

Studies on gene flow from GM herbicide-tolerant rapeseed (*B. napus*) to other species of crucifers

PU Huiming, QI Cunkou, ZHANG Jiefu, FU Shouzhong, GAO Jianqin, CHEN Xinjun, CHEN Song

Institute of Industrial Crops, Jiangsu Academy of Agricultural Sciences, Nanjing, 210014, China Email: fsz@jaas.ac.cn

Abstract

Frequency of gene flow from transgenic herbicide-tolerant (THT) rapeseed (*Brassica napus* L) to cruciferae plants was estimated in open pollinated (OP) population and artificial hybridization by using the THT rapeseed cultivar Q3 and HCN-19 as pollen donors. Results showed that the rates of gene flow from THT rapeseed to *B.oleracea*, *B.nigra*., *B.carinata*, *B.juncea*, *B.campestris* and *B.napus*, was 0%, 0.024%~0.243%, 0.028%~0.092%, 0.109%~0.951%, 0.479%~0.879%, 1.252~2.191% respectively. The frequency of gene flow from THT rapeseed to 7 cruciferae weeds was 0%. It indicates that no environmental risk could occurred for growing the THT rapeseed. However the frequency of gene flow from THT rapeseed to wild mustard (*B. juncea*) reached 0.885%. Owing to its wide spread in most of rapeseed production area in China and its various types, the wild mustard could be polluted easily in TNT rapeseed growing area. More attention should be paid to rapeseed for its herbicide-tolerant gene flow by pollen transmission from GM crops to its wild allies.

Key words: herbicide-tolerant rapeseed, Brassica crop, crucifer weed, pollination drift

1. Studies on the gene flow from herbicide-tolerant GM rapeseed to its close relative crops

The rate and its determining factors of gene flow between two herbicide-tolerant GM rapeseed varieties and seven varieties from six *Brassica* species was studied under the paired growth and natural pollination conditions. This study will provide scientific evidence for the evaluation of the ecological safety of growing GM herbicide-tolerant rapeseed crop.

At the year 2000/2001 season, the pollen donor glyphosate-tolerant GM rapeseed variety Q3 plants were grown in pairs with each of the seven pollen receptor varieties which are all close relative of the pollen donor. The row ratio is 4:2. The growth area for each pair is 20 m². Following natural pollination, the seeds from receptor plants were harvested and pooled for each variety. Seeds were sown in the following autumn in the field condition in order to screen and identify the individual progenies with gained glyphosate-tolerance. Seedlings at the 4-5 leaves stage were spread with 0.2% glyphosate at 450 kg/hm². Both dead and survived seedlings were counted 15 days later and the rate of gene flow through pollen drift from the glyphosate-tolerant GM plants was calculated. Among the six *Brassica* species studied, only *Brassica oleracea* var. Rowara was not contaminated by gene drift owing to the its incompatible flowering period, while the rest six varieties from five species all contaminated by herbicide resistance gene through pollen drift to some extent. The rates of gene flow to *B. carinata* var. Dodolla and *B. nigra* var. UNS are the lowest among the six pairs, 0.091% and 0.243%, respectively. The rate of *B. juncea* var. Liyang Kucai and *B. campestris* var. Xinghua youcai are relatively high, being 0.951% and 0.879%, respectively. The highest gene flow rates were observed in the two *B. napus* varieties, i.e. vars. Ningyou No.7 and Ningza No.1, with 1.252% and 2.191%, respectively (Table 1).

Table 1 The rates of CP₄+gox gene flow from THT rapeseed (*B. napus*) to the six *Brassica* species.

