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Variability in the Contribution of Male and Female to Cross-Fertilization in Faba Bean (*Vicia faba* L.)

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Abstract: The objective of this study was to determine the dispersal of own pollen and acceptance of foreign pollen in some faba bean (*Vicia faba* L.) lines using random amplified polymorphic DNA markers. It was carried out on four lines at Goettingen, Germany, in 2001 season. The lines' introgressive cross-fertilization (acceptance of foreign pollen) ranged from 18.9% to 46.9% with a mean of 31.2%. The mean dispersive cross-fertilization (i.e., contribution to introgressive cross-fertilization of the others) varied from 7.4% to 13.0%; indicating that there may be genetic differences as to these features. The importance of studying both pollen dispersal and pollen input in faba bean breeding programmes is discussed.

Key words: Faba bean; introgressive and dispersive cross-fertilization; RAPD markers

INTRODUCTION

Complete self-fertilization or complete cross-fertilization is rare in crops; there is, for example, some out crossing in groundnut, lentil and barley which are classified as self-fertilized crops. The breeding system of faba bean has long been known to be mixed selfing and crossing, i.e., partiall allogamy. Other genuine partially allogamous crops are cotton, rapeseed and pepper.

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In faba bean (*Vicia faba* L.), self- and cross-pollination is carried out by pollinating insects, mostly bumble-bees and honey-bees (Suso and Moreno 1999). The pollinating insects trip the flower and transfer foreign pollen to the stigma so that the own pollen and foreign pollen can germinate at the same time, and partial cross-fertilization occurs. This mixed mating system in faba bean leads to a complicated genetic structure of populations; they consist of groups of individuals differing in their inbreeding coefficient as well as in their heterozygosity. This variation has an impact on the choice between line cultivars (self-pollinated cultivars) and population cultivars. Hybrid cultivars in faba bean have not yet been developed due to instability of the cytoplasmic male sterility (Duc *et al*. 1992).

For the development of self-pollinated cultivars, individuals with a considerable degree of autofertility are needed (Stoddard 1986; Drayner 1959). Also, the closed-flower mutants discovered by Poulsen (1977), which reduce the degree of cross-fertilization, are possible aid to breed self-pollinated cultivars. Population cultivars, preferably bred as synthetic cultivars, are derived from a few, selected inbred lines having high degree of cross-fertilization (Metz *et al*.1994; Link *et al*.1994). For the production of such cultivars, not only lines with high introgressive cross-fertilization (C) are needed, but also knowledge of the dispersive cross-fertilization is crucial as it is a major component of gene flow in faba bean (Carré *et al*. 1998; Suso *et al*. 2006).

Many workers (e.g., Fyfe and Bailey, 1951; Link 1990; Metz e*t al*. 1994) used morphological markers to estimate C in faba bean genotypes functioning as pollen receptors, neglecting the capacity of genotypes to donate pollen to plants of other genotypes, i.e., dispersive cross-fertilization. One has to ask whether a high introgressive cross-fertilization may be attributed to a low production of low quality of own pollen, thus leading to a low dispersive cross-fertilization. If only such lines (high introgressive and low dispersive cross-fertilization) are chosen as parents for a population, this population would not show the expected high level of heterozygosity. Therefore, it is important to estimate both variables. The use of DNA markers has enabled breeders not only to estimate the proportion of cross-fertilization between genotypes, but also to study how individual genotypes function as pollen donors and pollen recipient, and the proportion of ovules fertilized by pollen from different sources. The objective of this study was to determine the dispersal of own pollen and acceptance of foreign pollen in some faba bean lines using Random Amplified Polymorphic DNA (RAPD) markers.

MATERAILS AND METHODS

Four faba bean lines; namely, Bulldog/1, Côte d'Or/2, Hiverna/2 and Webo/1-1, were used in this experiment. They were at least in the 8th selfing generation and were all homozygous for black hilum colour.

