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Breeding for heterosis and male sterility in faba bean

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SUMMARY - Floral biology in faba bean is partly allogamous and partly autogamous in most countries in which this crop is grown. This specificity raises the problem of how to choose the type of variety to be produced. Experiments conducted on spring– and winter–type faba bean have shown that hybrid vigor might be important in this species (over 30 to 50% above the mid-parent). Heterosis is dependent on the type of faba bean and the origin of the parents. In particular, it is more important in winter type faba bean and is dependent on genetic distance between the parents of the hybrid. Yield parameters involved in hybrid vigor are analyzed. Cytoplasmic male sterility (CMS) was discovered independently, both in Great Britain and in France ('447 cytoplasm' discovered by Dr. Bond and '350 cytoplasm' found by Dr. Berthelem). Both types of cytoplasms show an important instability resulting in disjunctions sterile/fertile in the course of the backcrossing generations or while multiplying female lines. Potential causes of this instability have been analyzed. The possibilities to correct this default are also shown, with particular emphasis on the most recent information available on the possible corpuscular structure of the '447 sterility'.

RESUME - "Sélection pour la vigueur hybride et la stérilité mâle chez la féverole". La biologie florale de la féverole est intermédiaire entre l'auto et l'allogamie dans la majeure partie des zones dans lesquelles cette espèce est cultivée. Cette particularité pose le problème du choix du type de variété à produire. Des expérimentations menées sur des féveroles de type printemps et de type hiver ont montré que la vigueur hybride pouvait être importante dans cette espèce (30 à 50% de supériorité par rapport au parent moyen). L'hétérosis est variable selon le type de féverole et selon l'origine des parents. Il est en particulier plus important chez les type hiver et est fonction de la distance génétique séparant les parents de l'hybride. Les différents paramètres du rendement affectés par la vigueur hybride sont analysés. La stérilité mâle cytoplasmique (SMC) a été découverte indépendamment en Grande Bretagne et en France sous deux forme différentes ('cytoplasme 447' et 'cytoplasme 350'). Ces deux types de cytoplasmes présentent une importante instabilité se traduisant par des disjonctions stériles/fertiles au cours des générations de rétrocroisement ou de multiplication des lignées femelles. Les causes possibles de cette instabilité sont analysées. Les moyens mis en oeuvre pour éventuellement la corriger sont également présentés en insistant en particulier sur les plus récentes informations relatives à la nature corpusculaire possible de la 'stérilité 447'.

Introduction

In most of the regions around the world the reproductive system of faba bean is intermediate between allo– and autogamy. Some differences may exist within types (in general, spring types are more allogamous than winter types) or within regions (southern have often more autogamous types than northern ones). In France the most common value found for allogamy is about 40% for winter and 60% for spring types. Nevertheless, these values are quite variable. At the present time we are breeding for strict autofertility in winter faba bean.

Variations in allo– or autofertility are often correlated with the occurrence of natural populations of pollinators (honey bees or bumble bees), natural selection in countries with high levels of pollinators leading in general to higher levels in allofertility.

These differences in floral biology incited breeders to define the best breeding method to be used for faba bean improvement. Different ways were explored to obtain high yielding commercial varieties, some using the autogamous side of the crop to produce pure lines, others exploiting its allogamous side to obtain varieties using partially (synthetics) or totally (hybrids) the heterosis arising from natural or controlled outcrossing (Picard *et al.*, 1982).

Heterosis in faba bean

The first attempts made in France to create pure lines in winter–type faba beans showed an important decrease in yield performances as long as homozygosity went along. In F_{10} yield was not higher than the check, and this observation convinced Berthelem in France to look for potential hybrid vigor, at the beginning through synthetic varieties and later, after the discovery of male sterility, by the total exploitation of heterosis in true hybrid varieties.

Heterosis in winter types of faba bean

Table 1 shows F_1 performances as compared to the top and the mid–parent of the crosses and to the French landrace 'Cote d'Or' used as winter type check.

From these results, it is clear that hybrid vigor is effective in faba bean and that satisfactory yield may be expected from hybrid combinations. As compared to the top parent, the gain to be expected from the mean of the F_1 is greater than 50% (2.0 t above the 3.4 t of the top parent) and this gain reaches 75% if the F_1 hybrid is compared to the mid–parent performance. When compared to the results of 'Cote d'Or', the mean of the F_1 exceeds the yield of the landrace by 70%.

