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# Gene dispersal from transgenic potatoes to conspecifics: a field trial

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**Abstract** Transgenic potatoes containing the marker genes NTP II and GUS were planted in the field and at varying distances (0–1, 1–2, 2–3, 10, 100 and 1l000 m) from them were patches of untransformed potatoes of another variety. All seeds produced by the untransformed potatoes were collected after the flowering season and screened for the presence of the marker genes. Gene dispersal was found to be highest in the immediate vicinity (72%). At the consecutive distances the presence of the gene was more or less constant ( 35%). Thus gene dispersal occurred both over large distances and to a higher extent than has been previously shown. Pollinator availability, as well as the foraging behaviour of the pollinators, are suggested to be important in this study. The plant material used is discussed in the light of sexually selected traits which could have contributed to the high gene dispersal.

**Key words** Solanum tuberosum · Pollen transmission Sexual selection · Pollinator behaviour

## Introduction

Gene technology is giving rise to a whole new set of possibilities in plant breeding (e.g., Goodman and Newell 1985; Ross 1986; Gasser and Fraley 1989). This new technique allows us not only to speed up the rate of acquiring the desired traits expressed within a species, but also opens up the possibility of combining traits of totally different origins. Field tests of transgenic plants are being conducted at an increasing rate in many parts of the world (Gasser and Freeley 1989; Karieva 1993). This, unfortunately, is not without risks, a matter that has been given a lot of attention in the last decade (e.g., Colwell et al. 1985; Williamson 1988; Tiedje et al. 1989; Karieva 1993). The major risk involved in field trials, and in the eventual conventional use of transgenic plants, is the loss of control of the inserted genes. This loss can be a result of either of two processes: (1) the transgenic plant itself develops into a weed which either affects agricultural practises or else invades natural habitats; (2) the genes are transferred by pollen to related species giving rise to hybrids expressing the engineered traits (Ellstrand 1988; Regal 1988; Crawley et al. 1993). Both of these cases can have serious economical and ecological effects. Due to its size, pollen is harder to control than the plants themselves, giving rise to the primary concern with the risks involved following pollen transfer (e.g., Colwell et al. 1983; Ellstrand and Hoffman 1990). It is important, therefore, to understand the processes involved in pollen dispersal and the subsequent fertilisation of the ovules in the recipient plant.

In this paper I present a study on the gene flow from transgenic potatoes to a conventional potato variety. The effect of ecological factors, such as species composition in the study area and pollinator availability, is discussed, as well as the effect of the sexual characteristics of the plants involved.

## Materials and methods

### Plant material

A potato (*Solanum tuberosum*) of the variety Desirée was used as the transgenic plant. It contained two linked marker genes, GUS and NTP II. GUS induces phosphorescence in plant tissues that can be seen in UV-light. NTP II codes for resistance against kanamycin. The receptor potato was of the variety Stina, which produces more flowers and fruits than is normally the case with cultivated potatoes.

### Design of field test

The transgenic Desirée was planted in a square field (121 $m^2$) containing about 700 plants. Surrounding this quadrant was a border of Stina (Fig. 1). The border was 1 m (two rows) on three sides and

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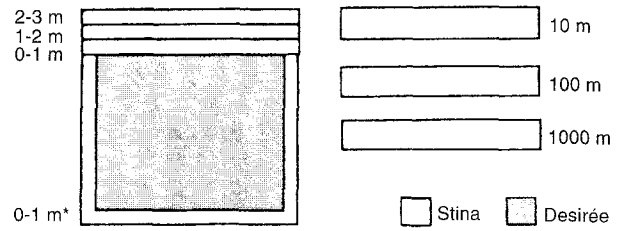


**Fig. 1** A schematic description of the field design. * The 0–1 m distance at the three sides of the transgenic field with only a 1-m border were treated separately from the 0–1 m distance at the fourth border

3 m (six rows) on the fourth. The latter border was divided into three distance groups of 1 m each. I treated the three sides with only a 1-m border separately from the 0–1-m distance in the 3-m border, since the number of recipient (Stina) plants in the immediate vicinity was higher in the latter case (Fig. 1). The total area of the border was 74 $m^2$. At 10, 100 and 1000 m distance from the edge of the transgenic quadrant were patches (20 $m^2$) of the receptor potato Stina. The field was situated in an area that was dominated by cereal crops. The nearest potato field was more than 2 km away.

