Theor Appl Genet (1996) 92: 505–508

A. J. Conner · P. J. Dale

Reconsideration of pollen dispersal data from field trials of transgenic potatoes

Received: 26 July 1995 / Accepted: 22 September 1995

Abstract During the initial field evaluation of transgenic plants, it is usual to isolate them genetically from other plants of the same species. Several field experiments on potatoes, using transgenes as markers, have shown that transgene dispersal by pollen to other potato plants is limited and very unlikely at distances over 10 m. In a recent study in Sweden, a frequency of transgene-containing progeny of over 30% is reported from non-transgenic potato plants grown at distances of 10–1 000 m from transgenic plants containing *nptII* and *gus* marker genes. Data from the Swedish study is discussed along with other relevant observations, and it is concluded that the high frequency of gene dispersal in that study results from a high frequency of false positives during PCR analysis of the *nptII* gene. From the data available in potato, it is concluded that a distance of 20 m is generally adequate for the initial field evaluation of transgenic potatoes containing novel gene constructs.

Key words *Solanum tuberosum* · Transformation · Transgene dispersal · Pollination distance · Risk assessment

Introduction

The initial small-scale field testing of transgenic crop plants is usually performed under semi-contained conditions to minimise the dispersal of transgenes into the environment. One of the concerns is transgene transfer by pollen to neighbouring crops of the same or closely related species (Dale 1993). When granting permits for field testing transgenic crops, regulatory authorities generally specify isolation distances from other plants of the same species. For this reason, it is important to determine the distances over which effective gene transfer can occur. From many years of experience of the production of seeds from traditional plant cultivars, isolation distances have become well established for most crops, and these are rigidly observed to maintain seed purity to defined legal specifications (Frankel and Galun 1977). However, for asexually propagated clonal crops, true seed production under field conditions is unimportant, and therefore there is comparably little experience and information.

Potatoes are one of the crops at the forefront of transgenic plant development, with many field trials having been performed around the world (Dale et al. 1993). Because it is an asexually propagated crop, there is very little accurate information on effective pollination distances (Conner 1994). Anecdotal information is available from plant breeders and farmers who occasionally observe berry formation on male-sterile cultivars growing beside a male-fertile cultivar. Such observations suggest that effective pollen dispersal in potato occurs over a few meters only. However, in the absence of quantitative data, regulatory authorities were initially very conservative when specifying isolation distances. During the first field trials on transgenic potatoes in the mid-late 1980s, isolation distances of 600 m and/or flower removal were required. In response to data on transgene dispersal by pollen, obtained during some of these trials, isolation distances have since been substantially reduced to 50 m and less (Dale et al. 1992). However, a recent report has concluded that transgene dispersal via pollen can occur at high frequencies at up to 1000 m from field trials of transgenic potato. In this brief paper, we critically compare and assess the reported data on pollen dispersal from field-grown transgenic potato plants. We also recommend isolation distances to avoid or reduce transgene dispersal by pollen during the initial evaluation of new transgenic lines of potato.

Communicated by J. Mackey

A. J. Conner (✉)
New Zealand Institute for Crop & Food Research,
Private Bag 4704, Christchurch,
New Zealand and Department of Plant Science, P.O. Box 84,
Lincoln University, Canterbury, New Zealand

P. J. Dale
John Innes Centre, Colney Lane,
Norwich NR4 7UJ, United Kingdom

Review of data on transgene dispersal by potato pollen

There have recently been several published reports that quantify the frequency and distance of transgene dispersal during potato field trials (Table 1). These studies have been performed independently in several different countries, using different cultivars and different transgenic phenotypes. All of the studies involved the screening of progeny, obtained from non-transgenic potato plants growing at set distances surrounding the transgenic plants, for the expression of dominant genetic markers transferred from the transgenic potatoes. The majority of these independently performed studies have reported very similar results; the very limited dispersal of pollen over very short distances near the edge of a trial (Tynan et al. 1990; Dale et al. 1992; Conner 1993; McPartlan and Dale 1994). In all of these studies, considerable care was taken to develop stringent screening methods to avoid the appearance of false positives and false negatives when assessing large progeny populations. Large populations of non-transgenic seedlings were screened to confirm the virtual absence of escapes through the selection schemes (false positives), and transgenic seedlings were used as positive controls to confirm the reliability of selection protocols (absence of false negatives) (Tynan et al. 1990; Dale et al. 1992; Conner 1993). Furthermore, the transgenic status of positive seedlings was verified by independent biochemical and molecular tests (see Table 1). In these studies, care was also taken to synchronise flowering of the transgenic plants and the non-transgenic pollen-trap plants by either using the same potato cultivar and/or the removal of flowers.