If we observe more closely the different combinations which can be obtained from different parents, we can notice that not only both, male and female parents, may influence heterosis expression, but that the origin of parents is also important. These observations fit the results which are to be reported below, and especially, the notions of general and specific combining abilities.

Hybrid combinations

Table 2 shows a comparison between Franco–English and entirely French hybrids. It is obvious that these results, together with those of Table 3, indicate that hybrids obtained through crosses between French and English lines (29D French line as female and S45 English res-

Table 1. Yield performance of F_1 hybrids (t/ha) in 3 locations.

	Rennes	Dijon	Cambridge	Mean
F_1 min.	5.02	6.40	4.45	5.29
F_1 max.	5.67	7.70	6.26	6.54
F_1 means	5.32	6.92	5.22	5.82
F_1-Top parent	1.83	2.83	1.35	2.00
F_1-Mid parent	2.29	2.99	2.22	2.50
Cote d'Or	2.78	5.06	2.41	3.42

Table 2. Role of the hybrid structure for heterosis.

Structure	Yield* (t/ha)	F_1 - Top parent		F_1 - Mid parent	
		Yield (t/ha)	%	Yield (t/ha)	%
Franco - English					
29-D x S45	6.21[a]	1.84	29.9	1.68	37.0
L6 x 6A	5.71[b]	0.65	14.3	1.00	24.0
Franco - French					
29-D x 6A	5.45[b]	0.48	9.6	1.05	23.9
29-E x 6A	4.67[c]	0.85	22.4	1.04	28.6
15-A x 6A	4.66[c]	0.37	8.3	0.60	14.8

*Values showing the same letter do not differ significantly

Table 3. Role of pollen parent in hybrid vigor; seed yield (t/ha).

Female	English pollen parent			French pollen parent			Average
	S45*	LCF*	Mean	6 A	23 B	Mean	
3-33	7.00[a]	6.48[b]	6.74	5.01	5.51	5.26	6.00
972-D	6.60[a]	6.22[b]	6.41	5.80	5.10	5.45	5.93
29-D	6.39[b]	5.97[b]	6.18	5.22	4.54	4.88	5.53
972-C	5.06[c]	5.27[c]	5.17	4.55	4.08	4.32	4.74
Mean	6.26	5.99	6.13	5.15	4.81	4.98	5.56

*Values showing the same letter do not differ significantly torer, as well as L6 as female and 6A French restorer) give better results than the hybrids with exclusively French origin. This seems to be a general rule, perhaps because of the higher genetic distance between French and English lines than that existing within French lines or because of a better individual value of the English lines when compared to the French ones.

Role of different parents

A top cross experiment was performed in order to compare both female and pollen parents in any possible combination. Results are shown in Table 3 (Berthelem and Le Guen, 1975).

These data are in good agreement with those reported formerly in relation with the pre–eminence of Franco–English hybrids over the French ones. They also show the specific role of each parent in the final expression of hybrid vigor. We will give below some more information about specific combining ability, but it is clear that both female and pollen parents have to be carefully chosen for optimal heterosis.

Role of genetic structure

Different genetic structures were compared over a 3–year period in the same site (Table 4) and it appears that complete hybrid structures (F_1 hybrids) produce at least 1.5 t/ha (31%) more than partial hybrid structures (synthetics). This increase in yielding ability is a justification to breed towards hybrid production in faba bean even if commercial seeds are more expensive for hybrids than for synthetics. The gain in yield is of the same order when comparing synthetics versus pure lines (30% more yield for synthetics than for pure lines).

As the rate of commercial seed production for F_1 hybrids is rather low because hybrid seeds are harvested on somewhat poor female lines, the potential performances of three–way hybrids were studied. As a matter of fact, seed production for commercial purposes increases considerably when seeds are harvested from F_1 hybrids and as a result, decreases in the same manner the cost of the sowing input. Experiments made at Rennes and at Dijon to test this assessment are summarized on Table 5, where the expected value for a three–way hybrid is the mean value of two F_1 hybrids obtained from the 3 parental lines involved in the three–way hybrid production (Berthelem and Le Guen, 1975).