An estimate of flowering duration and percentage was made once a week during the flowering season. At maturity all berries produced by the receptor potato were collected. The seeds were then frozen and allowed to hibernate for 14 months to ensure a high germination percentage.

### Pollinator studies

The pollinator abundance and species composition were recorded every 2nd day during the joint flowering season (11–31 July). Insects were collected from both potato varieties and preserved in alcohol (2×10 flowers per variety). Visiting insects to ten plants during 5 min were recorded visually. Two to three visual controls per variety were carried out each time.

### Germination tests

Surface sterilisation of the seeds was done through submergence in 70% alcohol for 2 min and then in 1% chlorine solution for 20 min. Afterwards they were rinsed in sterilised water three times. Petri dishes containing two sterile filter papers and 5 ml of kanamycin solution (500 mg kanamycin/l water) were used for the germination tests. In each dish I placed about 50 seeds. The seeds were then allowed to germinate for 3 weeks in 8 °C without light. As a control I used 5 ml of sterilised water instead of the kanamycin solution. This work was done under sterile conditions to lower the incidence of infection of the seeds.

### Gene transmission

Seedlings were tested for the presence of the marker genes with PCR techniques according to Appendix 1. Eight seedlings containing the NTP II gene were checked for the presence of the GUS gene.

## Results

### Flowering duration and percentage

Stina flowered earlier (4–31 July) than the transgenic Desirée (11 July–15 August). The joint flowering time was 3 weeks, giving ample time for pollen transmission. The flowering percentage of the transgenic plants and the surrounding border of Stina was close to 100. Only about 20% of the plants in the patches at 10 and 100 m flowered. At the largest distance (1000 m) 60% of the plants flowered.

**Table 1** Average number of pollinators found in ten flowers or seen visiting ten plants

| Species | Stina | | Desirée | |
|---|---|---|---|---|
| | In flowers | Flying visits | In flowers | Flying visits |
| *Meligethes aeneus* | 22.3 | 0.15 | 17.7 | 0.15 |
| *Diptera sp* | 0.1 | 5.4 | 0.45 | 5.4 |
| *Bombus sp* | 0 | 0.65 | 0 | 0.5 |

### Pollinator availability

By far the most common pollinator was *Meligethes aeneus*, which was present in all flowers in abundance all through the flowering season (Table 1). This insect is very small and it is possible that I sometimes missed seeing it during the visual registrations.

### Germination tests

The germination did not differ between seeds germinating on water and seeds germinated in kanamycin solution, [Table 2 (Spearmans ranking correlation, $r_s$=0.268; $P$>0.05)]. The only effect that could be seen was that the incidence of infection was higher in the Petri dishes containing kanamycin solution (18%) than in the dishes containing only water (2.5%). Infected dishes were not included in the germination comparison since the cause of infection has not been clarified.

### Gene transmission

The GUS gene was present in all eight seedlings tested. This indicates that the two genes are indeed linked, and further tests were only done on the presence of the NTP II gene. The highest gene dispersal (Table 3) was found to be to the potatoes growing in the immediate vicinity of the transgenic field (0–1 m). Here the marker gene was present in more than two-thirds of the seeds. The presence of the gene was lowered by a half at the next distance (1–2 m). Thereafter, at consecutive distances, the gene was more or less constant in occurrence, even including the patch 1000 m away (Table 3). This pattern resembles the gene dispersal found in *Raphanus sativus* by Klinger et al. (1991).

Note that at the closest distance more than half the seedlings were fathered by the transgenic plant, even though the mother plants were surrounded by Stina on three sides.

