

Gene flow from transgenic oilseed rape to Chinese conspecific landrace under field conditions and its implication for China

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Abstract

Quantifying pollen-mediated gene flow from genetically modified (GM) crops to non-GM crops or to related weedy species is vital for assessing the environmental impact of GM crops. This study quantifies the gene flow that occurs from transgenic oilseed rape (OSR) to nearby Chinese landrace Xiangyou15 in the agricultural fields of Jianli county, Hubei province, southern China, in 2004. Natural hybridization events between the two species were detected using GFP-meter in the field. The influence of the wind on pollen dispersal and gene flow were also evaluated. Preliminary results from this field showed that gene flow from transgenic OSR to nearby Chinese landrace crop occurred at a considerable rate. The maximum gene flow frequency observed was 16.17% at nearest to the common border. The minimum gene flow frequency observed was 0 or 0.03%. Moreover, frequencies of the gene flow decreased significantly with distance from the pollen sources. The results also indicated that the dominant wind of the region might strongly influence the gene flow rate between transgenic and non-transgenic plants. Therefore, isolation measures should be considered when deploying transgenic OSR in the coexist regions of non-GM OSR. The traditional agronomic system presented in this region could facilitate the gene flow as more pollen sources present, where crops are planted in many small patched farmlands adjacent to each other. It is suggested that the level of transgene escape (gene flow) will be high if transgenic OSR or any other cultivar is planted in such system. Management practices employed by the growers, as well as protocols followed for the isolation distances are needed to minimize gene flow.

Key words: Hybridization rate, *Brassica napus*, transgene escape, pollen source, winter oilseed rape area

Introduction

There are much considerable public and scientific debate for and against genetically modified (GM) crops. Among concerns of opponents to these crops are claims that pollen movement will cause unacceptable levels of gene flow from GM to non-GM crops or to related weedy species, resulting in genetic pollution of the environment. Therefore, quantifying pollen-mediated gene flow is vital for assessing the environmental impact of GM crops.

As one of the main GM crops, *Brassica napus* (oilseed rape) is now widely grown in North America, with proposed commercial release into Australia and Europe. *Brassica napus* is self-fertile, with pollen movement by both wind and insects (Williams, 1984; Williams et al., 1987). Reported gene flow in OSR although highly variable, generally shows that as the distance between the pollen source and the recipient population increases, the degree of outcrossing between populations is sharply reduced. Although the vast majority of *B. napus* pollen falls within a few meters of the plant, pollen flow from *B. napus* fields can be substantial, vary and extend over long distances mainly depending on the area of pollen source (Wei et al., 1999). Other factors that also affect the distance gene flow should be taken into account of the risk assessment, such as fertility of recipient plants, presence of insect vectors (Williams et al. 1987) and environmental conditions, such as temperature, wind speed and direction, relative humidity, etc.

Even in a country such as China where commercially planted transgenic oilseed rape has not been permitted yet, the potential risk of transgene flow has existed there from the importation of transgenic rapeseed for human consumption (ca. 60% was of transgenic origin). During transport and distribution (Thomas et al., 1991) seeds could be spilled, which could result in volunteer and feral population of *B. napus* and potentially increase the risk of transgenic introgression to other oilseed rape or wild relatives (Warwick and Stewart, 2005). Transgenic oilseed rape transformed with a Bt *cry1Ac* gene and a green fluorescent protein (*gfp*) gene as a marker was suggested as a model system for monitoring gene flow its consequences in the field (Harper et al., 1999; Halfhill et al., 2001). It has been proved that the hybrids formed between transgenic Bt OSR and Chinese conventional cultivar have similar insecticidal efficacy on the insect pest (Le et al., in revision), which could potentially enhance the evolution of insect pest tolerance in the field. A study was presented here to assess crop-crop gene flow with different distances and directions in adjacent fields of transgenic *B. napus* in Jianli county, Hubei province, southern China, in 2004.