In spite of the restriction that the results were obtained only by one year trials in two sites, Table 5 is interesting as it shows at least two main features. First, there is a quite good agreement between expected and observed values from F_1 to 3–way hybrids. When deviations occur, they generally underestimate the 3–way potential, two cases in which LCF is used as restorer excepted. Second, it appears that yielding ability of 3–way

Table 4. Role of genetic structure in heterosis expression.

Structure	Seed yield (t/ha)			Mean	Average
	Year 1	Year 2	Year 3		
Franco-English F_1	7.24	6.21	6.66	6.67	6.65
English F_1	6.99	7.00	5.86	6.62	
English synthetic	5.70	5.38	4.22	5.10	5.09
French synthetic	5.45	5.17	4.62	5.08	
French pure line	4.64	4.52	3.76	4.31	4.31

hybrids is as high as the potential of the corresponding F_1 hybrids. The decrease generally observed in other species between F_1 and 3–way hybrids does not appear in faba bean, and this is a quite interesting result for seed production.

Heterosis in spring types of faba bean

As most of the areas grown with faba bean in Northern Europe are represented by spring sowings, it seems necessary to test hybrid vigor in such a type of material, to try to develop commercial hybrid varieties. An experiment was conducted at Rennes and at Dijon in which F_1 and 3–way hybrids were compared to a common standard (variety 'Ascott' which was a few years ago the main cultivar grown in France). Table 6 presents the results obtained at Rennes. Two restorer lines (HG 115N and Ad20) and six female or non restorer lines (G7, G77, Ad23, G79, G78, 350/G58) were used for F_1 or 3–way hybrid production. As an exception, G58 is used in the '350 sterile–cytoplasm' discovered at Rennes; the other parents were in the '447 sterile–cytoplasm' discovered at Cambridge.

It seems clear from these results that heterosis is lower in spring type faba beans than in winter ones. In general, the mean increase over the standard is not higher than 25%, and this value is reached because of the performance of some lines (means of G7 with HG115 and of G7 with Ad20 were respectively 39% and 37% over 'Ascott'). It can also be observed that in spring types there is a good agreement between the yield of the F_1 and the yield of the 3–way combinations and that, as a rule, 3–way hybrids are at least equal and often superior to the F_1 hybrids.

Different restorer lines were compared as pollen parents in F_1 or 3–way hybrids in another trial (Table 7). The percentage of fertile revertants in the female lines fluctuated from 0 to 18%. From this experiment, it is obvious that heterosis value over the check ('Alfred') is

Table 5. Expected and observed yield values (t/ha) of 3-way hybrids.

	Rennes			Dijon		
	F_1 hyb.	3-way hybrids		F_1 hyb.	3-way hybrids	
		expected	observed		expected	observed
RESTORER S45						
L6	4.39	4.45	5.29	5.73	5.69	5.80
29E	4.50			5.63		
L6	4.39	4.75	4.74	5.73	5.62	5.73
972A	5.11			5.50		
L6	4.39	4.63	4.87	5.73	5.53	5.91
3-33	4.86			5.32		
Mean	4.82	4.61	4.97	5.49	5.61	5.81
RESTORER CF						
L6	4.61	4.29	3.97	5.63	5.50	5.39
29E	3.97			5.37		
L6	4.61	4.78	5.05	5.63	5.52	5.51
972A	4.94			5.40		
L6	4.61	4.30	4.35	5.63	5.40	5.40
972A	3.98			5.16		
Mean	4.30	4.46	4.76	5.31	5.47	5.43

Tabla 6. Heterosis in spring type faba bean (% of 'Ascott').

	F_1 hybrid	G7	G77	Ad23	G79	G78	Mean
RESTORER HG 115							
G7	134	–	143	132	148	133	139
G77	118	121	–	120	128	127	124
Ad23	102	116	108	–	111	111	112
350/G58	95	–	113	–	116	–	115
Mean	112	119	121	126	126	124	123
RESTORER Ad 20							
G7	123	–	140	130	143	134	137
G77	115	119	–	129	124	–	124
Ad 23	103	109	125	–	106	115	114
Mean	114	115	133	129	124	124	125

Table 7. Restorer lines in F_1 and 3-way hybrids[a]. Values expressed as % of cv. 'Alfred'

Female	Male				Mean female
	G - 58	G - 8	Ad23	HG-115	
447/Ad23 447/Ad23*123	112 (3.4) 119 (15.1)	113 (0) 124 (12.2)	116 (0) 110 (17.8)	111 (16.1) 110 (18.2) 125 (5.3)	113 113
Mean male	116	119	113	111	

