METHODS AND OBJECTIVES IN FABA BEAN BREEDING

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In Germany, faba bean is after lupine (38.000ha) and sunflower (27.000ha) one of the "small" crops; its acreage in 2005 was 16.000ha. The acreage of pea in Germany was much higher (111.000ha). Faba bean is as well a small crop in Poland (12.000ha) and Austria (4.000ha). In France we had a total of 105.000ha, amounting to one third of the French pea acreage. In UK, faba beans were grown to 188.000ha, this is double the area grown to pea plus lupins. In Spain we had in 2005 about 53.000ha of faba bean. These Mediterranean types are sown in late autumn. In parts of UK and France, where winters are relatively mild, autumn sowing of faba beans is to some extent

realized as well – using "true" winter beans, that can survive winters north of Pyrenee Mountains and Alpes. In UK, more or less half of the faba beans are such winter types. All types of faba bean can survive very mild frosts as young plants (until about minus 6°C). The breeding objectives for this crop are grain yield and grain yield stability and lodging resistance, and furthermore resistances against drought (and winter frost in case of winter bean breeding), and against fungi and further pathogens and pests. An additional objective is grain quality. Several present day cultivars are named in Table 1 for illustrative purpose.

OBJECTIVES

Normal faba beans show indeterminated growth, flowers and very young pods grow in competition with the vegetative apex of the stems. Several alternative growth types have been studied as a strategy to strengthen the pods as sinks for assimilates. The so called ti-type (terminal inflorescence, or "topless") and the so called stable type (st, somewhat stunted habit, very stiffy stem) were launched. Dwarfism is known as well. These phenotypes are all caused by a single recessive allele, respectively. Several corresponding cultivars have been bred, like 'Tina', 'Tinova' (ti) or like 'Boss', 'Mythos' (st); still, these are not widely used, no such type is present on the 2005 German List of Varieties. On the other hand, very recently Nadal et al. (2005) in Spain proposed the use of the ti-type as a solution in broomrape-infested conditions (production of young pods for fresh consumption).

Faba bean, in spite of its high importance in the Mediterranean Basin, is rather susceptible to drought. Its main mechanism to deal with this stress is a very early and sensitive stomata closure. Adapted material is escaping terminal drought by earlyness, whereas no powerful strategy is known to deal with unpredictable, intermittent drought. Recent data of Khan et al. (in preparation) show that several physiological traits may be used to assist in breeding for drought resistance. Germplasm from the ICARDA catalogue was used to specifically enlarge diversity. Frost resistance is mostly analyzed as a component of winter survival. Late frost during flowering and very early frost at maturity is extremely rare. Very few genotypes with outstanding frost resistance are known (Stoddard et al., 2006). Similar to drought, physiological traits might help in breeding, such as fatty acid composition of leaves (Arbaoui, and Link, 2006). No molecular tools are as yet available for these two topics.

Cultivar name	Sowing season		Prooder
	Autumn	Spring	Dieedei
ENRICO	+		Agro Service Spa, SAN SEVERINO MARCHE, Italy
GRIFFIN	+		Wherry & Sons Ltd, LINCOLNSHIRE, UK
REINA BLANCA	+		Semillas Fitó S.A. C., BARCELONA, Spain
FUEGO		+	NPZ Lembke KG, HOHENLIETH, Germany
LADY		+	Serasem, PREMESQUES, France
SALSA		+	Groupe Agr. Essonnois (G.A.E), MAISSE, France
VULCAIN		+	Serasem, PREMESQUES, France

Table 1.	OECD list of f	faba bean	varieties ¹	eligible for	or certification	on 05/06	(December	2005);
annex 2:	new varieties	http://www	v.oecd.org	g/dataoec	<u>d/1/26/3399</u>	9612.PD	<u>F</u>	-

¹Several further cultivars:

