Theor Appl Genet (1992) 84:303–306

THEORETICAL AND APPLIED GENETICS

Environmental variation for outcrossing rate in rapeseed (*Brassica napus*)

H. C. Becker [1], C. Damgaard [2], and B. Karlsson [1]

[1] The Swedish University of Agricultural Sciences, Department of Plant Breeding, S-26831 Svalöv, Sweden
[2] University of Aarhus, Department of Ecology and Genetics, Ny Munkegade, Building 550, DK-8000 Aarhus C, Denmark

Received November 25, 1991; Accepted December 19, 1991
Communicated by P. M. A. Tigerstedt

Summary. Rapeseed (*Brassica napus*) is a predominantly selfpollinated crop with about one-third outcrossing. The outcrossing rate may be influenced by environmental factors, and hence changes in the heterozygosity level of a variety may occur during multiplication. In an investigation on environmental variation in outcrossing, we estimated the outcrossing rate in the Swedish spring rapeseed cv 'Topas' by isozyme analysis and found that outcrossing varied from 12% to 47% over five locations in Sweden, Denmark and Germany. Among flowers at different positions on the same plant, average outcrossing varied from 11% at the top to 39% at the bottom of the plant. In conclusion, environmental factors can greatly influence the outcrossing rate in rapeseed, and an investigation therefore merit further studies.

Key words: *Brassica napus* – Outcrossing rate – Selfing rate – Mixed mating

Introduction

Rapeseed is a partially allogamous crop with an average outcrossing rate of between 20% and 40%. Nevertheless, rapeseed breeders mainly use the same breeding methods as for self-pollinated crops, and in many breeding programs pure line varieties are developed. As an alternative approach, Schuster (1969) and others proposed producing synthetic varieties by open pollination of mixtures from two or more lines. Such synthetic varieties can partly capitalize on yield heterosis, which can be more than 30% (for review, see Becker 1987; Downey and Röbbelen 1989). Synthetic varieties in rapeseed have been proven to be superior in both yield and yield stability (Léon 1991).

If synthetic varieties are pursued, a selection for a high amount of outcrossing should be very effective to gain a short-term increase in yield (Becker 1989). Such a selection should be possible, for there exists a considerable amount of genetic variation for the outcrossing rate (Olsson 1960; Rudloff and Schweiger 1984; Rakow and Woods 1987; Lewis and Woods 1991). The main problem when establishing synthetic varieties is that the environmental conditions during multiplication may influence the outcrossing rate and hence lead to unpredictable changes in the level of heterozygosity and yield. Information on the influence of environmental factors on the outcrossing rate in rapeseed is very limited. The only publication we know of is a report by Mündges-Christmann and Köhler (1990), who observed a difference in the outcrossing rate between 2 different years for one of three varieties investigated.

The objective of the study presented here was to investigate the variation in outcrossing rate in spring rapeseed under various environmental conditions. Two different types of environmental influences were investigated, (1) the effect of five different geographical locations, and (2) the effect of the within-plant flower position.

Material and methods

Materials

Outcrossing was estimated in the Swedish spring rapeseed cv 'Topas'. This cultivar showed the highest isozym polymorphism of a number of cultivars investigated; of 17 enzymes analyzed, 'Topas' was polymorphic for 3 of them (Damgaard 1990). The distribution of the various isozyme patterns for these three enzymes is shown in Fig. 1.

Correspondence to: H. C. Becker

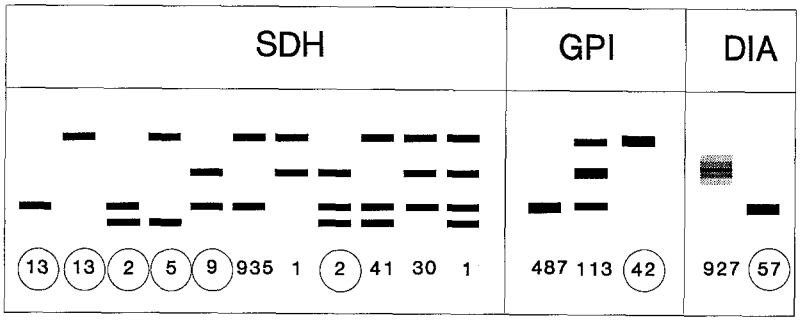

Fig. 1. Distribution (number of plants) of the various isozyme patterns within cv 'Topas'; classes used in progeny analysis are *encircled*

Field experiments

Outcrossing rate was estimated in plants grown in plots at a normal plant density and routine cultural practices at five locations in 1989. The locations were Kölbäck in middle Sweden (Östgötaland), Svalöv and Landskrona in southern Sweden (Skåne), Dyngby in Denmark (Jylland), and Natendorf in northern Germany (Lüneburger Heide). Plot size varied between 10×10 m and 20×20 m at the various locations. The plots were only separated by a broad surrounding path from other rapeseed, which has been shown to be sufficient to prevent outcrossing from outside the plot (Becker et al. 1991).