The experiment was conducted in 2001 at Goettingen, Germany. The lines were sown in equal proportions (1: 1: 1: 1). The four lines were sown in a plot of four rows (4.5 m²) with a seed rate of 21 seeds/m², 24 seeds/row (96 seeds per plot), 6 seeds/inbred line per row and a distance within the row of 20 cm and 30 cm between the rows. A balanced neighbourhood design (Morgan 1988) was used; hence, each inbred line had an equal chance to receive pollen grains from the other lines. The four-row-four-line plot was surrounded by one row (inner border). The distance between the holes within the inner border row was 20 cm and between this inner border row and the experimental plot was 60 cm. Moreover, the inner border row was surrounded by an outer one-row border. The outer row border was sown with 1: 1: 1: 1 from the ex-trial harvest of these inbred lines one year ago. The flowering time of the lines was synchronized by removing the earliest opened flowers as long as the latest line did not yet flower. Four random plants per each line were harvested and 20 offspring per plant were evaluated with RAPD markers.

DNA isolation was performed according to the method described by Doyle and Doyle (1990) with some minor modifications. The youngest leaves of the plant were crushed in liquid nitrogen. RAPD analysis was performed, essentially as described by Williams *et al.* (1990).

For primer screening, 88 primers were screened for polymorphism between the four inbred lines. Given RAPD to be dominant markers, a band should be present for three of the lines and absent for the fourth line, hence allowing the detection of a difference between this fourth line (female line) and its hybrids with the three others and identification of the paternal parent of any such hybrid. If necessary, i.e., if this type of polymorphism is not readily available from one primer, a combination of two primers could deliver the necessary polymorphism and information.

Five primers (Operon D20, C16, R06, AJ04, T12) were used to identify the progeny individuals whether they originated from self-fertilization (i.e. within a given female plant or between individuals of the same inbred line) or from cross-fertilization. In case of cross-fertilization, the parental pollen source, one of the three remnant inbred lines, was identified. Using these data the percentage of cross-fertilization (introgressive cross-fertilization), dispersive cross-fertilization and degree of self-fertilization were calculated as follows:

The degree of introgressive cross-fertilization= the number of progenies originating from the same and different pollen source (100%) − the percentage of progeny originating from the individuals of the same pollen source.

The dispersive cross-fertilization of the lines= sum (∑) percentage of the contribution of the line to introgressive fertilization of other lines.

The degree of self-fertilization= 100% − the percentage of progenies originated from individuals of different pollen source

RESULTS AND DISCUSSION

Figure 1 shows the RAPD fingerprint (Operon C16 primer) for 11 genotypes (as an example): Bulldog/1 as female plant (F^1), Côte d'Or/2 (Ma), Hiverna/2 (Mb) and Webo/1-1 (Mc) as males and seven progenies (P1 to P7). From the RAPD assay it is obvious that P2 and P6 progenies were originated from Webo/1-1 as pollen source, P4 and P7 progenies from Côte d'Or/2 (i.e., from cross-fertilization). On the other hand, P1, P3, and P5 progenies were not clearly identified, whether they originated from the female pollen source (Bulldog/1) or from Hiverna/2 as pollen source. Therefore, their results were identified based on second primer's results: D20, R06, AJ04, or T12 (Figures not given). Three hundred seventy-eight progeny individuals (Table 1) were identified.

The RAPD marker-based study on the degree of cross-fertilization (introgressive cross-fertilization) and dispersive cross-fertilization revealed differences among genotypes.The introgressive cross-fertilization varied between 18.9% for Côte d'Or/2 and 46.9% for Webo/1-1 with a mean of 31.2% (Table 1). The ability of the lines to donate pollen (dispersive cross-fertilization) varied from 7.4% for Hiverna/2 to 13.0% for Webo/1-1. These results indicated that there may be genetic differences as to the dispersive rate of cross-fertilization. Obviously, the expected degree of dispersive cross-fertilization of the lines, i.e., the contributions of any of the four lines to introgressive fertilization of the others, was different from the realized value. For example, Bulldog/1 "accepted" cross-pollen from Webo/1-1 for 22% of its progeny, whereas the expected value, taking the 63% of the self-fertilization of Bulldog/1 as given, was as low as (100%-63%)/3 = 12.3% (Table 1).

