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High crop barrier reduces gene flow from transgenic to conventional maize in large fields

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ABSTRACT

To test the effectiveness of high crop barriers in reducing gene flow, we conducted experiments in two large fields (around 750 m × 750 m) using yellow transgenic maize and white conventional maize in the growth season of 2013, with *Sorghum* as a high crop barrier in a shape of 5 m zone. *Sorghum* barrier affected pollen load at different directions, decreasing pollen number in further locations. It also decreased the cross-pollination incidence between transgenic and non-transgenic maize, with a average rate of 9.35% in the open site and 1.04% in the *Sorghum* site. But *Sorghum* barrier had little effect on the maximum distance of pollen flow and cross-pollination, which depends on wind direction and speed. The maximum distance of gene flow from transgenic to conventional maize was 300 m in the open site and 350 m in the *Sorghum* site. High crop barrier could be proposed as an effective method to reduce the frequency of gene flow from transgenic to conventional crops and to regulate their coexistence.

1. Introduction

As genetically modified (GM) crops are cultivated worldwide, one major concern that emerges over the ecological consequences is gene flow from GM crops to other plants, including their wild relatives and fertilized non-GM crops (Demont and Devos 2008; Liu et al., 2013a,b). This will lead to the adventitious presence of GM materials in non-GM crops (Devos et al., 2009). Without any regulations, farmers can choose to grow either GM or GM-free plants in their fields. The coexistence of GM and conventional crops depends in part on adopted coexistence policy, consumer choice and economic incentives (Demont and Devos, 2008; Jank et al., 2006). For example, in the European Union, the tolerance threshold of GM material is 0.9% in food, feed and organic products, while there is no a threshold officially in seed regulation (European Commission).

Previous studies have focused on gene flow from transgenic crops to landraces and wild relatives and the coexistence of transgenic and non-transgenic progenies (Liu et al., 2010; Liu et al., 2013a,b; Stewart et al., 2003). The coexistence of transgenic and non-transgenic crops, however, has been largely ignored (Hall et al., 2000; Liu et al., 2015). Moreover, ecological risk of crop-crop gene flow might be higher than that of a crop-wild system because no cross barrier exists between intraspecies crops (Hall et al., 2000). Breeding, agronomic and molecular strategies are employed to mitigate transgene flow (e.g. Liu et al., 2013). Isolation distance is in general considered as one physical mean to reduce gene flow from GM crops to conventional crops and thus to regulate their coexistence in agriculture production. The recommended separation distance for forage maize and sweetcorn is 80 m and 200 m respectively to maintain a 99% grain purity within traditional plant breeding fields (Ingram, 2000). In China, the obligatory isolation distance between GM and non-GM maize fields is 300 m (China Biosaftey Committee). However, it is unlikely to conduct an isolation distance of 200 m or 300 m in real fields, particularly in regions with small-sized fields, because this will harm the farmers' economic benefits.

Previous studies have found that an isolation distance of 40 m or even 20 m is adequate to keep GM presence below the legal threshold of 0.9% (Messeguer et al., 2006; Pla et al., 2006; Riesgo et al., 2010). In agricultural activities, mandatory distance limits the choice freedom of farmers to grow crops in farmlands (Devos et al., 2008). Thus, besides isolation distance, other physical means, e.g. cultivating gap or high barrier crops, are employed to decrease the isolation distance and reduce gene flow. However, gap crops (e.g. barley and sunflower) did not reduce the frequency of pollen-mediated gene flow between yellow kernel GM maize and non-GM white kernel maize (Langhof et al., 2013; Langhof et al., 2008).

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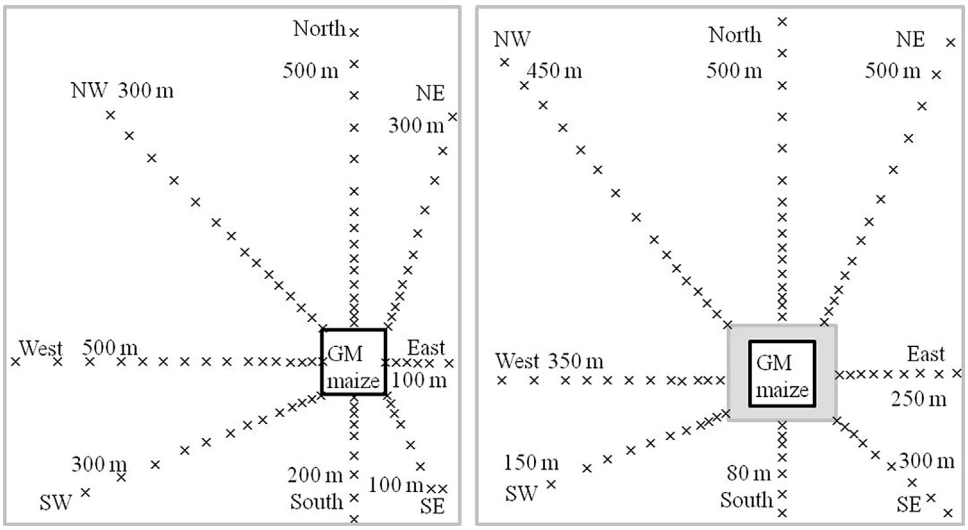

Fig. 1. Map of the open and *sorghum* barrier experiments. Left, open site; right, *sorghum* site. GM maize, transgenic phytase maize with 40 × 40 m cultivating area. Crosses represent the white conventional maize locations (2 × 5 m). Gray shadow around GM maize area is a 5 m zone of *sorghum* plants as high crop barrier.

