

Determination of the influence of the size of pollen donor field on the rate of GMO in a conventional maize field using SSR analysis

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Four different Bt yellow kernel maize hybrids were sown in the centre of a conventional white kernel maize field, each occupying increasingly larger surface areas. Yellow kernels in white cobs resulting from cross-fertilization were collected along a transect that traced the dominant downwind direction. Varietal identification was performed by means of simple sequence repeat (SSR) analysis. While changes in the size of the donor field clearly influence the % of GMO detected in the conventional field, this effect is moderate. Doubling the size of the donor field produces an increment of about 8% on the GMO rate in the receptor field, within the size range studied.

Introduction

Several factors influence the adventitious presence of GM material in conventional products. These include seed impurity, sowing equipment and practices, cross-fertilization between GM and non-GM crops, the presence of volunteers, and product mixtures occurred during harvesting. Of these, cross-fertilization is the major potential biological source of on-farm mixing in maize (Devos et al., 2008).

While field trials have been carried out on isolated fields of different sizes, as far as we know, no study has been carried out in which fields of different sizes were used simultaneously to study cross-fertilization rate in a given field.

We present a field trial aimed at establishing the effect of GM maize fields of increasing size on gene flow rate by means of simple sequence repeat (SSR) analysis. The SSRs are highly polymorphic, codominant markers that contain a short nucleotide sequence (1 to 3 bp) repeated in tandem (Hamada et al., 1982). The SSR markers have proven useful in genetic characterization of cultivars. They have been used as genetic markers for parentage analysis, varietal identification, and genetic map construction in plants such as *Arabidopsis* (Depeiges et al., 1995), melon (Oliver et al., 2001), *Fragaria* (Sargent et al., 2006), wheat (Plaschke et al., 1995) or maize (Senior, Heun, 1993; Smith et al., 1997).

Here, we used SSRs to identify the origin of maize pollen from a neighbouring nucleus formed by four varieties of yellow GM maize spatially distributed in fields of different size. Varietal identification of yellow kernels in a conventional white kernel cultivar was performed to demonstrate the influence of field size.

Material and Methods

Samples were obtained from a field trial conducted in Pla de Foixà (Girona) during the 2004 growing season. Four different insecticide resistant (Bt) yellow kernel commercial hybrids derived from Mon810 (Monsanto Co) were sown in the centre of the field, forming a rectangle of 4 hectares (ha) whose diagonal followed the dominant wind direction (Figure 1). The surface area covered by each variety was: Aristis Bt (Nickerson-Senasa), 0.25 ha; DKC6575 (Monsanto Co), 0.75 ha; PR33P67 and PR32P76 (Pionner Hi Bred), 1.25 and 1.75 ha, respectively. Non-transgenic white kernel maize hybrid PR32Y52 (Pionner Hi Bred) was sown around them, covering a total area of 27 ha. A neighbouring plot was sown with a conventional yellow kernel hybrid variety called Eleonora which had some influence in the field trial.

For the SSR study, a transect was drawn from the northwestern corner of the GM nucleus in the main wind direction (southeast), and samples were collected at different distances (0, 2, 5, 10, 20, 40, 80 and 120 m). Given that a 10 m path separated the sampled conventional field from the GM nucleus (Figure 1), each sampling distance was actually located 10 m further from the GM field, for example; samples at 0 m were in fact located at 10 m from the GM field.

Note that by maintaining a rectangular shape in the donor field and a constant distance from the GM field border to each sampling point, this field trial design allowed us to stress the influence of field size on gene flow. Therefore, we were interested in comparing the flow from Aristis Bt (0.25 ha), not with that from DKC 6575, but with the flow from Aristis Bt + DKC 6575 (1 ha),

Yellow grains in white cobs were sown in greenhouse conditions for two weeks, until plants reached an early vegetative stage. The DNA from these plants was extracted and analysed using SSR markers to detect their male parental origin.

We selected five primers to identify the five potential male parental hybrids: phi015, phi056, phi072, phi073, and phi085. As all samples were coming from white cobs, the female parental line was always PR32Y52. Markers were used in robustness order, determined by several identification probes performed with commercial seeds of each hybrid variety.