[a]Values in parenthesis correspond to the % of revertants in the female lines

not higher than 16% on average. Nevertheless, for the particular 3–way hybrids (Ad 23 x 123) x HG 115 we had two different female parents with different reversion rates to fertility, one with 18.2% revertants which yielded 10% over the check and a second one with only 5.3% fertile revertants whose yield was 25% higher over the same check. Thus, an increase of 13% in fertile plants in female parent leads to a decrease of 15% in yield potential. It is therefore obvious that there is a strict relation between the proportion of fertile plants (revertants) in the female parent and its ability to give high yielding hybrids.

Results of European trials

These trials were aimed to test different varieties or breeding accessions in a very large network including France, Holland, Great Britain, Germany, Denmark and Ireland. The main objectives of these trials are to define an 'ideotype' of faba bean well adapted to very different environmental conditions.

Table 8 gives the results for 19 varieties in 24 European locations during two years. Three French hybrids were included in these trials (A x C = Ad 23 x HG 115; B x C = G 58 x HG 115; (A x B)x C = (Ad 23 x G 58)x HG 115). These hybrids were not always the best cultivars in all the locations, but as average they behave correctly and when looking at the regression coefficients, the mean square of deviations and the variation coefficient for regression, they generally present the lowest values, indicating a good yield stability.

Table 8. European *Vicia faba* joint tests[a].

	Yield (t/ha)	r	D	CV (%)
Minica	4.78	1.07	68.1	17.3
Kristall	4.27	0.95	18.8	10.2
Alred	4.99	1.06	24.2	9.9
Troy	4.34	0.95	32.0	13.0
Optica	3.87	0.93	37.2	15.7
A * C	4.96	1.17	16.5	8.2
B * C	4.67	1.18	21.9	10.0
(A*B)*C	4.46	1.13	20.5	10.1
C	3.05	0.98	51.7	23.6
M5.1	4.83	1.19	17.0	8.5
F6	3.11	0.81	60.3	25.0
ST 47/2	3.76	0.96	26.4	13.7
Synt. A	4.82	1.06	29.2	11.2
Synt. B	4.86	0.97	14.0	7.7
TP667	3.57	0.82	19.9	12.5
Tricol	3.76	1.05	30.8	14.8

[a]r: Regression coefficient; D: Deviation mean squares; CV: Coefficient of variation of regression coefficients

Yield components involved in heterosis

Characters expressing heterosis were studied in several winter–type F_1 hybrids. The summary of these observations is shown on Table 9.

Tillering ability is largely increased when compared to both mid or top parents. The increase in seed production per plant is the consequence of an increase in the number of fertile nodes per plant, resulting itself in an increase of the total number of pods per plant. On the contrary, the number of pods per node and the number of seeds per pod are not affected by hybrid vigor.

Seed weight is intermediate between top and mid parent values. This result shows that it could be useful to produce hybrid varieties with a medium seed size emnating from small seeded and large seeded parents. Among vegetative traits, height of plants is positively affected by heterosis increasing the susceptibility of the hybrid varieties to lodging.

Table 9. Components of heterosis in winter types[a].

Characters	Deviation (%) from	
	Top parent	Mid parent
Tillers/plant	12.3(1)	22.3(1)
Fertile nodes/plant	29.0(1)	62.8(2)
Pods/plant	33.1(1)	68.4(2)
Seeds/plant	36.8(1)	77.5(2)
Pods/fertile node	1.0 NS	1.1 NS
Seeds/pod	1.3 NS	6.8 NS
Seed weight	-17.0	3.9 NS
Height	14.7(1)	16.7(1)

[a]NS: Not significant; (1): $0.01<p<0.05$; (2): $p<0.01$

Table 10. General and specific combining abilities for three yield components.

Yield component	G.C.A.	S.C.A.
Pods/plant	17.8	20.7
Seeds/plant	55.4	20.7
Seed weight	-0.105	-0.034
Yield	21.6	9.1

Genetical aspects

Many diallel analyses were performed within and between the different groups of *Vicia faba* (*V. faba major*, *V. faba minor* and *V. faba paucijuga*) from which some conclusions can be drawn. What seems to be fundamental, particularly for *V. faba minor*, is that as a rule, individual effects for general combining ability are superior to individual effects for specific combining ability. Some indications about these aspects are shown on Table 10 in relation with yield and three components of yield. If the character 'pods per plant' is excluded, the other two components as well as yield itself, show a G.C.A. 2 or 3 times higher than S.C.A. This observation, which of course is to be restricted to the material under study, is very important for breeding purposes as it means that the best expected hybrids are derived from crosses between lines possessing the best individual values.

