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Autofertility and rate of cross-fertilization: crucial characters for breeding synthetic varieties in faba beans (*Vicia faba* L.)

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Summary. To increase the level and stability of yield in faba beans (*Vicia faba* L.), heterosis must be exploited. Hybrids are not available because of the instability of male sterility. Synthetic varieties can and should be bred. Thus, we studied the reproductive behavior of this partially allogamous, insect-pollinated crop. Autofertility (AF) and the rate of cross-fertilization (C) were measured in 36 inbred lines and 28 crosses in F_1, F_2, and F_3 generations for 3 years at Hohenheim, Stuttgart, FRG. Heterozygosity strongly increased AF and decreased C. AF was negatively correlated with C. AF varied from 1% to 98%, and C varied from 7% to 82%. Heritability for both characters was high. For an optimum exploitation of heterosis, breeders should utilize lines with high C as variety components. It is often labor-intensive to multiply such lines, due to low AF. Hence, breeders tend to use autofertile lines with rather limited C. We showed that even in this case about 50% of the total heterosis, which equals a yield increase of at least 25% over the inbred line level, is realized. An increase in yield stability due to heterogeneity will occur simultaneously.

Key words: *Vicia faba* L. – Autofertility – Cross-fertilization – Outcrossing – Synthetic variety

Introduction

To overcome the acute problem of insufficient and instable yields in faba beans, heterosis must be exploited. Heterosis is the superiority in vigor and productivity of a heterozygous genotype over the mean of its homozygous parents. Heterosis for yield of faba beans is reported to be 50%–75% (Link 1988; Ebmeyer 1988; Scheybal 1988). Hybrid varieties that make full use of heterosis are not yet bred due to instability of the male sterility plasms available (Bond 1989). However, heterosis is used in population varieties. Due to its genetic heterogeneity, this type of variety is, to a certain extent, buffered against adverse environmental conditions (Becker and Leon 1988; Ebmeyer 1987, 1988; Fleck and Ruckenbauer 1989). Population varieties are preferably bred as synthetic (population) varieties, which are derived from a few, selected inbred lines. Consequently, the reproductive behavior of the crop must be thoroughly understood.

Vicia faba is an entomophilous species; bees (*Apis mellifera* L.) and bumblebees (*Bombus* sp.) visit the flowers. Both self- and cross-fertilization occur. For cross-fertilization, faba beans depend fully on insects. The rates of cross-fertilization (C) that have been reported vary around a mean near 30%–40% (Bond and Poulsen 1983). Consequently, only a markedly restricted part of the total heterosis is used in open-pollinated varieties.

Autofertile genotypes do not depend on pollinating insects for their self-fertilization. In contrast, pollen of autosterile plants can only self-fertilize after a mechanical stimulation of the stigma by visiting insects, so-called "tripping" (Drayner 1959). This can be imitated by hand, which is rather time-consuming work. Seed set of tripped, autosterile genotypes is more or less normal. Autosterility should not be confused with self-incompatibility! Autofertility (AF) is a quantitative character as is C. The present status of research on reproductive behavior is reviewed by Bond and Poulsen (1983) and by Lord and Heslop-Harrison (1984).

Breeding synthetic varieties in faba beans involves two successive steps and two crucial characters (Table 1). To breed and multiply homozygous lines at reasonable cost, these lines have to possess an adequate level of autofertility. On the other hand, the level of C determines

Table 1. Key points in the breeding of synthetic varieties of faba beans

	Step 1	Step 2
Aim	Select superior genotypes	Make use of heterosis
Means	Breed homozygous lines	Let several superior lines cross-fertilize
Pollination	Self; (bumble-) bees absent	Self- and cross-; (bumble-) bees present
Crucial character	Autofertility	Rate of cross-fertilization

to which degree heterosis is used in a synthetic variety. Therefore, high values for AF and C must be attained. The prevalent comment in the literature is that these two characters show a negative correlation (Bond and Poulsen 1983). Consequently, the main question for a breeder is whether a high expression of both can be combined.

The purpose of this work was to study the variability of autofertility and rate of cross-fertilization, their interrelation, and their significance for the breeding of synthetic varieties.

Materials and methods

From each of the well-known varieties Diana, Herra, Herz Freya, Kristall, Skladia's Kleine, and Kleine Thüringer, six homozygous inbred lines were derived. All 36 lines express the buff seed color. Eight of the lines (two from each of four varieties) were crossed in a diallel pattern, resulting in 28 crosses. The 8 parental lines and their 28 cross-progenies in the F_1, F_2 and F_3 generation were grown in 1985 and 1986 at Hohenheim, Stuttgart, to measure AF and C (F_3 only in 1986).

All 36 lines were grown in 1986, 1987, and 1988 at Hohenheim to measure AF, and in 1987 to score them for C.