Material and Methods

Trial sites

The sites were located at Jianli county (29°26'-30°12'N, 112°35'-113°19'E), Hubei province, southern China. This region represents the main winter oilseed rape growing area of a campagna physiognomy in Hubei province with a cropping system of small farmland holders, in which crops are planted in many small patches adjacent to each other.

Plant materials

Oilseed rape (OSR, *Brassica napus* cv 'Westar') was transformed with the pSAM 12 plasmid containing genetically linked GFP and Bt cassettes regulated by independent CaMV 35S promoters (Harper et al., 1999; Halfhill et al., 2001). Xiangyou no.15 (Chen et al., 2003), a Chinese rapeseed variety, was used as conventional crop in this study.

Experimental design

A 40×60 m plot of transgenic OSR was planted in center and surrounded by non-GM Xiangyou no.15 in the field of Jianli county, Hubei Province in October of 2003. The flowering time of OSR was adjusted by different sowing time to match that of Xiangyou no.15. Seedlings of transgenic OSR were transplanted into the experimental plot. Fields management was conducted similar to that of normal oilseed rape cultivation.

The mature seeds were randomly collected in the spring of 2004 from the landrace Xiangyou no.15 at a distance interval of five meter located at 0, 5, 10, 15, 20, 25, 30, 35 m along a transect perpendicular to the common border, respectively, at four different directions: South, North, East and West. At each distance interval, we collected three 0.5×0.5 m sampling points parallel to the common border. Seeds were kept in separate envelopes, and stored in a refrigerator at 4°C.

In 2005, seeds were sown in the field and seedlings were screened for hybrids with a gfp-meter instrument at the two-leaf stage (Shen et al., 2006). Approximately a total of 3000-5000 seedlings were screened at each distance interval. All government regulations were strictly adhered during all field experiments.

Data analysis

Frequency of gene flow for each distance interval was calculated as the number of detected hybrid plants divided by the total number of screened seedlings. The data of gene flow measured at four geographical directions (North, West, South, and East relative to pollen source) within 30 m apart from pollen source were analyzed using ANOVA to test the effect of wind direction and distance on gene flow. All analyses were performed using the SPSS for Windows software package (SPSS Inc., 1999).

Results

Levels of gene flow between transgenic OSR and surrounded non-GM xiangyou no.15, were estimated by frequency of occurrence of transgenic xiangyou no.15 plants. The mean level of gene flow from transgenic canola crop OSR to nearby Chinese landrace crop occurred at a considerable rate (Fig 1). The gene flow frequencies observed were ranged from 16.17% to 0.30% at the common border of transgenic OSR field of 0 m distance interval.

At 5 m distance, frequency ranged from 0.07% to 2.20%, whereas levels at 10m ranged from 0.10% to 2.83%. Gene flow decreased rapidly at the 15m sampling distance ranged from 0.03% to 0.07%, but not varied much between 20 and 30m, whereas there was no gene flow detected at 20m in the East direction. At 25, 30m interval, hybridization rate was 0.06–0.5%. The results show that, in most cases, gene flow via pollen movement occurs between OSR fields.

The results demonstrated that the recorded gene flow rate decreased as the distance increased from transgenic pollen source. There was a significant difference for gene flow frequency measured between different distances ($F_{3,21}=9.659$, $p<0.01$). There was also a significant difference ($F_{3,21}=4.798$, $p<0.01$) for gene flow frequency at four different directions. That meant that location of various direction of recipient plants significantly influence the rate of gene flow. The cross-pollination occurred at higher frequencies at North and South directions, while at a lower frequency at East and West directions. The obvious directional effects could be ascribed to wind or insect activity.