**Table 2** The germination results from seeds germinated with and without kanamycin

| Distance from transgenic field | Number of seeds tested | | Percent germinated seeds | |
|---|---|---|---|---|
| | Kanamycin | Water | Kanamycin | Water |
| 0–1 m | 3734 | 530 | 35.8 | 36.7 |
| 1–2 m | 2397 | 240 | 50.2 | 55.4 |
| 2–3 m | 417 | 195 | 30.2 | 46.6 |
| 10 m | 525 | 222 | 33.8 | 28.9 |
| 100 m | 915 | 233 | 22.5 | 19.72 |
| 1000 m | 1741 | 287 | 19.5 | 25.5 |

**Table 3** The presence of hybrids between the two potato varieties at different distances from the transgenic field

| Distance from transgenic field (m) | Presence of NTP II in the seedlings (%) | Number of tested seedlings |
|---|---|---|
| 0–1 | 72[a] | 86 |
| 1–2 | 32 | 71 |
| 2–3 | 39 | 18 |
| 10 | 34 | 29 |
| 100 | 36 | 29 |
| 1000 | 31 | 58 |

[a] This is a medium value of transmission to level 0–1 at the three sides of the 1-m border (67% of 45 seedlings) and to the same level at the fourth side with the 3-m border (78% of 41)

Assuming a situation that will give dispersal from Stina the highest chance, i.e., disregarding the effect of distance, Desirée constituted about 60% of the plants in the transgenic quadrant and the surrounding border of Stina. Even so, the presence of the gene differs significantly from the expected 60% given by the availability of pollen ($\chi^2$=5.24, $P < 0.025$). It is further remarkable that seedlings from plants at the 1000 m distance show such a high presence of the gene since there was an abundance of Stina surrounding these mother plants.

## Discussion

In this study genes were found to be transmitted both over larger distances and to a higher degree than has previously been shown for potatoes (Dale et al. 1990; Tynan et al. 1990). For gene transmission to take place, pollen needs not only to be dispersed, but must also compete with other available pollen to ensure fertilisation of the ovules (e.g., Willson and Burley 1983). Pollen deposition in this study could be influenced by ecological factors such as pollinator availability and species composition in the study area, while the plant material used could affect the fertilisation efficiency.

The proportion of fruits sired by a certain variety should equal the proportion of pollen received from that variety, given that there is no pollen competition. This proportion should further reflect the availability of different pollen varieties in the pollen source (Gliddon 1992). If there is no pollen competition one would expect a plant to produce fruits sired by a certain variety in equal proportion to the pollen deposited. The transgenic field and its surrounding border of Stina was a very large pollen source as compared to the patches at larger distances. This was an effect of both the relatively high flowering percentage and the high number of individual plants. It is thus likely that plants at the 10 and 100 m-distance received a high proportion of pollen from the transgenic field and its border, compared to what would have been the case if the closer pollen source had been large (i.e., if there had been a high flowering percentage in these patches).

The difference in pollen source, however, cannot by itself explain the large pollen flow from the transgenic field to the patch at the 1000 m distance. The effect of distance depends largely on the pollinator's foraging behaviour (Schmitt 1980; Levin 1991). When a pollinator moves only short distances between flowers, most pollen will be deposited in the immediate surroundings of the pollen source (Skogsmyr 1992). The flies and bumblebees found in potato flowers in this study are examples of pollinators that mostly fly short distances between flowers (Heinrich 1979). The most abundant pollinator, however, was *M. aeneus*. This is a small beetle that is usually found in large colonies. They emigrate from a patch in large numbers and can then fly over large distances (Sörenson personal communication). Specialised structures on the front legs allow *M. aeneus* to collect large amounts of pollen (C. Nilsson personal communication). The area of the field test was dominated by cereal crops which do not serve as a food source for this beetle. The potato plants were therefore the largest foraging patches available for the beetle. It is thus plausible that the beetles chose the potato patches when emigrating from the transgenic field.

### Pollen competition

Disregarding the effect of distance, the transgenic Desirée constituted about 60% of the pollen source for the border of Stina, implying that the NTP II gene should be present in an equal percentage of the seeds. The presence of the gene, however, was significantly higher than 60% at the 0–1 m distance. This is especially interesting since the recipient Stina plants were surrounded by Stina at least on three sides and, additionally, started to flower a week before Desirée, giving, if anything, an even higher (> 40%) expected pollen deposition from Stina. The presence of the gene in Stina plants was as high at the 0–1 m distance that had the highest availability of Stina pollen (compared to the 1–2 and 2–3 m distances) as the 0–1 m distance with lower availability (the other three sides). Thus more seeds were sired by the transgenic plant than would be expected from the mere availability of pollen, indicating that there is a difference in the fertilisation ability of the pollen grains between the two varieties.