Male sterility in faba bean

Use of heterosis was made possible in faba bean after the discovery of male sterility in this species. Two types of male sterility have been reported:

- Genetic male sterility known in faba bean under different forms (monogenic recessive or oligogenic; the latter, obtained from mutagenic treatments, is being used to improve outcrossing and gene randomization in both natural and breeding populations).
- Cytoplasmic male sterility (CMS) controlled through an interaction between cytoplasm and nuclear information. In Europe two countries devoted a lot of work to CMS: Great Britain (especially Dr. Bond at PBI, Cambridge) and France, where Berthelem and Picard's studies were followed by those of Le Guen, Duc and more recently by Lefebvre and Dulieu.

Cytoplasmic male sterility in *Vicia faba*

Genetic male sterility is quite difficult to use for hybrid production because the maintenance scheme implies only one half of fertile seeds. Only the dominant 'Diana' genetic male sterility is presently used for breeding purposes but not for commercial seed production.

Regarding CMS, two types at least are known in *Vicia faba*. The first one was discovered in 1957 by Dr. Bond at PBI Cambridge; it is known as '447' CMS. The second was discovered by Berthelem at INRA, Rennes, and is known as '350' CMS. Both of them were independently discovered in natural populations. They are different in the sense that they do not accept the same restorer lines, with the exception of the line HG115 which restores fertility both in '447' and '350' CMS. Until now, '447' CMS has been more intensively used than '350'.

Scheme for the classical use of CMS in faba bean

Fig. 1 shows a general method to use CMS for breeding and seed producing purposes, i.e., transfer of genotypes into a specific cytoplasm, maintenance of the CMS lines when converted and restoration of female lines to produce hybrids. This method is classically used in faba bean as well as in other species. Nevertheless, two specific features have to be mentioned in the case of *Vicia faba*: the so called 'permanent restoration' and the instability of CMS in the course of both backcrosses and seed increase generations.

Permanent restoration

Fig. 2 illustrates the specific behaviour of faba bean as compared to maize. What can be observed in faba

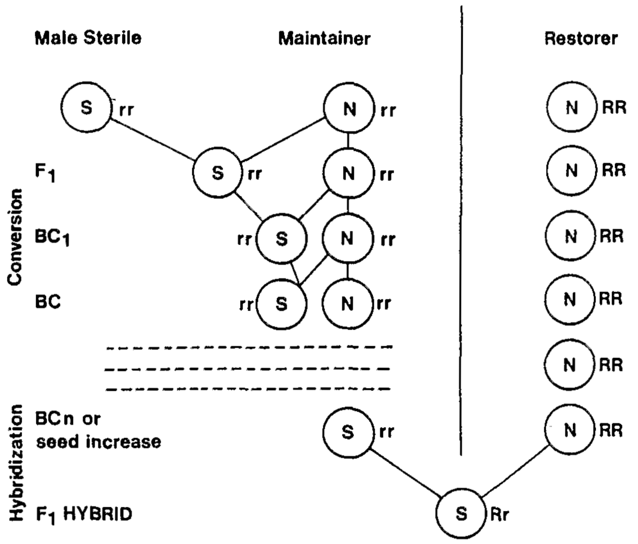

Fig. 1. General method to use CMS for breeding and seed production.

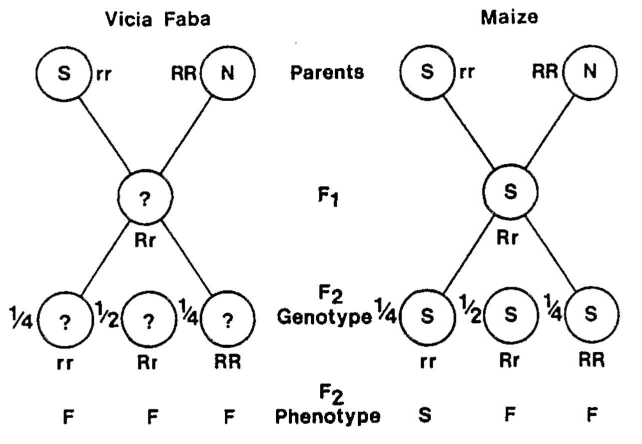

Fig. 2. Specific behaviour of faba bean as compared to maize.