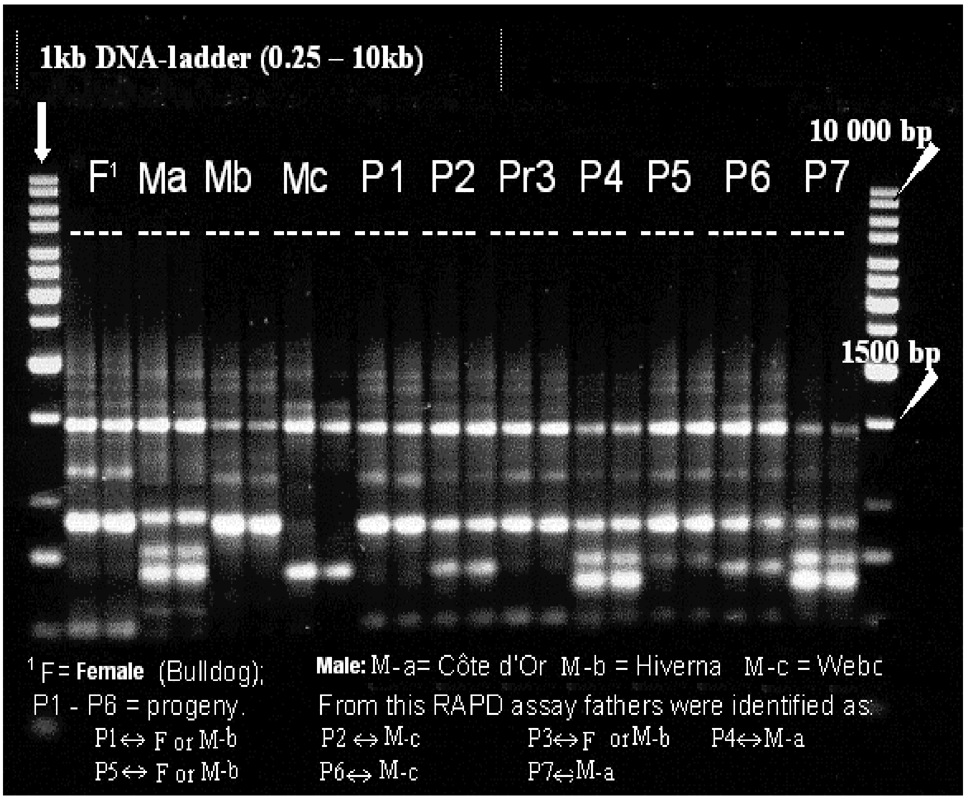

Figure 1. RAPD fingerprint (Operon C16 primer) of 11 faba bean genotypes: female, three possible males and seven progeny individuals. Progenies not clearly identified with one primer were identified based on the results of the second primer

Table 1. Percentage of introgressive and dispersive cross-fertilization of four faba bean inbred lines as assessed by RAPD polymorphism at Goettingen, Germany

Female	Male											
	Bulldog/1			Côte d' Or/2			Hiverna/2			Webo/1-1		
	N	Paternity (%)[1]	C[2]	N	Paternity (%)[1]	C[2]	N	Paternity (%)[1]	C[2]	N	Paternity (%)[1]	C[2]
Bulldog/1	59	63.0†	-	9	9.5	12.3	5	5.4	12.3	21	22.2	12.3
Côte d'or/2	5	5.3	6.3	77	81.1†	-	5	5.3	6.3	8	8.4	6.3
Hiverna/2	4	4.2	7.3	9	9.4	7.3	75	78.1†	-	8	8.4	7.3
Webo/1-1	15	18.8	15.6	16	16.7	15.6	11	11.5	15.6	51	53.1†	-
Mean dispersive fertilization	9.4[3]			11.9[3]			7.4[3]			13.0[3]		
Introgressive fertilization	37.0[4]			18.9[4]			21.9[4]			46.9[4]		

