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Estimation of Outcrossing Rates in Crambe (*Crambe abyssinica* Hochst. Ex. R. E. Fries) Using a Dominant Morphological Marker Gene

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(With 1 figure)

1. Introduction

Crambe (*Crambe abyssinica* Hochst. Ex. R. E. Fries), a new crop species of the Brassicaceae family, is currently considered to be a potential substitute of rapeseed in erucic acid supply for industrial uses (HIRSINGER 1989). In spite of high levels of erucic acid (C 22:1) in crambe seed oil (55 to 62%), the crop is not produced on a considerable scale at present. According to GILLIS (1988) this is due to marketing questions rather than agronomic performance. On the other hand a lack of competitiveness in terms of yielding ability of crambe in comparison with rapeseed necessitates an improvement of cultivars. Other drawbacks towards a broader use of crambe like high contents of glucosinolates in the seed meal, low oil content, or seed shattering also have to be lessened (KORSRUD et al. 1978, LESSMAN 1975, MCGREGOR et al. 1961, WEISS 1983).

In current breeding programs, crambe is treated as a primarily self-pollinated species (CAMPBELL et al. 1986). Leaf pubescence, which is governed by a single recessive gene (MEIER and LESSMAN 1973), has been used as a genetic marker in outcrossing experiments between *Crambe abyssinica* and *C. hispanica* types. Outcrossing rates in the range of 2 to 9 per cent have been reported (MEIER and LESSMAN 1973 a, BECK et al. 1975).

In the present contribution estimations of outcrossing rates between glabrous (dominant) and pubescent (recessive) genotypes of *C. abyssinica* are presented with respect to the type of plot and to overlapping in flowering period between pollinator and pollen acceptor.

2. Materials and Methods

Seed samples of *Crambe abyssinica* cultivar "Indy" (designated D1, glabrous leaf) and accession NU 52865 (designated r, pubescent leaf) were kindly provided by G. A. WHITE (USDA, Agric. Res. Center, Beltsville, USA). Seed material of crambe accession VIR 8 (= D2, glabrous leaf) was obtained from V. KRIVCHENKO (N. I. Vavilov All-Union Sci. Res. Inst. of Plant Industry, Leningrad, USSR).

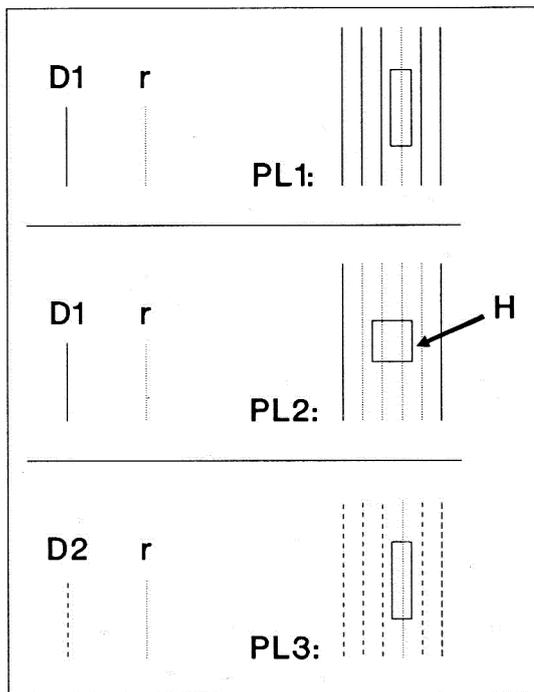


Fig. 1: Arrangement of dominant (D1, D2) vs. recessive (r) rows of genotypes in different types of plots (PL1, PL2, PL3) and the harvesting area (H) to determine outcrosses.