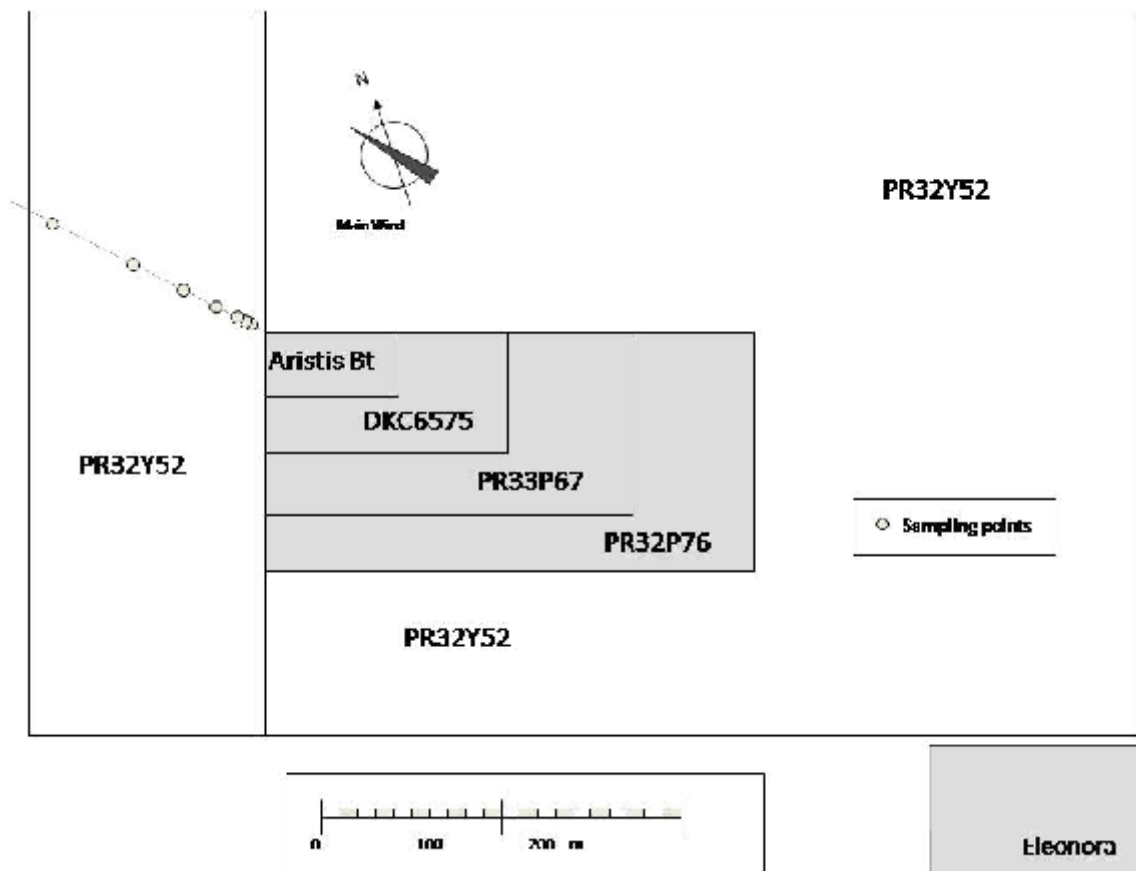


Figure 1. Plot schematic. The white kernel variety surrounding the four yellow kernel varieties (in the grey rectangle) and their relative surfaces are shown. The main wind direction and the relative position of the field sown with the conventional yellow kernel variety Eleonora are also shown.

Results and discussion

Table 1 shows gene flow values (% of yellow grains in white cobs) from the samples collected in this field trial. The percentage of cross-fertilization was very high at a 10

m distance from the donor field (at the conventional field border), whereas it decreased dramatically towards the centre of the field. This distribution follows a typical pattern, already described by several authors, in which the percentage of gene flow is high within the first 15 meters from the donor field but decreases below 0.9% at further distances (Della Porta *et al.*, 2006; Messeguer *et al.*, 2006; Weber *et al.*, 2007).

Likewise, we can observe the same pattern in Figure 2, where GM levels at 10 m from the donor field are much higher than those at 12 m or 15 m. Within the first 10 m all varieties greatly contributed to gene flow, especially Aristis Bt, which is responsible for 74.59% of the total gene flow in the receptor field at a 10 m distance (Table 1). As the distance increases, Eleonora influence becomes more important. However, since total cross-pollination reaches very low values (0.14% at 130 m), the significance of this variety in the total gene flow is very low.