ALBOREA (Spain), FAVEL (Portugal), DIXIE (France), GLORIA (Austria), HOBBIT (Germany), JÕGEVA (Estonia), KISVARDAI (Hungaria), KONTU (Finland), MERKUR (Czechia). The most important fungal foliar diseases are *Botrytis fabae*, *Ascochyta fabae* and *Uromyces viciae-fabae*; besides, root rot caused by *Rhizoctonia solani*, *Fusarium* species and further fungi occurs. *Botrytis* is seen in a wide range of growing conditions, it often is a very serious threat, and still no convincing source of resistance is known. Severe outbreaks are most common in the Nile delta, near rivers in China, rainy coastal areas of the Mediterranean Basin, and the more oceanic climate of western France and western UK (Tivoli et al., 2006). Differences in susceptibility follow a quantitative genetic pattern. Several less susceptible bean genotypes are known (e.g. ICARDA lines originating from Ecuador). For *Ascochyta* and *Uromyces*, specific resistances are known and even molecular markers were developed. The line 29H, amongst others, was repeatedly used to improve *Ascochyta* resistance. Bean rust (*Uromyces*) resistance is available in many cultivars. Qualitative resistance is common and widely used by breeders (Sillero et al., 2006). *Phoma* and mildew cause further, less well studied foliar diseases in *Vicia faba*. Few if any convincing sources of resistance against root rot are known. Zero-tannin material seems to be more susceptible at germinating and emergence than tannin-containing germplasm.

Viruses are not a frequent problem for the faba bean producer, still, virus diseases may occur as epidemic and become serious. The bean yellow mosaic virus, bean leaf roll virus, broad bean true mosaic virus and the broad bean stain virus must be named. Since no direct pesticide protection exists, the genetic strategy must hold. The two latter viruses are to some extent seed-transmitted and not aphid-transmitted. Yet, *Sitona lineatus* (see below) is spreading these two viruses early in the season (Rohloff, 1980). There has been promising breeding research prior to 1989 in GDR, but these programs were then abandoned (Lötsch, 1989). In 1992 and again in 1999, a new, aphid-transmitted virus (faba bean necrotic yellow virus) occurred at a devastating level in the Nile Valley. Meanwhile resistant genotypes have been identified (e.g., 'ILB132'; Khaled et al., 2000).

The most important pest is *Aphis fabae*, the black aphid. It occurs very often at significant levels, insecticides are used. In addition to the direct damage, it is spreading viruses. No useful resistance is known. Even earlier in the season than this aphid, the weevil *Sitona lineatus* feeds on the first, very young leaves. More important is the damage of its *larvae*, which is feeding on the root nodules and thus causes direct damage and probably increases root rot (Salt, 1983). *Bruchus rufimanus*, a seed-infesting weevil, is present in most faba bean fields and stocks. Additional *Bruchus* and *Callosobruchus* species live in bean seeds (mainly in the Middle East). The seed coat is a barrier to them, only about 50% of the larvae can enter and overcome it; there was no connection to the seed coat's tannin content. The present breeding of new cultivars with reduced vicine and convicine content of the seed can

favour the colonization of faba bean by additional weevils such as *Callosobruchus maculatus*, that does not infest normal-vicine faba beans (Desroches et al., 1995).

In addition to aphids and beetles, nematodes have to be mentioned. Faba bean may be infested by the stem nematode (Diteylenchus dipsaci) and the cyst nematode (Heterodera goettingiana). The stem nematode is widespread, and its "giant" race, common in North Africa, is a serious pest, especially in cases where nematode-infested seed was sown. Smallseeded beans are generally a poorer host, several resistant genotypes were identified, amongst them the Ascochyta-resistant line 29H. The cyst nematode is important in many temperate regions. Obviously no resistances have been found (Sharma et al., 1994). Broomrape, Orobanche crenata, is a parasitic plant, devastating pulses and other crops in the Mediterranean Basin and Nile Valley. Hand weeding, use of glyphosate, late sowing and breeding is used to fight it. Breeders have up to now not produced a bean with safe resistance. Screening is mostly done in fields where this parasitic weed occurs naturally, which is a difficult test situation. Evaluations in controlled environments are possible but expensive. These shortcomings make broomrape resistance a problematic trait. Partially resistant genotypes are available, the trait shows a quantitative genetic variation. The rather resistant geniteur F402 identified by Egyptians was repeatedly used. Several improved genotypes have been bred from this common source in Egypt ('Giza 402', 'Giza 429', 'Giza 674') and in Spain ('Vf1071', 'Vf136', 'Baraca'). Meanwhile, three QTLs for resistance were identified (Torres et al., 2006). Orobanche resistance is a trait that shows all features to make it an item of marker-assisted selection: very important topic, quantitative-genetic variation, difficult to phenotype the trait, one unique, common source of resistance ('F402'). Up to now the level of cooperation among breeders and scientists in the Meterranean Basin and Nile Valley does not yet meet neither the importance of this pest nor the promises hold by modern breeding techniques.