bean is the disappearance of male sterile plants in the F_2 generation after restoration of the fertility in F_1, whereas 25% sterile plants were expected. Moreover, sterility never reappears after selfing these fertile plants. Nevertheless, when a test cross on a sterile parent is performed with the progeny of a restored F_1, expected ratios (25% rr, 50% Rr and 25% RR) are really found, implying that the permanent restoration is not due to modifications in nuclear information but certainly due to a modification of the cytoplasmic structures by restorer genes (Berthelem, 1970).

Instability of CMS

– Phenotypic aspects of CMS (Benevent and Le Guen, 1976; Berthelem and Formal, 1973).

First reports on phenotypic aspects of CMS in faba bean by Bond *et al.* (1966 a,b) indicated large fluctuations in male sterility expression. Some sterile plants produced small amounts of normal viable pollen in some or in all the flowers of a plant. 'Normal' pollen yield was also variable, ranging from only some pollen grains to a large proportion of fertile grains in a flower. Given the importance of this phenomenon, both for fundamental and for practical reasons, it arose great interest in France. Conclusions deriving from the different experiments performed by several authors can be summarized as follows:

· Cytoplasmic male sterility in *Vicia faba* cannot be described simply in terms of strict male fertile or male sterile plants, because a continuous variation seems to exist between completely sterile to rather completely fertile plants occurring among expected sterile progenies.

· Instability (i.e. segregation into sterile and fertile plants in normally sterile progenies) appears depending on the genotype of the recurrent pollen parent.

· Chimeral structures, as for example 'tiller sterile' plants (one or more fertile tillers in a plant, the other tillers of the same plant remaining sterile), may occur.

· The phenomenon of reversion seems to be unidirectional from sterility to fertility.

– Instability through generations.

Classical use of CMS supposes 3 phases (Fig. 1):

· Conversion, i.e. the phase of introduction through backcrosses, of different genotypes into a male sterile cytoplasm.

· Seed increase, which in normal cases is the continuation of the backcrossing phase (sib crosses between isogenic N and S cytoplasm lines).

· Hybridization, or hybrid production.

In the course of the first stage of the scheme from F_1 to BC_n, crosses between the male sterile parent and the recurrent pollen partner give as a rule 100% male sterile progenies because of the maternal transmission of the cytoplasm. The disjunctions expressing cytoplasmic instability may appear in BC_1 or BC_2 but also in very late backcrosses (i.e, BC_6 to BC_8 or even more). Moreover, when segregations do appear the proportion of fertile segregants always increases from one generation to the next. This is the reason why from more than 150 genotypes tried on '447' CMS, only 3 or 4 remained sufficiently sterile in BC_{10} to be accepted for the second stage (seed increase). For example, 3 French lines (Ad 23, 658 and 196) stabilize sterility in '350' and '447' cytoplasms, permitting the production of hybrids.

As seed multiplication rate in faba bean is low, at least 5 generations are needed in order to obtain a convenient number of female plants for hybrid production. In spite of all the precautions taken to grow these 5 seed increase generations, we observed that the lines which had a good behaviour during the conversion cycle showed instability during the generations of multiplication (Fig. 1). Starting from 0% fertile plants in the female parent, we frequently observed a rate as high as 30 to 50% fertile plants in the female line in the fifth generation, indicating an exponential increase of fertility from one generation to the next.

– Possible explanations for instability.

Studies under field, glasshouse and controlled environment chambers showed that various environmental conditions may act on the phenotypic expression of male sterility. Among these, light and, especially, temperature play an important role (Fig. 2; Bouverat–Bernier and Le Guen, 1980).

Meiosis has been found to be very sensitive to environmental conditions for the phenotypic expression of male sterility. It has been shown, for instance, under controlled temperature conditions, that within a temperature range between 17 °C and 22 °C, the higher the temperature, the higher the proportion of fertile pollen in the flowers. We have also shown that, at constant temperature, there is an increasing fertility gradient from the bottom to the top of the stems and from the first to the last tiller (Le Guen *et al.*, 1984; Letouzey, 1981).