Distance	n. cobs	% cross	SE	Aristis Bt	DKC 6575	PR33 P67	PR32 P76	Eleonora	Off Type	TOTAL
10	15	4.85	0.60	74.59	9.90	9.24	4.29	1.65	0.33	303
12	20	1.30	0.12	69.12	7.35	14.71	2.94	4.41	1.47	68
15	44	0.79	0.21	64.00	4.00	26.00	2.00	4.00	0.00	50
20	46	0.50	0.05	70.89	7.59	15.19	3.80	1.27	1.27	79
30	69	0.35	0.04	59.72	12.50	18.06	5.56	4.17	0.00	72
50	86	0.29	0.02	54.88	10.98	25.61	2.44	2.44	3.66	82
90	470	0.10	0.03	36.81	12.09	35.71	6.04	7.69	1.65	182
130	374	0.14	0.10	28.57	18.37	39.80	5.10	8.16	0.00	98

Table 1. Cross-fertilization calculated as the percentage of yellow kernels found in the cobs. The cob is considered the experimental unit for calculating the standard error (SE). Gene flow and standard error of the samples at different distances from the donor fields are shown. In order to reach a minimum significant number of yellow kernels, an increasing number of cobs had to be collected as the distance increased. The percentage of kernels for each variety at each distance is also shown, as well as the total number of yellow grains analysed. Few samples did not fit to any of the expected patterns and were determined as Off type kernels, probably coming from other neighbouring fields.

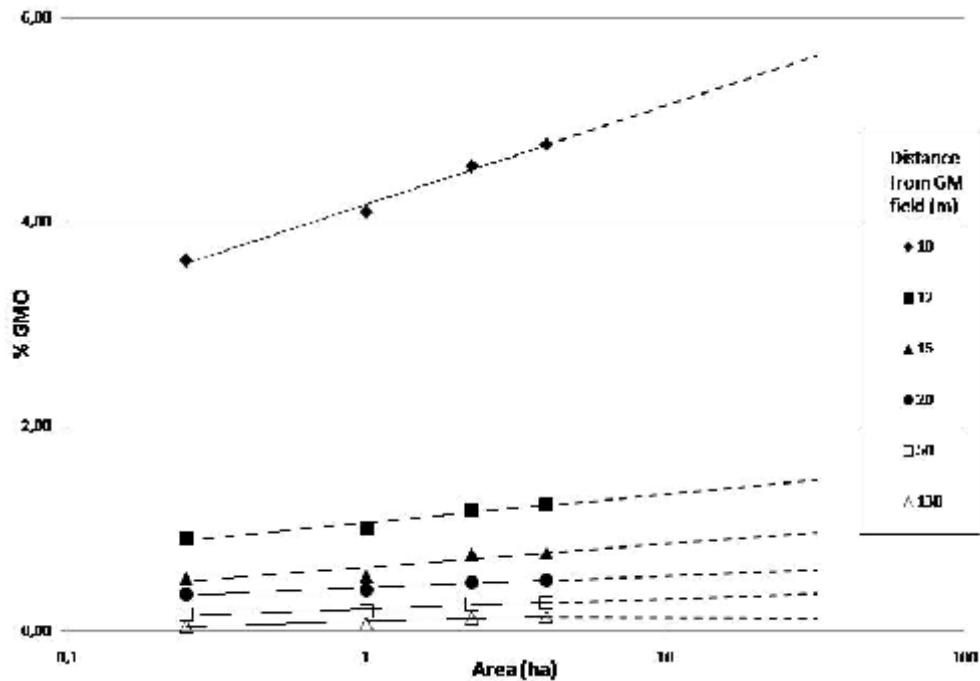


Figure 2. Percentage of GMO related to different donor field areas for each distance from the donor field obtained by SSR analysis. In the abscises axis, four points corresponding to four donor field areas (Aristis Bt = 0.25 ha; Aristis Bt + DKC 6575 = 1 ha; Aristis Bt + DKC 6575+PR33P67=2.25 ha; Aristis Bt + DKC 6575 + PR33P67 + PR32P76 = 4 ha) have been represented in a logarithmic scale. The six lines correspond to six representative distances from the GM field. Extrapolation of tendency lines traced over these points has been represented with discontinuous lines, until 32 Ha, which is the maximum size for maize fields in Spain.

As observed in figure 2, the origin of all regression lines tends to 0 as the donor field area decreases, indicating a very low background noise in the assay. Therefore, we can consider that all yellow kernels were product of cross-pollination with neighbouring fields.

Concerning the SSR analysis, results show that when doubling the donor field area, the GM content of the receptor field at short distances (10 - 20 m) increases 8%. Similar results appear in Figure 2: the GM content in the receptor field increases 16% when the donor plot surface area increases 4-fold (from 0,25 to 1 ha or from 1 to 4 ha).

The results from this assay contribute to understand the influence of GM field size on gene flow and will help to interpret and extrapolate results from future field trials.

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