Thus, this study aimed to test the effectiveness of high crop barrier (around 3 m high *Sorghum*) in reducing gene flow from GM yellow kernel to non-GM white kernel maize and the maximum distance of pollen flow and cross-pollination of maize in large fields. Maize is monoecious and diclinous and easy to cross-pollinated as it is protandrous, with pollen being shed before the pistils being receptive. Although maize pollen grains are relatively large and heavy, they can disperse long distance on the airflow in case of suitable meteorological conditions.

2. Materials and methods

2.1. Plants

Transgenic maize (*Zea mays* L.) conferring phytase gene (BV-LA430101 line), donated by Beijing Origin Seed Technology Inc., produces yellow seeds. Conventional maize (Haiyu-1 line), donated by Shandong Denghai Seeds Co., Ltd., produces white seeds. The inheritance of the seed (kernel) color can be considered to be a single gene, with one pair of alleles (yellow vs. white). The yellow allele is dominant, and the white allele is recessive. The two maize lines are medium maturing varieties, and the growth period is around 110 days. A local *Sorghum* variety (*Sorghum bicolor* L.) (Luliang-2 line) in Shandong province was chosen to be high barrier plants because its height achieves over three meters.

2.2. Experiment design

To detect the efficiency of *Sorghum* barrier in reducing gene flow from transgenic to conventional maize, open and *Sorghum* experiments were conducted in Jiyang county (36°58′N, 117°13′E), Shandong province. The experiments were carried out in two large fields (around 750 × 750 m each; Fig. 1), with one kilometers distance between the two fields. Since the wind during maize growth season is mainly blowing to north and southwest, transgenic maize was planted (0.5 × 0.25 m space) in a near southeast zone (40 × 40 m) to ensure the north distance up to 500 m that could detect the maximum distance of pollen flow and cross-pollination (Fig. 1). For the *Sorghum* experiment, *Sorghum* plants were cultivated (1.0 × 0.25 m space) around transgenic maize in a shape of 5 m zone (Fig. 1). Ten conventional maize plants were planted (0.5 × 0.25 m space) in two rows perpendicular to each direction at each location that was 1, 3, 5, 7, 10, 15, 20, 25, 35, 45, 60, 80, 100, 150, 200, 250, 300, 350, 400, 450 and 500 m away from the edge of transgenic maize zone. The location area was 5 × 2 m. The maximum distance of locations planting conventional white maize was different at each of eight directions, east (E), east-south (ES), south (S), west-south (WS), west (W), west-north (WN), north (N) and east-north (EN) (Fig. 1, Table 2). There were 134 locations in total at the open experiment and 139 at the *Sorghum* one.

In order to maximally overlap the florescence of transgenic and conventional maize, seeds of transgenic maize were sown directly in the field for three times, on June 30, July 3 and July 6, 2013. Seeds of conventional maize and *Sorghum* were sown on July 3, 2013.

Table 1
Mean (±se) values of pollen density and cross-pollination rate at each location in eight directions of the open and *sorghum* experiments.

	Pollen density (0.05 m^2)		Cross-pollination (%)	
Direction	Open	*Sorghum*	Open	*Sorghum*
East	2422 ± 839	934 ± 743	14.2 ± 6.95	3.83 ± 2.66
West	1107 ± 464	965 ± 577	7.90 ± 4.43	0.62 ± 0.27
South	917 ± 464	1861 ± 993	6.54 ± 3.71	0.46 ± 0.35
North	1054 ± 331	2233 ± 1226	13.3 ± 5.31	1.45 ± 0.80
NE	895 ± 425	4536 ± 2434	12.6 ± 6.09	1.45 ± 0.71
SE	468 ± 137	2567 ± 1395	10.6 ± 4.38	0.18 ± 0.11
NW	241 ± 121	272 ± 149	9.58 ± 5.43	0.11 ± 0.05
SW	1005 ± 387	885 ± 527	2.83 ± 1.84	0.12 ± 0.07
Total	1008 ± 1742	1791 ± 5434	9.35 ± 18.8	1.04 ± 3.62

2.3. Pollen collecting and cross-pollination analyzing

Pollen collecting was manipulated for three sunny days during the flowering period, September 5–7, 2013. Ten transgenic maize plants per direction at the full-bloom stage were randomly chosen to mark their pollen using brilliant blue staining. The marking time was at 6:30–8:00 a.m. every day. After 12:00 a.m., pollen grains were tallied on five sticky traps (glass slides, 0.10 × 0.10 m) coated with Tween20 at every location from 1 m to 500 m lied in eight directional transects (Fig. 1). Sticky traps were fixed on maize stems 1.2 m above the earth to match the height of flowering maize. Sticky traps were replaced every day. Meteorological data, including wind speed and direction, air temperature, precipitation, atmospheric pressure, and moisture were recorded in a micro meteorological station in the field. Pollen was counted using a haemocytometer and a light microscope under 10× magnification.