[1]Percentage of paternity, either selfing or crossing
[2]Expected crossing, e.g., of Côte d'or/2 with one male amounted to 6.3= 1/3 (100 – 81.1%)
[3]Mean dispersive fertilization, e.g., of Bulldog/1 amounted to 9.4% = ⅓(5.3%+ 4.2%+ 18.8%)
[4]Introgressive fertilization (cross-fertilization), e.g., of Bulldog/1 amounted to 37% = (100%-63%)
†Degree of self-fertilization

The data suggested that there are genetic differences due to actual combination of female and male genotypes. For example, Webo/1-1 rate of dispersive cross-fertilization was the highest (13%), yet this was not a general feature of Webo/1-1 but arose from the favourable combination of Webo/1-1 as pollen source and Bulldog/1 as maternal parent (22.2 % > 12.3%; Table 1). According to Becker *et al*. (1998), high introgression and high dispersal do not always coincide for a given genotype; genetic variation in introgressive cross-fertilization may simply depend on the different quantity of produced pollen. A genotype with poor pollen production is more apt to take in foreign pollen and less capable of donating its own pollen to others. This very likely contributes to a complex population structure of faba bean, a fact that renders its breeding and the prediction of yield difficult. This situation calls for a clear genetic switch of the mode of reproduction of faba bean to either full autogamy (as in pea) or to ~100% cross-fertilization (as in maize).

If the production of synthetic cultivars is the method of choice, the appropriate number of lines and their general and specific donor-receptor behaviour is of importance and should be considered in order to minimize inbreeding depression in the produced offspring. In this case, lines of high introgressive and dispersive cross-fertilization are needed. Therefore, Webo/1-1 could be selected for breeding synthetic cultivars. Also, as a consequence of the partial allogamy, breeding of self-pollinated cultivars is a possible choice. Here, lines with low introgressive and dispersive cross- fertilization abilities are needed and could be the basis for developing such cultiavars. In breeding self-pollinated cultivars, attention may be given to changing the flower structure to render the need for tripping unnecessary (Kambal *et al*. 1976).

However, the purely selfed seed produced by line cultivars will suffer from contamination with cross-pollen in open fields, unless spatial isolation or cages are used. As the consequence of the absence of pollinators, needed for tripping, in cages, yield of pure selfed seed in such cages is variable and mostly low. In cages, tripping can be performed manually to substitute for missing pollinators' mechanical stimulus of fertilization, thereby, allowing true selfing and high seed set, but this is a very costly procedure (Link 1990). It is, therefore, clear that the occurrence of self- and cross-fertilization in faba bean hampered its breeding progress.

In conclusion, the contribution of the dispersive cross-fertilization in faba bean mating process should be considered together with introgressive cross-fertilization in order to develop efficient and appropriate breeding and seed production strategies.

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التباين فى مساهمة الام و الاب فى عملية الاخصاب الخلطى فى الفول المصرى

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موجز البحث: جرى تقييم أربع سلالات نقية من نبات محصول الفول المصرى لمقدرة إنتشار حبوب لقاحها ودرجة قبولها لحبوب لقاح أجنبية (من السلالات الاخري)، وذلك باستخدام الواسمات الجزيئية (RAPDs). تراوحت نسبة الاخصاب الخلطي للسلالات (قبول حبوب لقاح اجنبية) من 18.9% إلــى 46% بمتوسط قدره 31.2%، فى حين تفاوت متوسط نسبة الاخصاب الخلطى الانتشارى للسلالات (المساهمة فى نسبة الاخصاب الخلطى للسلالات الاخرى) من 7.4% إلى 13.0% مما يوحي بوجود اختلافات وراثية بالنسبة لهذه الخصائص. نوقشت أهمية القدرة الانتشارية لحبوب اللقاح ومساهمتها في برامج تربية الفول المصري.

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