Estimation of outcrossing

Outcrossing was estimated by comparing the isozyme patterns of mother plants with their progeny. Plants (200–300) taken from the center of each plot were analyzed electrophoretically, and those with 'rare' patterns (encircled in Fig. 1) were harvested and their seedlings analyzed. The 'rare' diaphorase (DIA) and glucosephosphate isomerase (GPI) patterns were single banded; plants with such patterns were assumed to be homozygous and seedlings with a pattern differing from that of the mother plant were regarded as outcrossings. In mother plants with 'rare' shikimate dehydrogenase (SDH) patterns one of the two typical bands was absent, and seedlings from these mother plants showing both typical bands were regarded as outcrossings. The outcrossing frequency was corrected by taking into consideration the probability of undetectable outcrossings from plants with 'rare' patterns. In Dyngby, six to nine pods of each plant were analyzed, each with nine seeds; at the other locations three pods of each plant, each with ten seeds, were analyzed. All of these pods were taken from the middle part of the main stem. Additionally, in Dyngby, from each plant eight to ten pods were taken from the top, the middle and the bottom of the raceme, and one seed from each pod was analyzed.

Electrophoresis

The interplant outcrossing rate was estimated by isozyme electrophoresis on starch gels (12% Connaught). The following three enzymes were assayed: shikimate dehydrogenase (SDH, EC 1.1.1.25), glucosephosphate isomerase (GPI, EC 5.3.1.9), and diaphorase (DIA, EC 1.6.4.3). The isozymes were analyzed either in fresh young leaves taken from plants in the field before flowering or in young seedlings. Seeds were germinated on wet perlite for about 5 days in a growth cabinet at 24 °C. Cotyledons were placed in 50 µl homogenization buffer (TRIS-HCl, pH 7.2, containing mercaptoethanol, Soltis et al. 1983) and ground by means of a mechanically powered glass rod. Samples were loaded on paper wicks (Whatman 4). A modified buffer system 1 of Soltis et al. (1983) was used with 0.02 *M* histidine-HCl (pH 8.0) as gel buffer and 0.4 *M* citric acid (pH 7.0) as electrode buffer. The gels were run for about 4 h at a constant voltage of 125 V (approximately 80–90 mA). Gels were sliced horizontally into three layers and stained according to Soltis et al. (1983) for SDH and GPI (agar overlay) and according to Vallejos (1983) for DIA (liquid stain).

Table 1. Outcrossing rate (±95% confidence interval) at different locations

Location	Number of plants	Outcrossing rate [%]
Svalöv	8	32.3±12.1
Landskrona	10	46.8±10.0
Kölbäck	10	12.2± 8.6
Dyngby	7	32.2±20.4
Natendorf	9	45.1± 7.4

Table 2. Influence of flower position on outcrossing rate

Plant	Outcrossing rate [%]		
	Bottom	Middle	Top
1	60	60	20
2	56	60	10
3	60	10	0
4	0	0	10
5	38	20	0
6	30	30	0
7	30	50	40
Mean	39.1	32.9	11.4
P[a]	0.29		0.002
	0.0002		

[a] Probability values for non-significant difference between the means, Fisher's exact test

Results

The outcrossing rate in the cv 'Topas' was estimated at five locations in 1989; the results are given in Table 1. The most northern location, Kölbäck, showed only 12% outcrossing, a value that was significantly lower than those

for all other locations. The other locations had outcrossing rates between 32% and 47% and did not differ significantly from each other.

At one of the locations, Dyngby, the outcrossing was estimated separately for seedlings raised from flowers from different parts of the plant. Table 2 shows that flowers at the top showed a significantly lower outcrossing rate than flowers in the middle or lower parts of the raceme.

Discussion

The outcrossing analyses in this paper are based on phenotypic rather than genotypic frequencies. As a consequence of this, the outcrossing rate is slightly underestimated, for not all of the outcrossings from heterozygous pollen parents are detectable. However, this should not affect the comparison between different environments.

More reliable estimators can be expected from multilocus approaches based on genotype frequencies (Brown et al. 1990). Rapeseed is an allotetraploid species, however, and in such species no unambiguous relationship exists between genotype and electrophoretic pattern. For example, a genotype with the three-banded pattern in GPI (see Fig. 1) can be either homozygous for two different alleles at two loci located on the homoeologous chromosomes or heterozygous at one or both of these loci. In completely homozygous doubled-haploid rapeseed lines three-banded GPI patterns are quite common (unpublished results).

