

POLLEN-MEDIATED TRANSGENE FLOW IN TOMATO: A CASE STUDY ON VIRUS-RESISTANT HYBRIDS

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TSWV, virus resistance, field test

There is concern that release of transgenic plants into the environment could allow transfer of the inserted transgenes to neighboring crops. Although tomato is a mostly self-pollinating crop, we determined the flow of transgenic DNA through pollen by analyzing the progeny obtained from control nontransgenic tomato plants present in field tests.

In 1999 two experimental releases of genetically modified (GM) tomato hybrids were performed under notification No. B/IT/99/05. The fresh tomato line under investigation contained two transgenes: the nucleoprotein (N) gene of the tospovirus TSWV-It (*Tomato spotted wilt virus-Italian isolate*), conferring virus resistance; and the neomycin phosphotransferase II gene (*nptII*), conferring resistance to kanamycin. The field tests aimed also to evaluate virus resistance and agronomic performance of the GM hybrids (2).

The first experiment was performed in Central Italy in a plastic greenhouse where no pollinating insects were present. For the second trial, an area in Liguria, Northern Italy was chosen, where TSWV had been present for several years: this experiment was in the open, with no barriers to insects. In both cases fruit were collected from the nontransgenic plots included in the experimental field and from border rows, and seed (about 10,000 in total) were used to estimate the flow of the transgene via pollen. Seedlings were sprayed with kanamycin, then those apparently resistant to kanamycin spraying were analysed by PCR: only 16 plants, originated from the open field, contained transgenic DNA.

These results indicate that: 1) no gene flow was observed in the protected crop, where no pollinating insects were present; 2) in the open, pollen derived from GM plants pollinated nonGM ones at a frequency of 0.32%: this figure is low, considering that all tomatoes were in the same plot, without any distance or barrier between donors and acceptors; 3) this cross-pollination is likely to be due to pollinating insects, since it increases through the summer.

In conclusion, these preliminary data indicate that there is apparently no risk of gene transfer through pollen in tomato when grown as a protected crop, while in the open limited transgene flow occurs. The transgene flow detected is in agreement with the percentages (0.5 to 10%) of cross-pollination reported for tomato. Further and more focused experiments are needed to establish the safety distances or barriers required for transgenic tomato crops in order to minimize risks and assess appropriate safety procedures. The ban on antibiotic resistance genes in transgenic plants imposed by the new regulation in the European Union (EU directive 2001/18) could complicate future work on transgene flow, particularly in species like tomato with low cross-pollination rates.

(1) Accotto *et al.* (2000) - In: *Plant Genetic Engineering: Towards the Third Millennium*. A.D. Arencibia (Ed.), Elsevier Science B.V.2000

(2) Accotto *et al.* (2005). *Phytopathology* 95:800