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Lecture

Feral Growth of Genetically Modified Oilseed Rape Around Harbours in Japan and its Impact on the Environment

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Session

Ecological Risk Research

From Organisms to Landscapes

Nagoya

7. - 9. October 2010

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Introduction

Japan imports more than two million tons of oilseed rape (OSR) a year for food oil, of which 90 percent are from Canada. In 2009, about 90 percent of OSR in Canada was genetically modified, most of which was herbicide tolerant. As a result, many volunteers of GM-OSR are found around harbours in Japan. We have surveyed the feral GM-OSR around harbours to consider how we can conserve the domestic Brassicaceae from introgression of modified genes since 2004, because commercial cultivation of GM-OSR is not carried out in Japan. The main survey areas are the ports of Yokkaichi and Nagoya. The concerns of gene transfer from GM-OSR to other Brassicaceae are realistic because many experiments and natural surveys show genetic crosses between *B.napus* and other Brassica species and Brassicaceae genera (Chevre et al. 1997; Brown et al. 2005; Beckie et al. 2006). Our research shows that gene introgression has occurred in domestic crops and natural weeds in Japan over these years.

Materials and Methods

The samples were collected around the roads from ports to seeds processing factories. Thirty surveys were carried out around Yokkaichi port since 2004. Many feral *B.napus* are growing and sometimes flowering year round at the road sides, although most domestic Brassicaceae plants grow annually from winter to spring. *B.napus* and domestic species of *B.rapa*, *B.juncea* and *B.oleracea* were analyzed. Plants from a different genus, *Sisymbrium sp.,* were also collected in 2009 and 2010 in Yokkaichi region. The samples were analyzed by simple lateral flow test. The method used immuno-chromatography to detect the proteins produced by herbicide tolerant genes. Two types of herbicide tolerance, glyphosate and glufosinate, were checked for. Glyphosate tolerance was detected by the CP4EPSPS protein from *Agrobacterium tumefaciens* and glufosinate tolerance was detected by the PAT protein from *Streptmyces hygroscopicus*. Transgenes were also detected by PCR methods if necessary.

Results

A lot of feral *B.napus* were discovered around Yokkaichi harbour in July 2004. They were found also outside of the Yokkaichi port area, along roads to an oil factory 40 km away from the harbour. The cause of feral OSR was spilling of the seeds during truck transportation. Roadside OSR sometimes bloomed and were germinating or producing seeds year round. This means that the feral OSR became perennial, although most domestic Brassicaceae are usually annuals. The season independent growth of *B.napus* may increase the chance of hybridization with related domestic cultivar species and wild *B.juncea* and other Brassicaceae. As a result of perennial growth of *B.napus*, sometimes large feral plants were observed. Some of the OSR developed to large populations and others grew as single plants in this area. Generational alterations of feral OSR were also observed. Feral OSR scatter seeds, giving rise to offspring around themselves. So, even if efforts were successful in stopping trucks carrying GM-OSR from spilling seeds, this would not effectively influence the situation.

The contamination rate of GM-OSR has increased considerably over these seven years. More than 70 percent of feral OSR were herbicide tolerant in 2009. There were two kinds of OSR: glyphosate and glufosinate tolerant. In the surveys of 2008 and 2009, we found stacked GM-OSR, that show tolerance to the two herbicides, glyphosate and glufonsinate. The samples

were analyzed by PCR method and confirmed the existence of the two kinds of herbicide tolerant DNA sequences. This means genetic crosses have occurred naturally between the two kinds of GM-OSR by co-existent growth.By sympatric co-existence of herbicide tolerant OSRs and other domestic Brassicaceae, possibilities of out crossing increase between them. We found some hybrid plants between glyphosate tolerant *B.napus* and wild *B juncea*, and another hybrid plants between glyphosate tolerant *B,napus* and *B.rapa* in Toyokawa city in Aichi prefecture in 2008 and 2009, near an OSR processing factory. The factory produces machine oil from imported OSR, which was contaminated with fungi or soil during the shipping procedures and could not be used for food oil. There are big populations of *B.juncea* and *B.rapa* on riverbanks near the factory. In addition, we found glyphosate tolerant broccoli (*B.oleracea*) on the roadside in Yokkaichi region in 2009.