Variety type	Cultivar	Total number of tested plants	Number of plants transformed by gene flow	Frequency of gene flow
<i>B.napus</i>	Ningza No.1	11773	258	2.191%
	Ningyou No.7	12385	155	1.252%
<i>B.campestris</i>	Xinghua youcai	10576	93	0.879%
<i>B.juncea</i>	Liyang kucai	12302	117	0.951%
<i>B.carinata</i>	Dodolla	8739	8	0.092%
<i>B.oleracea</i>	Rowara	687	0	0
<i>B.nigra</i>	UNS	5761	14	0.243%

The same methodology was used to measure the rate of gene flow from glyphosate-tolerant GM rapeseed HCN-19 and its close relative crops in the growing season of 2001-2002. The row ratio was 2:2 and the growth area for each pair was 30m². Mature seeds from each pollen receptor variety were harvested and sown in Autumn to evaluate the rate of gene flow from the glyphosate-tolerant GM pollen donor. The seedlings at 4-5 leaves stage were spread with 0.2% glyphosate at 450 kg/hm². Except that *B.oleracea* var. Rowara, all the rest six varieties of five species have been contaminated by the gene flow from glyphosate-tolerant GM pollen donor. The rate of gene flow to *B.carinata* var. Dodolla and *B. nigra* var. UNS was relatively low, being 0.028% and 0.024%, respectively. *B.juncea* var. Liyang kucai and *B.campestris* var. Xinghua youcai ranked in the middle, being 0.109% and 0.479%, respectively. The highest rate of gene flow was observed with *B.napus* var. Ningyou No.7

and Ningza No.1, up to 1.698% and 1.724%, respectively (Table 2).

Table 2 The rates of bar gene flow from THT rapeseed (*B. napus*) to the six *Brassica* species.

Variety type	Cultivar	Total number of tested plants	Number of plants transformed by gene flow	Frequency of gene flow
<i>B.napus</i>	Ningza No.1	16877	291	1.724%
	Ningyou No.7	15545	264	1.698%
<i>B.campestris</i>	Xinghua youcai	20108	100	0.479%
<i>B.juncea</i>	Liyang kucai	34054	37	0.109%
<i>B.carinata</i>	Dodolla	17910	5	0.028%
<i>B.oleracea</i>	Rowara	573	0	0
<i>B.nigra</i>	UNS	20847	5	0.024%

The current research across two consecutive growing seasons has produced consistent results in relation to the trend of gene flow of two different herbicide-tolerance genes from rapeseed to its close *Brassica* relatives. It is clearly demonstrated that the rate of gene flow from rapeseed to other *B. napus* varieties is higher than to other *Brassica* species. It was also observed that the rate of gene flow from herbicide-tolerant rapeseed to *B. juncea* varieties or *B.campestris* varieties is higher than to *B.nigra* varieties or to *B.carinata* varieties. The true transgenic identity of the plants contaminated by gene flow has been further examined by PCR using primers derived from the respective herbicide-tolerance gene and all of these plants were confirmed to be positive. This clearly confirmed the high reliability of the current data.

The current research also demonstrated that the flow rate of herbicide-tolerant gene from GM rapeseed to its *Brassica* relatives is determined by multiple factors, among which the sexual compatibility between the pollen donor and recipients, synchronous flowering time are very important. It is the authors' opinion that the rapeseed could transfer herbicide tolerance gene to its *Brassica* relatives through pollen drift and therefore special consideration and care should be taken for growing GM rapeseed.

2. The studies on gene flow from GM herbicide-tolerant rapeseed to Cruciferous weeds.

Eight common Chinese Cruciferous weeds were grown in pairs with the glyphosate-tolerant GM rapeseed variety Q3. The frequency of gene flow from the herbicide-tolerant GM rapeseed to these Cruciferous weeds was studied under either natural or manual pollination conditions. This study is to provide scientific evidence for the evaluation of the ecological safety of growing herbicide-tolerant GM rapeseed.