Discussion

Oilseed rape pollen can be transported over large distances (Rieger et al., 2002) and a transgenic oilseed rape cultivar could pollinate rape intercrops and volunteers growing in neighbouring fields, as well as related weedy species. The multiple pollinating agents (wind and insects) of OSR and the size of the source may contribute to the randomness of pollination events (Wei et al. 1999). Preliminary results indicate a high gene flow rate which can be influenced by distance and wind direction. A strong asymmetric distribution of the gene flow was detected in different directions. The dominant wind of this region may strongly influence the gene flow rate between transgenic and non-transgenic plants. High hybridization rate was found in the samples collected near the border of transgenic OSR in this study. Edge effects have been consistently observed in all previous pollen movement works, with cross-pollination occurring at higher frequencies closer to the source field. The variety of OSR may be a contributing factor in random pollination events at distance. Varieties have differences in flowering period, which will affect pollination events. Roaming insects may target single plants flowering early or late in a field, resulting in sporadic pollen movement. However, insects are more likely to remain in a single field if sufficient resources (e.g., flowers) are readily available thus that may result in a limited distance of pollen flow.

Oilseed rape is the main crop in this region, where landraces are widely grown. The traditional agronomic system presented in this region is of small farmlands, where crops are planted in many small patches adjacent to each other. It could facilitate the gene flow as more pollen sources in such system. Moreover, small farmland-holders may select or exchange their seeds for desirable traits and then replant them, while the adequate communication guidelines for the isolation distances needed between different cultivars are generally lacked to growers. Management practices employed by the growers, as well as protocols followed for the isolation distances are needed to minimize pollen movement. As the widely grown varieties are open-pollinated, they are subject to cross-pollination (i.e. gene flow) from similar varieties or non-local varieties.

The decrease of average gene flow with distance between transgenic and non-transgenic plants, and the clear influence of

the wind could enable the design of a mathematical model. This could be applied to predict the gene flow rate and to establish strategies to have it minimized. In addition, the use of transgenic plants has proven to be an effective tool to quantify the gene flow to other cultivars of the same species or to wild and weedy relatives. The potential risk of gene flow has to be assessed by case, and it is necessary to be cautious when making general conclusions.

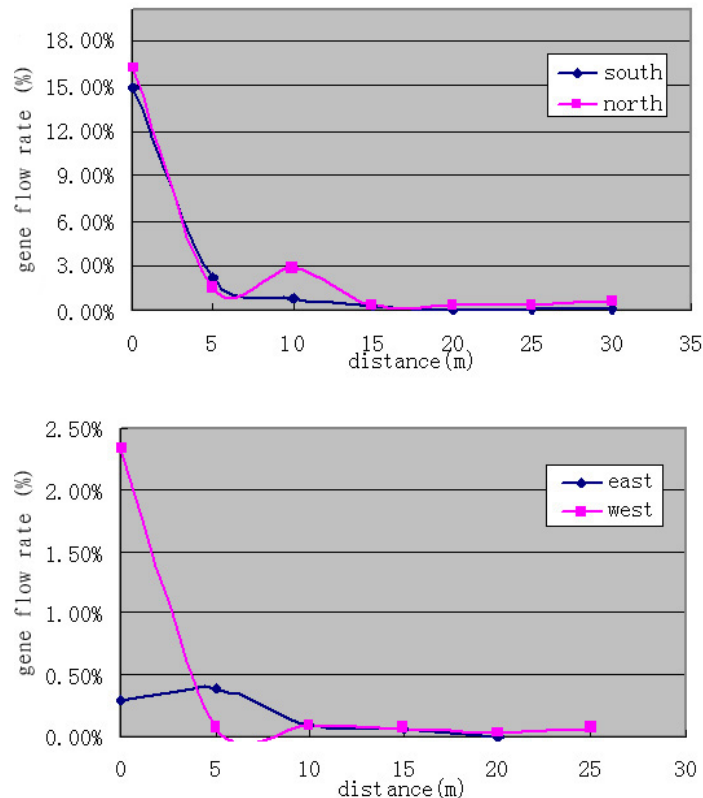


Fig.1 Frequencies of the gene flow at various distances and directions.

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