Light and temperature shocks have also been found to increase fertility, and it has been demonstrated that growing conditions from sowing to anthesis affect the stability of the progeny (Duc, 1980, 1978).

Recent programs and future projects

CMS instability prevents its use for hybrid seed production at a commercial scale. For this reason efforts were made to improve present CMS types or to discover a new, more stable, type than those already known.

New CMS type

– Mutagenic treatments.

Mutagens have included EMS (ethyl methane sulfonate), EB (ethydium bromide) and mixtures of both of them. Different commercial varieties ('Ascott' and 'Diana') and pure line accessions (29 H) were treated without any success if numerous genetic male sterilities are excluded (among these the oligogenic one previously mentioned).

Treatments on '447' CMS seeds were performed in order to modify the cytoplasmic determinants to get a better stability. The same mutagens mentioned above were used on this material. In order to verify in the progenies of the treated seeds that the sterile plants were different in the type of sterility than the original ones, two tests were performed:

· Non permanent restoration: recovery of sterile plants in the progeny of selfed restored F_1 plants would indicate deviation from the initial '447' CMS type.

· Differences in restorer lines: HG 115, which restores fertility both in '447' and '350', was used in crosses involving sterile plants found in the progenies of treated seeds.

In this way, at least two 'new' cytoplasms were found to be different from both '447' and '350' CMS. They were named '417' and '421' and are presently under study. There is no indication yet wether they are more stable than the former ones.

– Interspecific hybridization.

Attempts to cross *Vicia faba* with *Vicia narbonensis* were made with two main purposes: first, to introduce the high level of resistance to chocolate spot (*Botrytis fabae*) from *V. narbonensis* into *V. faba,* and second, to try to induce cytoplasmic male sterility as it was successfully done in some other species.

It appeared that compatibility between *V. faba* and *V. narbonensis* was very scarce and that in spite of the successful fertilization of ovules, embryos were never obtained. In fact, the abortion of the young embryo occurred generally less than 72 hours after fertilization because of incompatibility between maternal and hybrid tissues. In vitro culture of embryos was attempted without success.

– Somatic or 'zone' selection (Bouverat–Bernier and Le Guen, 1980).

As variations in pollen fertility have been found both between and within plants of the same progeny, a selection was performed based upon a choice, after microscopic observation of stained pollen, of the best plants for male sterility, and within these best plants for the best sectors (nodes with the lowest levels of viable pollen). Only seeds on these sectors were harvested and sown the next generation. The magnitude of the work involved in the process limits the possibilities of its use.

New perspectives

The discovery by Edwarson *et al.* (1976) of specific particles in the '447' cytoplasm (cytoplasmic spherical bodies) permitted a new approach to male sterility in faba bean. These particles were found to contain double stranded RNA particles of $12x10^6$ daltons surrounded by an unitary cellulosic membrane; they are 70 μm in diameter. As they look like viruses they are now designated as VLP (virus like particles).

Scalla *et al.* (1981) found that there was a close relationship between the total amount of these particles in a cytoplasm and its level of sterility, the higher the number of particles the more sterile the cytoplasm. Starting from these results, it was attempted to improve a method to purify the VLP and to evaluate accurately their amount with the purpose of finding a simple early test to predict the male sterility level of faba bean individuals. A method of purification and concentration of the particles was achieved allowing for the preparation of a specific antiserum. Following these results, an ELISA test was developed to test antibodies, purified from this antiserum, against crude extracts of faba bean leaves containing VLP in very small amounts. It is expected that by using this method a better way to select good male sterile plants will be achieved (Duc *et al.* 1984).

Conclusions

In spite of the high level of hybrid vigor in faba bean, especially in winter types, no commercial variety is actually available in France nor in other European countries. The inability to produce such varieties at a commercial scale arises from the instability of the type of cytoplasmic male sterility found which at present makes conversion and multiplication generations impossible.

Much work has been done but more is needed to understand and control this phenomenon. Even if synthetic varieties give rather satisfactory yields, the main objective in faba bean breeding is still to obtain good hybrid varieties which could allow for regular stable high yields in different countries. Great hope is centered in the new techniques to bring some answer to this problem. However, we think we have to remain reasonably optimistic for the future because new information is becoming regularly available. It should, nevertheless, be added that hybrid varieties are presently on trials in European tests with satisfactory results.

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