Even though potatoes are self compatible, they show characteristics that indicate previous selection for outcrossing. By this I mean that they produce a lot of pollen

and conspicuous flowers, both of which are efficient in attracting pollinators. Plants outcross to ensure new gene combinations in their offspring (Thompson and Barret 1981). The less related the pollen is, the higher is the genetic difference. It is thus common for outcrossing plants to prefer pollen from less-related individuals. Plants can regulate fertilisation by suppressing growth of unwanted pollen (Willson and Burley 1983). All the receptor potatoes were from the same clone, and thus from a genetic point of view the same individual. It is possible that pollen grains from the transgenic Desirée were preferred since they were genetically more different from the mother individual than the pollen grains coming from Stina. It is a further possibility that the pollen from Desirée had a higher competitive ability, i.e., had a higher germination rate or survival. This would also lead to more seeds fathered by the transgenic plant.

In the dispersal study from potatoes made by Dale et al. (1990) the same variety was used as transgenic and receptor potato, which would not have given rise to the factors discussed above. In the eventual use of transgenic potatoes for commercial purposes, it is plausible that the closest field of conventional potato is of another variety with pollen of a different competitive ability.

Tynan et al. (1990) studied the gene dispersal from transgenic potatoes to wild potato. It is possible that the pollen grains of the wild variety has a higher competitive ability than the cultivated varieties, since cultivated potatoes have been selected to invest resources in potato tubes, rather than in pollen competitive ability. In that case the pollen from the transgenic plant would have difficulty in competing with pollen from the wild variety.

The germination tests did not seem to have any selective effect. This could be the result of the relatively short time allowed for the seedlings to grow, since they were placed on a water solution containing kanamycin rather than on medium. At the beginning of germination the seedlings use resources stored in the seed. Only later do they start resource uptake from the environment. It is also possible that the resistance towards kanamycin is dose dependent so that, even if the seedlings did have a certain uptake of kanamycin during the germination time allowed, it was not large enough to have an effect. To ensure an effective treatment of kanamycin selection it is necessary, therefore, to place the seedlings on medium containing kanamycin.

The results from this study show that under certain circumstances the transmission of genes can be very high as well as occurring over long distances. Even though these circumstances could be unusual, they must be taken into account when evaluating risks involved with transgenic plants. With a fuller understanding of how pollen is dispersed under different circumstances and the mechanisms involved in sexual selection in plants, we should be able to avoid perilous circumstances as well as making better evaluations of risks.

## Appendix 1

DNA preparation

One seedling was crushed in a 1000-µl Eppendorph tube with a glass stick. Four-hundred microliters of PEB buffer (pH 8.0) were then added to each sample. Between each sampling the glass stick was rinsed in 95% alcohol and burnt. The samples were individually marked and treated in sequence throughout the rest of the DNA preparation and PCR reaction to allow detection of eventual contamination. The tubes were centrifuged for 1 min at 13 000 rpm. Three-hundred microliters of the substrate were transferred to a new 1000-µl Eppendorph tube, 300-µl of isopropanol was then added, and thereafter the samples were incubated at room temperature for 2 min. After centrifuging for 5 min at 13 000 rpm, the supernatant was poured off and the pellet was washed in 70% alcohol; then the tubes were centrifuged again for 1 min at 13 000 rpm. The pellet was dried in a vacuum centrifuge, which after 100 µl of TE was added.

PCR reaction

All through the PCR reaction, and the subsequent electrophoresis, new pipette tips were used for each sample. Filter tips were used for the PCR reaction. Thirty-nine and a half microliters of PCR master mix and 10 µl of DNA extract were added to a 600-µl siliconised Eppendorph tube and the samples were placed on ice; 0.5 µl of *Taq*-polymerase and a drop of mineral oil was added to each sample. They were then directly transmitted to the PCR machine. After the PCR reaction the samples were frozen.

| PCR master mix (five reactions) | PCR program |
|---|---|
| 25 µl 10* *Taq* buffer | 94 °C 4 min |
| 15 µl 25 mM MgCl | 60 °C 2 min |
| 20 µl 2.5 mM dNTP | 72 °C 2 min, 1 cycle |
| 1 µl primer 821 | 94 °C 45 s |
| 1 µl primer 822 | 60 °C 45 s, 30 cycles |
| 2.5 µl 10% (v/v) Triton X-100 | 752 °C 7 min, 1 cycle |

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