DARCOFenews

Newsletter from Danish Research Centre for Organic Farming • September 2004 • No. 3

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Contamination of oilseed rape varieties by pollen and seeds

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Oilseed rape (*Brassica napus*) is a common crop in Denmark and mainly cultivated for its content of oil used for food and feed. *B. napus* originated from *B. oleracea* (genome C) and *B. campestris* (genome A) as described by U (1935). Oilseed rape (OSR) crossbreeds easily with its closest relatives as demonstrated several times (e.g. Hansen et al., 2001).

Furthermore, the species is often genetically modified (GM), e.g. to herbicide tolerance or insect resistance. Spread of the inserted genes is likely to occur not only to the species itself, but also to its close relatives.

The **TOPRO project** was initiated in 2002 and one of the objectives has been the detection of dispersal of pollen and seed from GM oilseed rape under field conditions in order to suggest measures to limit the GM dispersal to organic fields.

Dispersal of pollen and seed purity

Self-pollination as well as insect- and wind-pollination occur in OSR. The flexible pollination mode, and the easiness of the plant to incorporate alien genes, makes the species an excellent candidate for studying the spread of genetically modified genes.

Field trials with GM plants have largely been limited to the field trials required before marketing (C-notifications). Information on pollen dispersal from more extensive cultivation of GM-OSR in Europe is lacking. Alternative methods as modelling and as checking purity of conventional OSR varieties have been the options. Modelling based on field observations revealed that pollen dispersal is limited if the distances separating the GM- and the non-GM OSR fields are from 100 to 200 metres (Kjellsson and Damgaard, 2003).

Regarding the purity of the seeds, only morphological traits have been accepted as criteria for variety separation among breeders. Yet, DNA based methods might be a stronger tool for variety identification. Therefore, a study using molecular markers was initiated with the major aim to detect pollination from an alien variety and to check for purity of the resulting crop. The results would indicate the probability of adventitious presence of alien pollen or seed in non-GM OSR cultivated under the specific conditions (e.g., field size and separation distance), assuming that the processes involved in pollination and fertilisation was similar between GM and non-GM

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Study sites

In the summer 2002, an experimental field for testing new varieties was chosen and visited at harvest time. The field was a part of the national field trials in Denmark performed by the Danish Agricultural Advisory Service, National Centre, Aarhus. The field consisted of a central part with different OSR varieties surrounded by the variety Canberra. Among the varieties tested the variety Express and Canberra were selected.

By random amplified polymorphic DNA (RAPD) using the primer B20 from Operon it was possible to distinguish one Express seedling among 30 Canberra seedlings. However, it was not possible to perform the analysis directly on seeds despite many extraction methods tested. The company, who owned the right to produce Express went bankrupt later, so further experiments with Canberra and Express was out of the question.

With help from LandboCenter Midt (Ib Møller Jensen), two new fields adjacent to each other were selected, one with the variety Canberra (owner Niels Åge Kastbjerg) and one with the variety Royal (owner Kurt Stilling). The two fields were separated by a road, so that the upper right corner of the Canberra field was adjacent to the lower left corner of the Royal field.

A random sample of Canberra leaves from the upper right corner was collected in May 2003 and similarly, a sample from the Royal field was collected from the lower left corner. The two fields were similar in size, approximately 5 ha. Just before harvest in July 2003, seed samples from each of 100 plants were collected from both fields. Certified seeds of Canberra and Royal were obtained from Monsanto and Sejet Planteforældling, respectively.

Suitability of test methods

Certified seeds had nearly the same distribution of the RAPD phenotypes in the two varieties **(Table 1)**. In order to separate the varieties, another analytic technique was therefore applied, inter simple sequence repeat (ISSR) using the primer UB#9, 888 (Reddy et al., 2002). This technique was better suited for distinguishing the two varieties as shown in **Table 2**.

Contamination of Canberra in the field

Based on the ISSR data, two groups were assumed for statistical testing of Canberra: phenotype 2 and the others combined (phenotype 1, 3-6).

Certified seeds compared to plants from the field and certified seeds compared to seeds from the field crop both revealed highly significant deviations **(Table 3)**. This indicates that the crop in the field may have been contaminated by other seed sources (e.g., OSR seeds surviving in the soil) and potentially also by pollen from other OSR fields. However, the distribution of phenotypes in Canberra plants from the field was homogenous with the distribution seen in harvested seeds from the field. This indicates that the plants and the seed crop may be regarded as coming from the same population and no evidence of contamination by pollen from other fields is found.

Homogeneity in Royal

For the Royal field, it was not possible to compare the plants in the field with the harvested seeds, as no variation was observed among the plants in the field. All samples contained phenotype 3, which was the common phenotype **(see Table 2)**. Comparing certified Royal seeds with Royal

seeds from the field crop revealed homogeneity between the two samples **(Table 3)**. However, the two rare phenotypes 2 and 6 found in the certified seeds were also observed in the seeds from the field crop **(Table 2)**.

The Royal seeds revealing phenotype 2 might originate either from the Royal field itself by the fact that certified Royal seeds revealed phenotype 2 or from the Canberra field. Additional analysis of seeds from these two plants was carried out and the distribution was listed in **Table 4**. However, the data did not allow further analysis for differentiation between the two hypotheses.

Conclusion

The present study did not reveal any significant pollen dispersal between the two fields with different OSR varieties. However, based on the relatively small sample sizes, a low level of cross-pollination between fields cannot be excluded.

The study showed a significant difference between phenotypes in the certified seeds and phenotypes in plants and in seeds in the field. The reason for this might be the presence of OSR volunteers from a previous crop surviving in the soil seed bank. This type of contamination is affected by e.g., harvest and post-harvest handling procedures (Fargue et al., 2003) and can consequently be reduced.

More studies on homogeneity (i.e., genetic variation) between field crop plants and certified seeds may contribute to the detection of adventitious presence of GMO in organic crops and the routes of contamination.

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