Firstly, we studied the gene flow from glyphosate-tolerant GM rapeseed to Cruciferous weeds under natural pollination conditions. Eight Cruciferous weed species including *Capsella bursa-pastoris*, *Crdamine hirsuta*, *Descurainia sophia*, *Orychophragmus violaceus*, *Rorippa palustris*, *Thlaspi arvense*, *Rorippa montana* and *B.juncea* were grown in pairs with glyphosate-tolerant GM rapeseed variety Q3. The row ratio was 3:3. The seeds of the selected eight weeds were sown three times, each with a 20-days gap. The first time was 20 days earlier, the second period was at the same time and the third period was 20 days later when compared to the sowing date of rapeseed. This is to ensure that at least some plants from each weed species could be at the synchronized flowering period with rapeseed. The planting area of each pair is 30m². All plants were allowed to pollinate naturally. Each weed species was harvested separately after maturity. The screening of glyphosate-tolerant weeds contaminated by the pollen drift of GM rapeseed was carried out in field conditions in the following autumn. Seeds were pretreated with GA₃ to break dormancy. Seedlings at 3-4 leaves stage were spread with 0.15% glyphosate at a rate of 450kg/hm². The survival rate of spread weeds were investigated 15 days after the spread and the rate of gene flow through pollen drift from rapeseed were calculated. The results demonstrated that under natural pollination conditions, there was no gene flow from glyphosate-tolerant GM rapeseed to seven out of the eight weed species examined. These weeds are *Capsella bursa-pastoris*, *Crdamine hirsuta*, *Descurainia sophia*, *Orychophragmus violaceus*, *Rorippa palustris*, *Thlaspi arvense* and *Rorippa montana*. The total sample size of these seven weeds was 240,000 plants, none of which survived the herbicide spread. In contrast, 205 out of 23,157 *B. juncea* plants examined survived the herbicide spread, and the rate of the gene flow through pollen drift was estimated as 0.885% (Table 3).

Table 3 Gene contamination of transgenic herbicide-resistant rapeseed to cruciferae weeds

Weed type	Total number of tested plants	Number of plants transformed by gene flow	Frequency of gene flow
<i>Capsella bursa-pastoris</i>	44277	0	0%
<i>Crdamine hirsuta</i>	52826	0	0%
<i>Rorippa montana</i>	62307	0	0%
<i>Rorippa palustris</i>	43908	0	0%
<i>Descurainia sophia</i>	30000	0	0%
<i>Thlaspi arvense</i>	3655	0	0%
<i>Orychophragmus violaceus</i>	2886	0	0%
<i>B.juncea</i>	23157	205	0.885%

Secondly, we have also studied the gene flow from glyphosate-tolerant GM rapeseed to Cruciferous weeds with the assistance of manual pollination in addition to natural pollination. The seven cruciferous weed species were grown in pots,

about 10-20 plants in each pot. The weeds-growing pots were surrounded by glyphosate-tolerant GM rapeseed Q3 plants which were sown in three time periods each with a 15-days gap to ensure the synchronized flowering between the rapeseed plants and each of the weed species. During the whole flowering season, in addition to natural pollination, manual pollination of each of flowering weed plants using rapeseed pollen was carried out between 9 am to 4 pm daily. The harvested mature weed seeds were screened for glyphosate-tolerance in the following autumn. Seeds were pretreated with GA₃ and germinated in Petri dishes prior to sowing to the field. Weed seedlings at 3-4 leaves stage were spread with 0.15% glyphosate herbicide at 450 kg/hm². None of the 5234 weed plants survived the glyphosate spread, indicating that gene flow by pollen drift from glyphosate-tolerant rapeseed to the seven cruciferous weeds examined did not occur, even with the assistance of manual pollination (Table 4). This is perhaps because of the high level of sexual incompatibility between rapeseed and each of the seven weed species examined. This clearly demonstrated the biosafety of growing GM rapeseed in relation to the seven cruciferous weeds examined in this study.

Table 4 Gene contamination of CP₄+gox gene to cruciferae weeds

Weed type	Total number of tested plants	Number of plants transformed by gene flow	Frequency of gene flow
<i>Capsella bursa-pastoris</i>	727	0	0%
<i>Crdamine hirsuta</i>	357	0	0%
<i>Rorippa montana</i>	483	0	0%
<i>Rorippa palustris</i>	1145	0	0%
<i>Descurainia sophia</i>	860	0	0%
<i>Thlaspi arvense</i>	649	0	0%
<i>Orychophragmus violaceus</i>	1013	0	0%

However, there is a relatively high level of sexual compatibility between rapeseed and the wild *B.juncea* and subsequently the rate of gene flow through pollen drift reached as high as 0.885% in the current research. The wild *B.juncea* is a common Cruciferous weed in China. It is highly divergent and distributed widely. Therefore, the spread of transgenes to wild *B.juncea* from the large scale growth of herbicide-tolerant GM rapeseed needs particular consideration.

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