Three kinds of plots (PL1, PL2, PL3) of 8 meters length and 6 rows of 20 cm row spacing were sown according to different arrangements of pollinator and pollen acceptor rows illustrated in Fig. 1 at Gerasdorf (Vienna) on May 16, 1989, with a single row sowing machine. A harvesting area (H) of approximately 1 m² in rows of plants carrying the recessive character (r) was either surrounded by rows of one of the dominant pollinators D1 or D2 (plots PL1 and PL3) or by two different numbers of rows of dominant genotype D1 (plots PL1 and PL2, respectively). Genotype D2 (planted in plot PL3) was known to flower significantly later than D1 and r. Each kind of plot was replicated twice in a completely randomized design. In order to minimize the possibility of pollen movement between different plots, single plots were arranged one after the other in a line, and no other crambe plants were allowed to be present in the experimental field. Beginning of flowering was recorded when at least 10 per cent of plants of a genotype showed their first open flower on top of the main branch. Dry seeds from the main branches (racemes) of 14 different plants per plot, which had been selected randomly from the harvesting area (H) of pubescent rows (r), were collected separately by hand at the time of maturity (on August 11) and bedded in perlite in the greenhouse. After three weeks, the first true leaf of each seedling was checked visually for the glabrous leaf character marking an outcross. A total number of 1297 progenies (about 15 seedlings from each plant harvested) was evaluated.

Outcrossing rates were calculated as percentages of glabrous plants among the total number of seedlings evaluated from each plot. The significance of differences in outcrossing between the three types of plots was examined using a chi-squared statistic (2 × 3 contingency table). The outcrossing rates determined as described above are underestimates of total cross pollination, since intragenotypic pollinations between recessive plants are not considered. Therefore, an adjustment towards a maximum likelihood estimate of the total outcrossing frequency

(HARDING and TUCKER 1964, JAIN 1979) was attempted by dividing outcrossing percentages by the pollen pool allelic frequencies. Assuming equal plant density and equal amount of pollen production in different rows of a plot, pollen pool allelic frequency equals the frequency of dominant pollinator rows of a plot. Therefore, pollen pool allelic frequencies of 5/6, 2/6 and 5/6 were used for plots PL1, PL2 and PL3, respectively, to calculate adjusted outcrossing rates from the observed outcrossing percentages.

Glabrous plants obtained from plots PL1 and PL3 were grown to maturity in order to verify their hybrid nature. The 2 different F₂-populations (all F₂-seeds of glabrous plants from the same type of plot were grown in a bulk) were checked separately for 3:1 (glabrous vs. pubescent leaf) segregation ratios in the field.

3. Results

The date of first flowering of the main raceme of the genotypes involved in the experiment is given in Table 1. The overlapping of flowering period between the main raceme of r-plants and D2-plants was 61.5 per cent only, which is due to the late flowering of D2.

Table 1

Date of first flowering and percentage of overlapping of flowering period between dominant pollinators (D1, D2) and the main raceme of recessive (r) crambe plants (end of flowering of the main branch of r was recorded on July 6)

Genotype	date of first flowering	% overlapping with r
D1	June 25	92.3
D2	June 29	61.5
r	June 24	

The number of glabrous seedlings among the total number of progenies from pubescent plants, the outcrossing rates resulting from these figures and the adjusted outcrossing rates are given in Table 2 for the different plots. Average outcrossing rates from glabrous to pubescent plants were in the range of 4.8 to 10.7 per cent, depending on the type of plot. In the 84 single plant progenies investigated, outcrossing rates were between 0 and 23.5 per cent, and the highest rates were found in the replications of plot PL1. Considering both outcrossing between dominant pollinators and recessive plants as well as cross pollination within recessive rows, maximum likelihood estimates of the overall cross pollination rates (adjusted outcrossing rates) were 12.9, 14.3 and 9.0 per cent in plots PL1, PL2 and PL3, respectively.

Table 2

Extent and estimations of outcrossing in crambe with respect to the type of plot and to replication

Plot	repl.	No. of plants		% outcrosses	st. dev.	adjusted outcrossing rate
		glabrous	total			
PL1	1	16	162	9.9	7.3	11.9
	2	25	216	11.6	7.2	13.9
PL2	1	9	186	4.8	6.4	14.5
	2	12	255	4.7	6.7	14.1
PL3	1	17	233	7.3	6.4	8.8
	2	19	245	7.8	8.0	9.3

According to a chi-squared statistic comparing observed and expected numbers of glabrous vs. pubescent progenies, differences in the rate of outcrossing referring to different types of plots are significant at the 1% level (chi-square = 10.78, df = 2).

