Naturally occurring flower mutation in off-spring of a large fruited raspberry chance seedling

Dora Pinczinger, Magda-Viola Hanke, Marcel von Reth and Henryk Flachowsky
Julius Kühn Institute, Institute for Breeding Research on Fruit Crops, Dresden
E-mail of corresponding author: dora.pinczinger@julius-kuehn.de

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 to eight sepals and petals, with stamens and carpels present. Type 3 has sepaloid and carpeloid structures, but no petals and stamens.

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

The ABC-model of flower development suggests that type 3 phenotypes are caused by perturbation of class B genes. In other Rosaceae plant species similar phenotypes are caused by mutation of the *PISTILLATA* (*PI*) gene, as *APETALA3* has several homologs and is thus more robust against impairment. We defined MADS- and K-box containing genes from *Rubus occidentalis* (black raspberry) by Hidden Markov Model search. A neighbor joining tree was produced through amino acid sequence homology. Although no homolog for PI was found initially, a BLAST search detected a non-annotated sequence with high homology to *Arabidopsis thaliana PI*.

A PCR with type 1 and 3 genomic DNA was performed. Since fragments were of the same size, we speculated that the difference between type 1 and type 3 is on the sequence level. Indeed, cloning and sequencing of type 1 and 3 cDNA and type 3 gDNA revealed that type 1 translates into 212 and type 3 into 207 amino acids. Both sequences differ in 54 amino acids. This makes impairment of PISTILLATA in type 3 plants a plausible explanation for type 3 phenotypes.

An RT-PCR on type 1 and type 3 whole flowers and individual whorls was conducted. There was substantial reduction of *PI*-levels in type 3 samples. As expected, whole flower as well as petals and stamens displayed the highest *PI*-levels. Moreover, we plan complementation of the *A. thaliana pi-1* mutant with type 1 and type 3 alleles.

To analyze the impact on fruit size, we crossed type 1 and type 3 plants with two tester cultivars. Fruit size evaluation will be carried out in the F_1.

This project will also lead to the development of molecular markers for fruit size, which might benefit current raspberry breeding programs.

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Kontaktadresse/ Contact
Dr. Anja Hühnlein
Julius Kühn-Institut (JKI)
Bundesforschungsinstitut für Kulturpflanzen
Informationszentrum und Bibliothek
Erwin-Baur-Straße 27
06484 Quedlinburg
Germany

Telefon +49 (0) 3946 47-123
Telefax +49 (0) 3946 47-255

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