Juliane Würdig et al.

## Generation of cisgenic apple (*Malus x domestica* BORKH.) with a biotic resistance to apple scab caused by *Venturia inaequalis*

Juliane Würdig, Henryk Flachowsky, Magda-Viola Hanke Julius Kühn-Institut, Institute for Breeding Research on Horticultural and Fruit Crops juliane.wuerdig@jki.bund.de

The cultivated apple (Malus x domestica Borkh.) is one of the economically most important fruit crop worldwide. Among hundreds of excising apple cultivars only a handful of them are favoured by consumers due to their appearance, quality, flavour and storability. These cultivars are all susceptible to different plant diseases like apple scab caused by Venturia inaequalis. The introduction of the natural occurring scab resistance gene HcrVf2 from Malus floribunda 821 by classical breeding is time consuming and expensive, because of selfincompatibility, heterozygosity and a long juvenile period of 5 to 12 years. Genetic engineering offers the opportunity to overcome these limitations. The introduction of HcrVf2 into the genome of existing cultivars via Agrobacterium-mediated plant transformation requires an efficient selection as realized by the neomycin phosphotransferase II marker gene in apple. Such marker genes conferring resistances to antibiotics are not accepted by the consumer and need to be eliminated. A vector was developed allowing the site-specific excision of all unwanted DNA sequences after selection, mediated by a heat-shock inducible expression of the FLP recombinase. The vector contains beside HcrVf2 under its own regulatory elements a recombination cassette flanked by direct repeated flp recognition target sites. The recombination cassette comprises of two marker genes *nptll* and *dao1*, both driven by a CaMV 35S promoter, as well as the flp recombinase gene under control of the heat-inducible *Gmhsp17.5-E* promoter. The second marker gene dao1 codes for the DAAO protein, which converts the nontoxic amino acids D-valin and D-isoleucin to plant toxic products. A further selection of gene modified cells in which the FRTflanked box was removed by recombination is possible. Using this vector gene modified lines were produced and investigated by PCR, RT-PCR. First results will be presented.