In progenies of glabrous plants originating from pubescent plant rows of plots PL1 and PL3, segregation ratios of 126:41 and 82:28 were found for the glabrous vs. pubescent leaf characters, respectively. Both segregations closely fit to a 3:1 ratio which demonstrates the hybrid nature of glabrous plants found in pollen acceptor rows.

4. Discussion

In order to obtain information about the breeding system of a crop species, outcrossing measurements have been undertaken in various fields of crop research. These data are used to design optimum procedures for recurrent cycles of recombination and selection, to estimate population genetic parameters, or simply to gain insight into requirements of isolation necessary during the development and maintenance of pure lines. Besides morphological markers widely used to detect outcrosses in progeny of recessive genotypes (JAIN 1979), several alternative procedures including isozyme marker loci (BICKELMANN 1988, SCHMIDT-STOHN et al. 1986), erucic acid content (HÜHN and RAKOW 1979) or inbreeding depression (WRICKE 1979) have been used to determine outcrossing rates.

Outcrossing in crambe has been reported so far between *C. abyssinica* and *C. hispanica* types using leaf pubescence as a genetic marker (MEIER and LESSMAN 1973 a, BECK et al. 1975). Since the two genotypes involved in these studies hybridized easily and produced fertile progeny after natural cross pollination as well as artificial hybridization, MEIER and LESSMAN (1973, 1973 a) suggested to classify both introductions into the same species (*C. abyssinica*). This view is also supported by the present outcrossing experiments between *C. abyssinica* genotypes resulting in outcrossing rates similar to those found in the studies mentioned above. The current results demonstrate that the frequency of off-types can be reduced significantly by arranging isolation rows or border plots between different genotypes (plots PL1 vs. PL2). A decrease of outcrossing from 10.7 to 7.5 per cent (plots PL1 and PL3) also is achieved when the overlapping in flowering period between dominant pollinator and recessive pollen acceptor is reduced from 92.3 to 61.5 per cent. The latter also might be partly due to differences between pollinators other than flowering time (e.g. amount of pollen production), as reported in other species.

Referring to the adjusted outcrossing rates given in Table 2, it has to be pointed out that the total amount of outcrossing could have been slightly underestimated by the maximum likelihood method particularly in plot PL2, because this method is based upon a homogeneous distribution of dominant and recessive parent plants within plots. If parent plants are arranged in separated rows, pollen pool allelic frequency ("pollen density" of the dominant pollinator) is higher around pollinator rows but lower in rows of recessive pollen acceptors. Therefore, the overall outcrossing rate will be underestimated.

Summary

A dominant marker gene controlling leaf pubescence has been used to measure outcrossing in crambe (*Crambe abyssinica* Hochst. Ex. R. E. Fries), a cruciferous crop species producing a seed oil with high content of erucic acid. In experimental plots, outcrossing rates within the range of 4.8 to 10.7 per cent have

been determined in different types of plots between dominant and recessive genotypes. Overall outcrossing rates of 9 to 14.3 per cent considering both inter- and intragenotypic cross-pollination, have been calculated.

**Bestimmung von Fremdbestäubungsraten bei Crambe
(*Crambe abyssinica* Hochst. Ex. R. E. Fries) mittels eines dominanten
morphologischen Markergens**

Zusammenfassung

Mit Hilfe eines dominanten Markergens für Blattbehaarung wurden Fremdbestäubungsraten bei Crambe (*Crambe abyssinica* Hochst. Ex. R. E. Fries), einer zur Produktion von Erucasäure geeigneten Art aus der Familie der Brassicaceae, bestimmt. Innerhalb von Prüfparzellen wurden dabei Auskreuzungsraten von dominanten zu rezessiven Genotypen im Bereich von 4,8 bis 10,7 % in unterschiedlichen Parzellentypen festgestellt. Daraus wurde ein Gesamtanteil an Fremdbestäubung von 9 bis 14,3 %, in welchem auch die nicht direkt nachweisbaren Fremdbestäubungen zwischen Pflanzen desselben Genotyps berücksichtigt sind, errechnet.

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