Autofertility (AF)

The plants were grown in bee-proof isolation cages, covering 60 m^2 each, with 13 plants per m^2. In a pilot study we either reduced the flower number of the first 12 inflorescences to 1, 2, 3, 4, or 5 flowers per inflorescence or left the inflorescences intact (Table 2). Our genotypes produce about 6–9 flowers per inflorescence.

In the main experiments, we had ten plants per plot and two replications. At the first 12 inflorescences, flower number was reduced to 2, resulting in a total of 24 flowers per plant. Other inflorescences and tillers were removed. All flowers were left untripped. The assumption is that all fertilized flowers develop to pods. Rate of fertilization was calculated as (number of seed containing pods/24) × 100. In this way, we indirectly scored the relative proportion of flowers that self-fertilized without being tripped, thus measuring autofertility (Drayner 1959; Table 2).

Rate of cross-fertilization (C)

The genotypes were sown in the open field, ten seeds per plot, with two replications. They were individually surrounded by eight seeds of a stock that is homozygous for the dominant black seed color and shows an appropriate flowering time. The resulting plant density was 20 plants per m^2. Only the plants with buff seeds were harvested. Their progenies were grown in the following years. The number of offspring of each genotype with buff seeds as well as the number of offspring with black seeds at harvest time was counted. An offspring arising from a buff seed that yielded black seeds is a result of cross-fertilization. At least 1,000 offspring from each genotype were screened. C was calculated according to Eq. (1)

$$C = \frac{(\text{no. of black seed. prog.})}{(\text{total no. of scored prog.})} \times 100 \times (1+R), \qquad (1)$$

where R is the realized quotient of buff-seeded plants to black-seeded plants in the generation in which the cross-fertilization occurred.

AF and C are on a percent scale. For both characters only rank correlation coefficients due to Spearman were calculated.

Results

Table 2 shows that the relative proportion of seed-containing pods that developed from untripped flowers is consistently within a range of one to three untripped flowers per inflorescence. We conclude that in this range all fertilized flowers develop to pods. The value decreases in the treatments with five or more flowers per inflorescence. In these cases fertilized flowers are obviously also aborted. As a result of this pilot study, the number of flowers per inflorescence was reduced to two in the main experiments.

Figure 1 contains the results of the characters AF and C, given as average values of the generations per year. The F_3 generation could only be scored in 1986. Obviously, heterozygosity increases AF and decreases C. In 1986, AF was lower, whereas C was higher than in 1985.

The correlation coefficients between the results in the two years are relatively high (Table 3). The low value for C in F_2 results from a small genetic variation between the genotypes in that generation. The F_1s varied for AF between 50% and 100%, but most F_1 values are densely clustered near the maximum, leading to a coefficient of only 0.70.

In Table 4, the correlations within and between both characters are given. Again, the generations are regarded separately. A strong relationship between AF and C is evident only as long as C in F_1 is considered. Correlation coefficients between parents and progenies are high for AF as well as for C. An exception is the correlation between parental inbred lines and their F_1s for C, where the correlation coefficient is only 0.43.

Figures 2 and 3 show the relationship between AF and C. AF versus C is plotted for the eight lines; furthermore, AF is given as the parental mean of each cross, together with C of each cross in the generations F_3, F_2, and F_1. Five parental lines are more or less autosterile, whereas three lines are rather autofertile. Consequently,

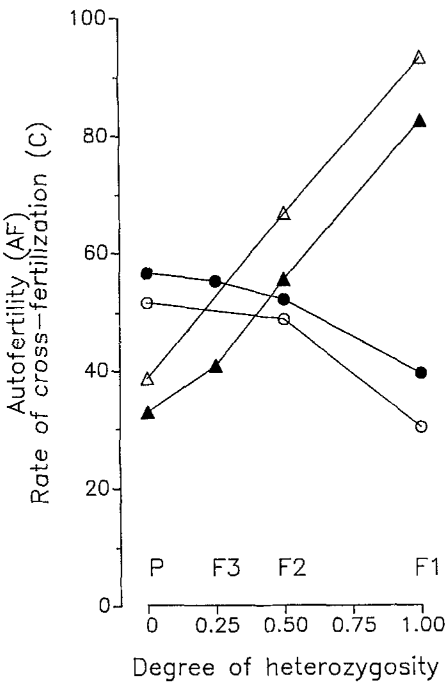

Fig. 1. Generation mean values for AF (△ = 1985; ▲ = 1986) and C (○ = 1985; ● = 1986)

Table 2. Results of the pilot study concerning the method of measuring AF (numbers indicate pods per flowers, given as percent)