Breeding for improved quality means increase of the seed protein content and protein quality. Protein content could easily be increase to over 30%, still, as long as there is no economic incentive to do so, breeders do not "spoil" their anyway restricted selection intensity for this trait. Protein quality is mainly limited by a low sulphur content (content of methionine and cysteine). Classical methods are not promising, since the variation is low and there is a negative genetic correlation between seed protein content and sulphur content of this protein (Link et al., 2006). Quality moreover depends on the content of antinutritive factors. Zero-tannin cultivars do exist (e.g. 'Gloria'), the recessive monogenetic segregation and pure white flower as pleiotropic effect of this gene make the trait easy to handle. Low vicine cultivars do as well exist (e.g., 'Mélodie'). The trait is again monogenetic recessive, a morphological

marker (white hilum) and even molecular markers are available. Further antinutritive compounds are not of importance in faba beans (Duc et al., 1999).

TECHNIQUES and METHODS

Several in-vitro-techniques would very useful for faba bean breeding. By means of protoplast fusion and regeneration or by embryo-rescue assisted interspecific crossing, e.g. resistance to black bean aphid, as occuring in the related species *Vicia johannis* (Birch, 1985), could probably be introduced to *Vicia faba* – still, these techniques are not yet available for faba bean. The same is true for any approach to produce doubled haploid lines. Genetic transformation based on *Agrobacteria* is possible, it has been reported twice (Böttinger et al., 2001; Hanafy et al., 2005). The procedure was used to e.g. improve the S content of the seed protein. It is a tedious approach with very low success rate; faba bean is a very recalcitrant crop as to in-vitro technologies.

Classical breeding in faba bean looks back on very marked improvements, e.g. for nonshattering, yield, yield stability, lodging resistance. Still, breeding progress is hampered by the partial allogamy of the bean. Pollinators are bumble-bees and honey-bees (Suso et al., 1999). The degree of cross-fertilization is about 50%, with a large genotypic and environmental component of variation and with marked heterosis; the heterozygous types show on average less outcrossing. Inbreeding depression for grain yield is marked, F1-hybrids outyield their inbred parents mostly by more than 30%. Nevertheless, line breeding is one of the applied methods. An important bottle-neck in line breeding is the production of purely selfed seed. In open field situation, seed production suffers from contamination with cross-pollen, unless spatial isolation and pure lines were used. In cages, pollinators can be excluded and pure self-fertilization can be enforced. Still, without pollinator visit, most genotypes admit a need of tripping (tripping is a mechanical stimulation of the stigma, caused by the pollinator visits; tripping induces successful pollination and fertilization). As a consequence of absence of pollinators, yield of purely selfed seed in cages is variable and mostly low. In Southern Europe there seems to be a lower or no need of tripping. In the cages, tripping can be done manually, to substitute for the missing pollinators, thereby allowing true selfing and high seed set, but this is a very costly procedure. As alternative to pure line cultivars, synthetic cultivars are bred, and successfully so. Still, due to the limited degree of natural outcrossing, only about half of the potential hybrid vigour is used in a synthetic cultivar. Hybrid cultivars are not yet bred because none of the several published CMS-systems (CMS447, CMS350,

CMS297, CMS199) allows to be employed in practical breeding, mostly due to instability and spontaneous reversion to pollen fertility (Link et al., 1997).

To summarize, the most important and urgent shortcomings seem to be powerful resistance genes and tighly linked molecular markers to allow breeding against *Botrytis*, root rot and *Orobanche*. As well very helpful would be to identify alleles that increase drought and frost resistance as well as increase seed methionine content. Furthermore, a clear genetic switch of the mode of reproduction to either ~100% of cross-fertilization or ~100% of self-fertilization would make breeding as straightforward as e.g. in case of corn or pea. Any success with interspecific crossing would open the gate to new, very welcome allelic diversity.

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