In marked contrast to these reports of negligible dispersal of transgenic pollen, a more recent study from Sweden has reported high frequencies of transgenic pollen dispersal up to 1000 m from a field trial of transgenic potato (Skogsmyr 1994). The Swedish report includes elaborate explanations, involving sexual selection in plants and selective foraging behaviour of pollinating beetles, to account for the much higher than expected frequency of pollen dispersal over very long distances. However, a close examination of the experimental methods used in this latter study raises serious doubts over the validity of the data. Since this study was published in a reputable international scientific journal, authorities in regulatory agencies may accept the conclusions without question. A critical assess-

Table 1 Summary of studies on transgene dispersal from field trials of transgenic potatoes

Reference	Tynan et al. 1990	Conner 1993	Conner 1993	Dale et al 1992 McPartlan and Dale 1994	Skogsmyr 1994	Erjefält unpublished[a]
Details of field trial						
Location	Lincoln, NZ	Lincoln, NZ	Lincoln, NZ	Cambridge, UK	Svalöv, Sweden	Svalöv, Sweden
Year	1988/89	1988/89	1989/90	1989	1991	1991
Transgenic cultivar	Iwa	Rua, Iwa, Ilam Hardy	Iwa	Désirée	Désirée	Désirée
Transgenes	*nptII, gus, als*[b]	*nptII*	*nptII, gus, als*	*nptII, gus*	*nptII, gus*	*nptII, gus*
Non-transgenic pollen-trap cultivar	Iwa	Mixed breeding lines	Mixed breeding lines	Désirée	Stina	Stina
Genetic marker screened	Chlorsulfuron resistance	Kanamycin resistance	Chlorsulfuron resistance	Kanamycin resistance	PCR for *nptII*	Tuber colour
Confirmation of transgenic status	GUS activity	NPTII enzyme assay	GUS activity	Southern analysis	Not performed	Not performed
Frequency of transgenic progeny in pollen-trap cultivar						
Distance from transgenic plants:						
Adjacent rows (less than 1 m)	1.14%	0.19%	0.046%	23.64%	72%	1.3%
Up to 3 m	0.04%	0.00%	0.000%	2.07%	34%	0.5%
3–9 m	0.02%	0.00%	0.008%	–	–	–
10 m	0.00%	–	0.000%	0.02%	34%	–
20 m	–	–	–	0.00%	–	–
100 m	–	–	–	–	36%	–
1000 m	–	–	–	–	31%	0.00%
Total number of progeny screened	61,381	45,640	306,532	29,412	291	523

[a] The data from Erjefält was from a naturally occurring tuber-colour marker gene present in the transgenic variety 'Désirée' used in the study

[b] *nptII*, Neomycin phosphotransferase; *gus*, beta-glucuronidase; *als*, chlorsulfuron resistance

ment of this paper is therefore important, as the results may be used to establish government policy and guidelines for isolation distances of field trials with transgenic potatoes.

Assessment of the Swedish study

In the study by Skogsmyr (1994), there are a number of features associated with the experimental design, and the assay used for screening the potato seedlings that suggest the results are incorrectly interpreted. Some of these points are not apparent in the paper, but have become evident upon subsequent communications with the author and with the company that developed the transgenic potatoes and performed the field trial.

