing of PI in type 1 raspberry is planned as proof that the affected gene is indeed PI. The development of markers suitable for marker assisted breeding following progress made in this project could be beneficial in breeding aimed at large fruited raspberry cultivars.