Year	Genotype	No. of (untripped) flowers per inflorescence						
		1	2	3	4	5	[a]	LSD*
1984	1	80	79	78	–	–	43	
	2	92	–	92	–	–	–	7
1985	1	–	80	80	76	68	49	
	3	–	1	1	0	0	0	5
1986	1	76	79	–	65	–	48	
	4	2	2	–	3	–	1	12
	5	60	66	–	55	–	53	

* LSD for comparison of treatments, $P = 0.05$

[a] In this treatment the number of flowers was not reduced (i.e., 6–9 flowers per inflorescence)

Table 3. Phenotypic rank correlation coefficients due to Spearman for AF and C scores over two years (1985 and 1986)

	Autofertility (AF)	Rate of cross-fertilization (C)
P	0.83**	0.69**
F_2	0.81**	0.50**
F_1	0.70**	0.78**

** Significant for $P = 0.01$

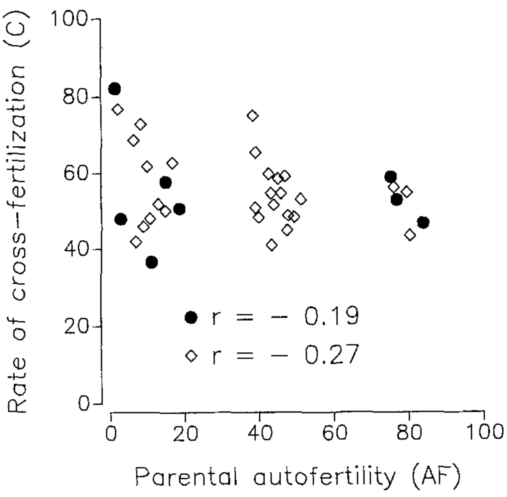

Fig. 2. Parental AF and C of lines (●) and F_3s (◇)

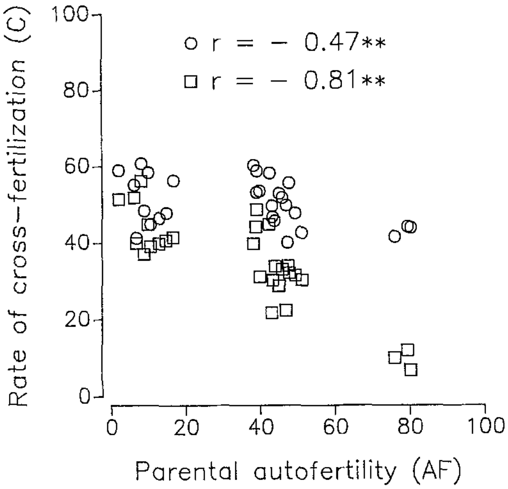

Fig. 3. Parental AF and C of F_1s (□) and F_2s (○)

there are three crosses within the three autofertile lines, ten crosses within the five autosterile lines and 15 crosses among both groups. The correlation coefficient for the eight lines of $r = -0.19$ can be compared with the one for all 36 lines (AF from 3 years, C from 1987) of $r = -0.37$.

Discussion

The number of seeds per untripped plant is influenced by the percentage of flowers that are self-fertilized without tripping. This is what we consider autofertility (Drayner 1959). Other factors are the number of flowers per plant, the number of ovules per flower, and the productivity of the genotype. Nevertheless, AF is distinctly the main factor for the propagation of untripped faba beans. According to our observations, an AF-level of 40% is sufficient to ensure a reasonable propagation in untripped conditions, provided that other growth factors are not limited.

Table 4. Phenotypic rank correlation coefficients due to Spearman between AF and C

		Autofertility (AF)				Rate of cross-fertilization (C)			
		P	F_3	F_2	F_1	P	F_3	F_2	F_1
AF	P	–	0.89**	0.82**	0.71**	−0.19	−0.27	−0.47**	−0.81**
	F_3	–	–	0.95**	0.82**	−0.26	−0.33	−0.41*	−0.74**
	F_2	–	–	–	0.86**	−0.18	−0.24	−0.35	−0.67**
	F_1	–	–	–	–	−0.19	−0.25	−0.17	−0.66**
C	P	–	–	–	–	–	0.82**	0.83**	0.43*
	F_3	–	–	–	–	–	–	0.71**	0.44*
	F_2	–	–	–	–	–	–	–	0.60**
	F_1	–	–	–	–	–	–	–	–

*, ** Significant for $P = 0.05$, 0.01, respectively

We used a rather easy approach to measure AF. It should be tested with other material as well. In our experiments the method yielded meaningful data. To some extent it is comparable to the method of Lawes (1974). He also reduced the number of flowers, but he tripped half of the inflorescences of a plant to compare the pod set of tripped and untripped inflorescences.