In 2009 and 2010, we found another example of cross hybridization between GM-OSR and a plant from a different genus, *Sisymbrium sp.*, a Brassicaceae weed, which grows all around Japan. The hybrid plant shows different morphology from Brassica species, but exhibited herbicide tolerance. Usually *Sisymbrium altissimum* grows near the hybrid, which may be one of the parents of the hybrid. Both glyphosate and glufonsinate tolerant weeds were observed. A stacked hybrid weed tolerant to both glyphosate and glufonsinate was also found in Yokkaichi region. These were confirmed by the existence of nucleotide sequences via PCR. Most of these *Sisymbrium* weeds do not produce seeds and show sterility. However, sometimes, small amounts of seeds are found which are different from *Sisymbrium's*. One weed was found in 2009, but 13 were found in 2010. 92.5 percent of the weeds are herbicide tolerant, which shows an abnormally high rate of the modified genes.

Discussion

Japan imports over two million tons of OSR seeds a year, mostly from Canada. In 2009, more than 90 percent of OSR from Canada was genetically modified. Reflecting the situation in Canada, the feral OSR around Japanese harbours contains a considerable rate of herbicide tolerance, as described in the results. The Japanese situation was reported by Saji et al. (2005) and Kawata et al. (2009). The possibility of hybridization between OSR and its wild relatives has been discussed by many authors since the development of genetically modified crops, because of the concern about potential introgression of genetically modified genes into cultivated or wild relatives (Mikkelson et al. 1996; Timmons et al. 1996). Brown et al.（1996）carried out extensive experiments on pollination by herbicide tolerant *Brassica napus* with its wild relatives in field conditions, before commercial cultivation of GM-OSR. They suggested that transportation would give rise to volunteer weeds by seed spillage, pollen movement would be affected by wind direction, hybridization would occur between GM canola and its wild relatives in field conditions, and bridge crosses between the hybrid and its relatives could play a major role in the movement of herbicide resistant genes into the natural weed population. FitzJohn et al. (2007) reviewed many articles on hybridization within Brassica species and allied genera and estimated potential transgene escape. They reported at least 23 Brassicaceae that can hybridize successfully with *B.napus,* including *B.rapa*, *B.juncea* and *B.oleracea*. Sixteen different genera containing *Sisymbrium* can also hybridize with *B.napus*. Hansen et al. (2001, 2003) demonstrated extensive introgression between *B.napus* and *B.rapa* in the natural population and persistence of the introgressed gene in the following generations. Transfer of modified genes from *B.napus* to *B.rapa* were genetically analyzed in details under Japanese conditions by Lu et al. (2002).

Perennial growth of GM-OSR increases the chance of hybridization and gene transfer to related cultivars and weed plants. We have many agricultural cultivars for food of *B.rapa* and *B.oleracea* in Japan. Wild *B.juncea* grows around many riverbanks. Our findings of hybrids bet-

ween GM-OSR and *B.rapa*, *B.juncea* and *B.oleracea* show that the possibilities have become reality. The occurrence of the herbicide tolerant weed, *Sisimbrium sp.*, is the first case reported in the Japanese natural environment. Although most hybrids are sterile, some of the plants have seeds. If the hybrid seed grows and back crosses with the parent weed, gene introgression may occur into the environment, which influences the conservation of biological diversity.

Conclusion

Unintended dispersal of GM-OSR around Japanese harbours was surveyed, especially around Yokkaichi port since 2004. Many glyphosate and glufonsinate herbicide tolerant feral OSRs were discovered. Perennial growth and generational alteration of the OSR were confirmed. The cause of volunteer growth of OSR was transportation and spillage of the seeds from trucks moving from the import harbour to an oil factory and to another seed processing factory. Stacked gene herbicide tolerant OSRs were also discovered. This means that natural pollination and hybridization between two kinds of GM-OSRs have occurred. We have discovered several hybrids between herbicide tolerant *B.napus* and cultivars (*B.rapa* and *B.oleracea*). In addition, hybrids were also discovered with weeds, *B.juncea* and with a different genus, *Sisymbrium sp.* in 2008 and 2010. The modified gene transfer to weedy plants may influence the natural conservation of biological diversity.

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