14. Gene Transfer by Cross-Pollination and Persistence of DNA in the Soil of Test Fields with Transgenic Oilseed Rape

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A risk of transgenic plant release that is frequently discussed is the possibility of gene transfer or gene escape. In Wörrstadt (Germany) a field trial with glufosinate-resistant oilseed rape containing the synthetic phosphinotricin acetyltransferase gene (PAT-gene) (Donn and Eckes, 1992) is being performed over a three-year period (1994 - 1996). The plants are growing on a 960 m^2 center-plot surrounded by a 3 m wide grass border followed by a 8 m wide non-transgenic oilseed rape frame as pollen trap. The objectives of our studies are 1) the estimation of the frequency of *PAT*-gene transfer by pollen dispersal, and 2) the stability of transgenic DNA in the soil and the probability of its uptake by any microorganisms under field release conditions.

1. Pollen dispersal

To estimate the frequency of cross-pollination, seed samples (F1) of the surrounding non-transgenic plants were harvested at a distance of 6 - 8 m from the transgenic rape field. The DNA from individual seedlings was extracted (Edwards *et al.*, 1991) and tested for the presence of the transgene by PCR. A statistically relevant number of ca. 1,000 seedlings were analyzed. Various primers (18mers) were used in PCR amplification of different parts of the PAT-gene-35-s-CaMV-promoter construct, giving rise to DNA fragments between 431 bp (P2/T2) and 514 bp (P1/T1). The amplification of the endogenus rape gene for the phosphoenol-pyruvate-carboxylase (*PEPC*)-gene was used as control. Duplex PCR was performed with primer pairs P2-T2 and PEP1-PEP2. The results shown in Table 1 indicate a rate of outcrossing of 0.9% at a distance of 6 - 8 m, which is significantly higher than previously reported (Scheffler *et al.*, 1993). The difference might be due to differences in the size of the donor plot or to the ratio between donor and recipient plot sizes. Another reason might be the genotypic method of transgene detection.

2. DNA persistence in soil after field tests with transgenic oilseed rape

DNA from transgenic plants released to the soil might be taken up by other organisms and then lead to horizontal gene transfer. The longer transgenic DNA survives in the environment, the higher the probability for such a horizontal gene transfer might be. Therefore, we investigated the persistence of the *PAT* transgene construct as well as of the *PEPC* gene as control in the soil after harvest of the transgenic oilseed rape. Soil samples were collected from the test field at intervals of 4 - 6 weeks. Total DNA was extracted from the soil, purified according to the method of

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Smalla *et al.* (1993) and then analyzed by PCR using *PAT* gene-specific and *PEPC* gene-specific primer. The resulting amplified DNA fragments were tested by gel electrophoresis, dot-blot-hybridization and DNA sequencing. Transgenic and non-transgenic rape DNA was repeatedly detected in soil samples collected up to 102 days after harvest. The PCR results are summarized in Table 2. The prolonged persistence of the rape DNA in soil may be ascribed to different factors : 1) seeds or fragments of seeds may be present in the soil samples, 2) parts of sprouted F1-seedlings may be present, 3) unrotten plant materials may still persist, 4) the DNA may be bound to minerals and thus be protected from degradation or 5) plant DNA may have been taken up by microorganisms,which is unlikely.

The DNA of the soil samples collected at different intervals after harvest of the transgenic oilseed rape plants was tested by PCR with various primer combinations for the presence of plant DNA. In the first column the tested rape genes are shown. In the second column the primers used for the different PCRs are listed (rubisco = ribulose-1,5-bisphosphate carboxylase). "+" = DNA fragment readily detectably after PCR "+" = DNA fragment only present at a low concentration after PCR, "O" = repeatedly negative. The time intervals are shown along the bottom axis. The arrows indicate the soil sample collection times in days after harvest. Oilseed rape DNA persisted in the soil samples up to 102 days after harvest, irrespective of which probe was used for detection.

References

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Table 1 Determination of the outcrossing rate by PAT-gene specific PCR

Number of F1 oilseed rape seedlings from the nontransgenic frame tested	1010
DNA extracts without PCR-result	23
Number of extracts used for the calculation	987
PAT-positive extracts	9
Outcrossing rate in %	0.9

Table 2 Presence of DNA in soil samples