Heterozygosity clearly increased AF and decrased C (Fig. 1). This is in good agreement with published results (Drayner 1959; von Kittlitz 1982). For AF, the overall mean of the F_2 generation is very near to the average of the lines and the F_1s (Fig. 1). For the rate of cross-fertilization (C), there is obviously no linear relationship to the degree of heterozygosity. The results of the F_2 and F_3 generations are rather similar to that of the inbred lines. In contrast to this, the F_1 generation showed a markedly decreased C. The effect is not as drastic as reported from Drayner (1959) and von Kittlitz (1982). An exception are Poulsen's data (1980) where C for F_1s is as high as for inbred lines.

From Fig. 2 it can be seen that high AF can already be found at the homozygous level. This is in contrast to Drayner (1959) and Stoddard (1986). Stoddard proposed the introduction of exotic material to increase the AF level.

The reliability of our data can, to some extent, be deduced from the comparison of the results of two years (Table 3). Heritability in the broad sense, i.e., the reproducibility of the results in different environments, is high. This can be regarded together with the high correlation coefficients between parents and progenies (Table 4), the latter indicating narrow-sense heritability. For both broad- and narrow-sense heritability, similar results were found.

For the breeder, the correlation between the two characters is of crucial importance. The more heterozygous a genotype, the more C is reduced by high AF (Table 4). Only a very high AF, based on a high inbred value plus heterosis for AF, leads to a clear decrease of C (Fig. 3). Poulsen (1980) stated that a highly autofertile genotype will self-fertilize before anthesis, so that insects with foreign pollen will come too late to cause crossbred progeny.

Table 5. Panmictic index (P) and yield in syn-2, influenced by C of parental lines and of F_1s

C of lines	Autofertile lines C of F_1s = 0		Autosterile lines C of F_1s = C of lines	
	Panmictic index (P)	Yield[a] (tons/ha)	Panmictic index (P)	Yield (tons/ha)
30%	0.360	4.72	0.405	4.81
50%	0.500	5.00	0.625	5.25
70%	0.560	5.12	0.805	5.61

[a] Calculated as: 4.0 t/ha + $P \cdot 2.0$ t/ha

The correlation of C in F_1 to the parental AF is stronger than that to the AF of the F_1s themselves. The reason is probably the higher variation for AF between the lines than between F_1s.

The correlation coefficient for C between P and F_3 is high. It is lower when the F_1 generation is included. C in F_1 is, in fact, influenced to a larger extent by AF of the parents than by C of the parents.

Regarding Fig. 2, there seems to be a smaller variation for C between autofertile genotypes than between less autofertile ones. Nevertheless, an AF of at least 40% and a C of over 50% can be combined.

The farmer should grow the syn-2 or syn-3 generation of a given synthetic variety, because in syn-1 the optimum use of heterosis is not yet realized (Wright 1977; Link and Ruckenbauer 1987). According to the presented results, a synthetic variety built up from autosterile lines will express a rather stable C during its propagation, in spite of an increasing degree of heterozygosity. In contrast to this, a variety synthesized from autofertile lines will yield heterozygous offspring that show a drastically reduced C (Fig. 3).

For Table 5 we assumed C of the F_1s to be either equal to C of the lines (autosterile lines) or to be zero (autofertile lines). Thus two extreme situations are demonstrated. Equations (2) and (3) are used. For convenience, we considered synthetics bred from a large number of lines. The panmictic index P ($P=1$ – coefficient of inbreeding) was calculated for generation syn-2. To demonstrate the possible effects on yield, we applied an inbred minimum of 4.0 t/ha and a heterosis of 2.0 t/ha (Link and Ruckenbauer 1987).

$$P=(1-C)\cdot C+C\cdot 0.5 \qquad (2)$$

(autofertile lines)

$$P=(1-C)\cdot C+C\cdot (C+(1-C)\cdot 0.5) \qquad (3)$$

(autosterile lines)

In Table 5 is shown how the indirect effect of the parental AF on C of heterozygous offspring may influence the productivity of a variety. The higher the level of cross-fertilization in syn-0 (C of lines), the greater is the part of F_1 plants in syn-1, and the more important is their specific reproductive behavior for syn-2.

Whether a breeder can really use lines with low autofertility as components for a variety is, nevertheless, a question of practicability of line breeding and line propagation.

We never found a relationship between AF or C and agronomic characters, e.g., yield, therefore choice is free in this respect.

Components for synthetic varieties should add favorable agronomic characters to:

- an adequate and reliable yield per se,
- a high rate of cross-fertilization per se,
- a high general combining ability for yield,
- a high general combining ability for C.

Since general combining ability for yield is reported to be positively correlated to yield per se (Link and Ruckenbauer 1987; Ebmeyer 1988), after yield per se the rate of cross-fertilization per se should be measured next.

We could show that AF and C are relatively easy to handle genetically due to high variation, high heritability, and low negative correlation. We conclude that the prerequisites for the systematic breeding of synthetic varieties are present.

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