During the Swedish study, the 'Stina' non-transgenic potato cultivar used as a pollen-trap (or pollen recipient) flowered for a period of 27 days, of which the last 20 days coincided with flowering of the transgenic 'Désirée' plants. Since the field trial was isolated 2000 m from the nearest other potatoes, all of the berries forming on 'Stina' during the first 26% of its flowering period would be expected to result from self pollination within this cultivar. Assuming an even spread of flowering and seed set during the season, this would amount to 26% of all the seeds developing on 'Stina' plants. On the basis of this reasoning, the maximum possible frequency of 'Stina' derived seeds resulting from pollination by transgenic 'Désirée' plants is estimated to be 74%. The Skogsmyr (1994) study reports a frequency of 72% transgenic progeny from the pollen-trap 'Stina' plants within 1 m of the transgenic plants. This means that virtually every seed developing on the 'Stina' plants during the period of synchronised flowering would have arisen from the transgenic 'Désirée' pollen. Since 'Stina' is highly self-fertile, this seems exceedingly unlikely; even for elaborate hypotheses involving specific foraging behaviour by pollinating insects, or highly selective pollen competition of outcrossed over selfed pollen.

The transgenic 'Désirée' plants growing in the field trial were maintained clonally since transformation. Consequently, the plants in the field trial would have been hemizygous for the transgenic loci, and the progeny derived from these plants would segregate for the transgenic trait. Neither Southern analysis nor inheritance studies were performed on the transgenic 'Désirée' plants, so the number of T-DNA inserts in the field-grown plants is unknown. However, the simplest model involves a single insertion locus into an autotetraploid potato cultivar to give an allelic configuration in the simplex state (Conner 1994). Upon outcrossing this would result in 50% of the progeny without the transgenes. The comparable figures for two, three and four unlinked transgene copies would be in 25%, 12.5% and 6.25% respectively, of progeny without the transgene. When this is coupled with the calculated 74% of 'Stina' progeny that could have resulted from outcrossing with transgenic 'Désirée', the maximum possible frequency of transgenic progeny in the pollen-trap cultivar is 37% (one copy), 56% (two copies), 65% (three copies) and 69% (four copies). In each case, this is a lower percentage than the reported 72% of transgenic progeny from the pollen-trap plants growing close to the transgenic 'Désirée' plants.

On the basis of these considerations and others to be considered later, the reported results are likely to be a consequence of a high frequency of false positives. This is not uncommon when using such a highly sensitive assay as the polymerase chain reaction (PCR). When performing PCR reactions molecular biologists generally recognise the absolute care required to avoid false positives arising from contamination of solutions, the sample, or the environment.

Unfortunately, there were no controls in the Skogsmyr (1994) study to determine the frequency of false positives in the PCR reactions. In particular, the screening of a large number of self-pollinated pollen-trap seedlings would have been a critical control. False positives could have easily arisen from the samples, since many of the seeds were contaminated with microbes (Skogsmyr 1994), and the *nptII* gene is widespread in many microbe communities (Nap et al. 1992). This is highly possible given the extremely sensitive nature of PCR, and the fact that the *nptII* primers used for the PCR reaction were the 821 and 822 sequences described by Hamill et al. (1991), which are based on the coding region of the *nptII* gene.

Of the 291 potato seedlings screened by Skogsmyr (1994) for the *nptII* gene by PCR, 68 were reported to be positive. However, there was no confirmation of the transgenic status of these seedlings using a different independent assay such as Southern analysis or gene expression. This could have been very simply performed by a histochemical assay for GUS expression or assessment of tuber colour (see below). Eight seedlings containing the *nptII* gene were also found by PCR to contain the *gus* gene. However, these seedlings arose from the transgenic 'Désirée' plants, and not from the pollen-trap 'Stina' plants.

Re-interpretation of the Swedish results

Given the above arguments, we propose that the frequency of false positives in the Skogsmyr (1994) study is likely to be about the level reported at 10–1,000 m from the transgenic plants (31–36%). If this background level is subtracted from the reported figures, then the Skogsmyr (1994) data can be corrected as follows: approximately 38% of potato progeny in rows adjacent to the transgenic plants resulted from transgenic pollen, with no effective gene flow by transgenic pollen dispersal over longer distances. When the experimental error associated with the very small sample sizes used by Skogsmyr (1994) is taken into consideration, this corrected result is not dissimilar to the results reported in other studies (see Table 1).

