6. Summary

The aim of this work was the risk assessment of genetically engineered apple trees in the case of a potential release. One part of the investigations concerns the relative frequency of gene flow from transgenic to non-transgenic apple cultivars in the environment. Another objective deals with the stability of integration and long-term expression of transgenes in vegetatively propagated in vitro plants and after grafting of ex vitro plants in different scion/rootstock combinations. Furthermore the transport of transgene products in grafted ex vitro plants was analysed.

To determine the vertical gene transfer by pollen from one apple cultivar into surrounding populations the pollen transfer rate in dependence of the distance of a pollen dispenser plant was analysed by using molecular and morphological markers. Within an existing apple orchard, 60 apple trees were selected as pollen receptor plants, representing 38 apple cultivars. 15 plants of a hybride of *Malus sieversii* var. *sieversii f. niedzwetzkyana* Dieck. named ‚TNR 31-35' had been selected as pollen dispenser.

This species carries a dominant gene, which is responsible for red pigmentation of the leaves and other parts of the plant. Test-crosses between ‚TNR 31-35' and the pollen receptor cultivars with subsequent phenotypic and molecular rating of the seedlings by SSR-analyses confirmed the dominant inheritance of the red marker gene.

For the assessment of the pollen transport rate, in the years 2003 and 2004, a total of 11058 open pollinated seedlings were phenotypically scored for leaf pigmentation. On the basis of the number of red-coloured seedlings the gene flow frequency was determined for different distances to the dispenser plants. The highest frequency of red-coloured seedlings was present within a radius of 5 to 10m. With increasing distances to the pollen dispenser plants less pigmented seedlings were found. However, the diminishment was not proportional to the distance. Furthermore the results indicated that the pollen transport frequencies appear to be influenced by the bee flight, the position of the bee hive and the orientation.

The investigations on the stability of transgenes were performed on 60 transgenic apple plants, propagated from 14 independent *in vitro* lines. These had been genetically modified with four different gene constructs to increase bacterial resistance. All lines have been positively tested for their integration and expression of the T-DNA after regeneration and were transferred to the green house. Two to four years after regeneration, selected *ex vitro* plants were grafted onto a non-transgenic rootstock or were used as rootstocks for a grafted non-transgenic scion. Following on, the

greenhouse plants were analyzed for transgene integration by PCR and Southern blot analysis. The transgene expression was investigated by RT-PCR and ELISA assay.

Eleven of the investigated lines showed a stable integration of the T-DNA. In five plants of the line T267 the T-DNA was not longer detectable. The loss of the transgenic character of these plants could be caused by propagation of chimera during the in vitro culture. In all plants of line T211 and one plant of line T136 the gene of interest could not be detected by PCR. However the detection of the gene of interest by Southern-blot analysis was possible. This might be a result of rearrangements of the T-DNA.
After southern-blot analysis only two lines had a single copy integration of the T-DNA. For the remaining 12 lines multiple copy of the T-DNA with up to four integration sites were detected. In three lines the copy number between gene of interest and marker gene differed. Size differences of the T-DNA fragment were detected in plants of two lines. The variation in T-DNA size and number of integrations could also be founded in a rearrangement of the T-DNA during the in vitro or ex vitro culture. Possibly, these variations were caused by segregation of chimeric tissue.
For all plants with stable T-DNA integrations also a stable transcription of the transferred genes was observed, except for the plants of line T363. Plants of this line showed a normal transcription of the gene of interest, but for the *nptII* marker gene only weak bands were amplified during RT-PCR. The reduced expression of the *nptII* marker gene is probably a result of methylation of the *nos* promoter site.
For all plants with a stable transcription of the *npt*II markergene, the NPTII protein was also detected by ELISA assay. But for several plants a great variation in the NPTII amount within one line was determined. Continuative analyses of plants of the line T211 showed that the NPTII amount varied within one plant depending on the investigated leaf stage. This result indicated either chimeric plants of T211 or a correlation between the physiological leaf age and the NPTII amount.
In conclusion in this work no correlation between grafting and the stability of transgenes was found within the investigated material. The results indicate that in the course of cultivation of transgene apple plants repeated investigations on the stability of T-DNA integration and expression are necessary.
The analysis on the transport of transgene products was performed for 22 grafted apple plants from eight different lines. These lines were both grafted as transgenic scions and as rootstock in combination with either non transgenic scions or rootstocks. Following on the presence of the transgene product (NPTII- and GUS-protein, respectively) was analysed for both transgenic and non-transgenic parts of the plants.

The presence of the transgene product was only observed in the transgenic part of the graftings. The absence of a transport of transgenic protein is mainly founded in the cytosolic expression of both genes. The used gene constructs had no signal peptide which accomplishes the transport of gene products out of the cell. In this case the transgene expression is controlled by *nos*- or CaMV35S-promoter without a signal peptide, and no transport of transgene products in grafted apple trees was detectable.

The present study on the environmental compatibility of genetically engineered apple plants shows, that the risk of a vertical gene transfer from transgenic apple trees could be decreased by the compliance of safety distances to neighbouring orchards. However, on the basis of this study it is not possible to make a statement in which distance no pollen transport takes place. The analyses on the stability of T-DNA showed that the main part of the investigated apple plants had stably integrated and expressed T-DNA over years. In most cases, a potential loss of the transgenic trait can be detected with the available molecular techniques. By this a selection of stable transgenic plants is possible before a release.
An improvement of the environmental compatibilty of transgenic plants concerning the vertical gene transfer by the combination of transgenic and non transgenic grafting components was not verifiable for the analysed plants.