The average outcrossing rate of plants from the five locations was 34%; this agrees well with what we expected from the literature. However, locations do exist with values significantly below and above this rate, and it has to be confirmed whether a similar variation exists between different years. A large unpredictable environmental variation in outcrossing rate would be a severe obstacle for a reproducible seed production of synthetic varieties.

The outcrossing rate was also greatly influenced by the position of the flower on the plant. In rapeseed flowering starts with the lowest flower of the main raceme and ends with the flowers at the top of the side branches. Two alternative interpretations of the influence of flower position are possible: this effect can either be a result of an accidental change in climatic conditions towards the end of the flowering period in this particular environment, or there may be a general tendency for outcrossing rates to change during the flowering period, as observed in other species (Brown et al. 1990). These two possibilities can not yet be specified, and our results demonstrate that determination of the overall level of outcrossing in the population requires a carefully randomized sampling.

For practical breeding purposes it may be interesting to manipulate the amount of outcrossing, e.g., by changing plant density or adding bee hives. To our knowledge nothing is known about the relative contribution of the three possible mechanisms for outcrossing in rapeseed: insect pollination, wind pollination, and direct mechanical contact among flowers from different plants. A better understanding of the biological basis of outcrossing may explain the large variation between various environments, and we have begun experiments to investigate this question.

In summary, we conclude that environmental influences affect outcrossing rate in rapeseed to a large extent and thus merit further studies. A better understanding of factors influencing outcrossing is of interest for all categories of rapeseed breeding: for the breeding of pure lines, it is interesting to reduce undesirable outcrossing; for the breeding of synthetic varieties, it is important to increase the outcrossing rate, and for breeding hybrids a reliably high amount of outcrossing is a prerequisite for seed production.

Acknowledgements. Thanks are due to Vibeke Simonsen for introducing to us the electrophoresis technique, to the seed companies Svalöf AB, W Weibull AB, Pajbjergfonden and Semundo Saatzucht GmbH for help with the field experiments, to Carita Lannér-Herrera for comments on the manuscript, and to the Swedish Plant Breeding Board, the Swedish Council for Forestry and Agricultural Research, and the Danish Natural Science Research Council for financial support.

References

Becker HC (1987) Quantitative Genetik und Zuchtmethodik bei Raps – Versuch einer Literaturübersicht. Ber Arb Tagung 1987 AG Saatzuchtleiter, Gumpenstein, pp 67–82

Becker HC (1989) Breeding synthetic varieties in partially allogamous crops. Vortr Pflanzenzücht 16:81–90

Becker HC, Karlsson B, Damgaard C (1991) Genotypic and environmental variation for outcrossing rate in rapeseed. In: McGregor DI (ed) Proc GCIRC 1991 Congr. Saskatoon Canada pp 1454–1459

Brown AHD, Burdon JJ, Jarosz AM (1990) Isozyme analysis of plant mating systems. In: Soltis DE, Soltis PS (eds) Isozymes in plant biology. Chapman and Hall, London, pp 73–86

Damgaard C (1990) Evolutionen af parringssystemer hos hermafroditiske planter – med eksempel i raps. Inst Genet og Økologi, Århus Universitet

Downey RK, Röbbelen G (1989) Brassica species. In: Röbbelen G, Downey RK, Ashri A (eds) Oil crops of the world. McGraw-Hill, New York, pp 339–362

Léon J (1991) Heterosis and mixing effects in winter oilseed rape. Crop Sci 31:281–284

Lewis LJ, Woods DL (1991) Outcrossing in summer oilseed rape (*Brassica napus* L.) under Peace River, Alberta conditions. In: McGregor DI (ed) Proc GCIRC 1991 Congr. Saskatoon Canada, pp 1528–1531

Mündges-Christmann H, Köhler W (1990) Enzymmarker zur Bestimmung der Fremdbefruchtungsrate und der genetischen Struktur bei Winterraps. Mitt Ges Pflanzenbauwiss 3:163–166

Olsson G (1960) Self-incompatibility and outcrossing in rape and white mustard. Hereditas 46:241–252

Rakow G, Woods DL (1987) Outcrossing in rape and mustard under Saskatchewan prairie conditions. Can J Plant Sci 67:147–151

Rudloff E, Schweiger W (1984) Eine Methode zur Schätzung der Fremdbefruchtungsrate bei Qualitätsraps (*Brassica napus* L.). Arch Züchtungsforsch 14:327–334

Soltis DE, Haufler CH, Darrow DC, Gastony GJ (1983) Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. Am Fern J 73:9–27

Schuster W (1969) Vergleich von zwei Zuchtverfahren in der Erhaltungszüchtung bei Winterraps. Z Pflanzenzücht 62:47–62

Vallejos CE (1983) Enzyme activity staining. In: Tanksley SD, Orton TJ (eds) Isozymes in plant genetics and breeding, part A. Elsevier, Amsterdam, pp 469–516