This interpretation of the published results is supported by the unpublished data of Lennart Erjefält from Svalöf Weibull (see Table 1), which were obtained from the same field experiment and seed samples used by Skogsmyr

(1994). These data were based on the red-skinned tuber colour in 'Désirée' as a dominant genetic marker. In contrast, the pollen-trap cultivar Stina has yellow-skinned tubers, and self pollinated Stina plants never have red-skinned tubers. Controlled crosses between 'Stina' (seed parent) and 'Désirée' (pollen parent) resulted in 332 progeny with red-skinned tubers and 352 progeny with yellow-skinned tubers (chi square=0.58, $P \approx 0.5$ for a 1:1 segregation ratio; L. Erjefält, unpublished results). These segregation results confirm the value of using red-skinned tubers as a dominant genetic marker for gene flow in potatoes. They also confirm the heterozygous basis of the tuber colour locus in 'Désirée', which is present in the simplex state (Aaaa). This is the same allelic configuration expected for a single transgenic locus in clonal potatoes (Conner 1994).

The use of red-skinned tuber colour as a genetic marker to monitor pollen flow from the Swedish field trial on transgenic 'Désirée' produced virtually identical results to the other studies from New Zealand and the United Kingdom (Table 1). Since these results are based on the same seed samples used in the Skogsmyr (1994) study, they substantiate our assertion that this latter study is based on a high frequency of false positives rather than on actual gene flow.

Conclusion

After careful consideration of the published data on effective pollen dispersal of transgenes from field trials of transgenic potatoes, we recommend that isolation distances of 20 m are sufficient to mimimise pollen-mediated escape of transgenes from potato. We anticipate that once initial field trials have established that the transgenic phenotype has negligible environmental impact, this recommended distance can be further relaxed during scale-up field trials and commercial release of transgenic cultivars.

Acknowledgements We thank Io Skogsmyr for answering some queries regarding her paper, Per Hofvander of Svalöf Weibull for information on the transgenic potatoes and the field trial used in the Skogsmyr study, and Lennart Erjefält of Svalöf Weibull for permission to incorporate his unpublished data into this paper.

References

Conner AJ (1993) Monitoring "escapes" from field trials of transgenic potatoes: a basis for assessing environmental risks. In: Seminar Sci Approaches Assessment Res Trials Genet Modified Plants. Organisation for Economic Co-operation and Development, Paris, pp 34–40

Conner AJ (1994) Analysis of containment and food safety issues associated with the release of transgenic potatoes. In: Belknap WR, Vayda ME, Park WD (eds) The cellular and molecular biology of potatoes, 2nd edn. CAB Int, Wallingford, pp 245–264

Dale PJ (1993) The release of transgenic plants into agriculture. J Agric Sci 120:1–5

Dale PJ, McPartlan HC, Parkinson R, MacKay GR, Scheffler JA (1992) Gene dispersal from transgenic crops by pollen. In: Casper R, Landsmann J (eds). The biosafety results of field tests of genetically modified plants and microorganisms. Proc 2nd Int Symp, published by: Biol Bundesanstalt Land- und Forstwirtschaft. Braunschweig, Germany, pp 73–78

Dale PJ, Irwin JA, Scheffler JA (1993) The experimental and commercial release of transgenic crop plants. Plant Breeding 111:1–22

Frankel R, Galun E (1977) Pollination mechanisms, reproduction and plant breeding. Springer, Berlin Heidelberg New York

Hamill JD, Rounsley S, Spencer A, Todd G, Rhodes MJC (1991) The use of the polymerase chain reaction in plant transformation studies. Plant Cell Rep 10:221–224

McPartlan HC, Dale PJ (1994) An assessment of gene transfer by pollen from field-grown transgenic potatoes to non-transgenic potatoes and related species. Transgenic Res 3:216–225

Nap JP, Bijvoet J, Stiekema WJ (1992) Biosafety of kanamycin-resistant transgenic plants. Transgenic Res 1:239–249

Skogsmyr I (1994) Gene dispersal from transgenic potatoes to conspecifics: a field trial. Theor Appl Genet 88:770–774

Tynan JL, Williams MK, Conner AJ (1990) Low frequency of pollen dispersal from a field trial of transgenic potatoes. J Genet Breed 44:303–305