



GM and non GM supply chains: Their CO-EXistence and TRAcability

Outcomes of Co-Extra

Pollen containment by cleistogamy in oilseed rape

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The diversification of farming production systems with the apparition of transgenic crops, as well as the specialization of crops cultivars for different markets, require measures to prevent adventitious presence in productions at the field, storage and refinement level. For instance, in oilseed rape crops, such means are necessary to allow the coexistence of productions requiring different fatty acid composition. In fields, adventitious presence in adjacent fields is mainly due to pollen flow, which have to be reduced to make possible the co-existence of different crops. Pollen flow between adjacent fields may be reduced by physical ways: by putting separation distances between fields grown with the same crops or by surrounding the crop of which the pollen is considered as contaminant with a buffer crop strips. Biological ways of containment, such as male sterility or cleistogamy, may also be used depending on the species. One simple way to prevent pollen flow between oilseed rape is to ensure that their flowers do not open. Cleistogamous plants do not exist naturally among the genetic resources of the oilseed rape species, but different lines of cleistogamous oilseed rape were obtained by chemical induced mutagenesis at INRA Rennes (Patent FR 97 15768). The cleistogamous trait is controlled by one gene (Renard and Tanguy 1997) and would be a good way of securing biocontainment, on condition that this trait is stable during the flowering period and under various environmental and agricultural conditions. One aim of our study was to test the stability of the cleistogamous trait in the field under several environmental conditions. In this goal, the flower opening level was observed at different dates during the flowering period, on two cleistogamous genotypes tested in three locations, during two successive years and under two treatments (with or without the application of a growth regulator at the vegetative restarting at the end of winter). The second aim was estimate the rate of adventitious presence of cleistogamous lines by allo-pollen under several environmental conditions. In this goal, the allo-pollination in seed sets collected on Clg1 plants was tested in three locations using erucic acid as a marker during two successive years.

Material and Methods

The stability of the cleistogamous trait was assessed for two rapeseed cleistogamous lines, Clg1 and Clg2, corresponding to the lines 17046 and 16960, respectively, provided by INRA-Rennes (Patent FR 97 15768). Control cultivars were used in each site. In each site, a split-plot field design, using a randomized block design, in four replications, was carried out, with elementary plot having areas between 22.5m² and 47,5 m². The development of the crop was characterized by notations of the dates when key development stages were reached and the plant height at maturity. During the flowering period, the stability of the cleistogamous trait was assessed visually by scoring of opening level on mature flowers of the inflorescence with a three-level scale: the first class corresponded to the full opened flowers, the second class of the totally closed flowers that appeared like a big yellow bud, and the last class of the partially opened flowers. Ten plants were scored per plot, with notation of at least five flowers on the main stem and on one secondary stem.

The allo-pollination was assessed for one rapeseed cleistogamous line (Clg1, corresponding to the lines 17046 provided by INRA-Rennes (Patent FR 97 15768)). As a pollinator cultivar a high erucic acid rapeseed line (Markant) was used in each site. The trial was isolated by at least 500m from other rapeseed fields. The trial was composed of 2 neighbouring plots :The first plot was sown with a mixture of 99% of Marcant (erucic line) seeds and 1% of Clg1 (cleistogamous line) seeds.The second plot was sown with the cleistogamous line Cleisto1. Each plot was 50m long and 50m large and the sowing rows had the same direction as the limit between the two plots, and as the dominant wind. Correlations between rates of seeds derived from crosses with the erucic line and the erucic acid content in seed sets were established in each site according to the erucic acid content of seeds produced by manual crosses between Clg1 and the erucic line.

Results

The first experiment showed that flowers of cleistogamous lines are mostly totally closed, but a variable proportion of flowers were observed as partially open. The average percentage of totally closed flowers (Clg1 and Clg2) reached 72.03% at location 1 (2007), 80.91% at location 2 (2007), 85.05% at location 3 (2007), 86.96% at location 2 (2006), 88.91% in at location 1 and 89.69% at location 3 (2006), with standard deviations of 26.6, 24.3, 19.3, 9.54, 7.9 and 6.6, respectively in each site x year. Global analyses of all the data from the six site x year combinations revealed that the environment (site x year) had an effect on the stability of the cleistogamous trait, as differences among sites and years were observed. The main effect of genotype (Clg1 or Clg2) explained 33% of the variability of the percentage of totally closed flowers. This statistical result reflects the difference of mean and of variance showed by the two genotypes: in each environment, Clg1 showed a high stability level for the cleistogamous trait, whereas Clg2 showed a higher and more variable rate of partially open flowers. Finally, a low but significant difference was also observed between the notations done on the primary or on secondary stems, and the application of growth regulator had no significant effect.

The second multi-site experiment showed that the environment (site x year) had an effect on the allo-pollination, as differences among sites and years were observed. Allogamy rates of Clg1 under a high pressure of allopollen (Clg1 sampled in erucic block) vary in three locations between 4.4% and 16.2%. The samples collected on open pollinated cleistogamous plants (Clg1 sampled in Clg1 block) at different distances from the erucic plots showed that the percentage of allogamy rates rapidly dropped over the initial meters around the pollen source and decreased as the distance from the pollen source increased. In samples (location 2) collected on plants at 0m from the erucic plot, the erucic acid content reached at mean 1.64%, but at 6m, we observed only 0.26% of erucic acid. However, erucic acid was also detected in samples collected at 48m from the erucic plot, showing that adventitious presence, at low rates (less than 0.2%) may occur at large distances.

Conclusions

The main result from our various studies is that cleistogamy has a major potential for limiting crosspollination due to the strong reduction of the pollen cloud. We suggest that isolation distances implemented for oilseed rape could be dramatically reduced when using cleistogamic oilseed rape as a containment strategy.

References:

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