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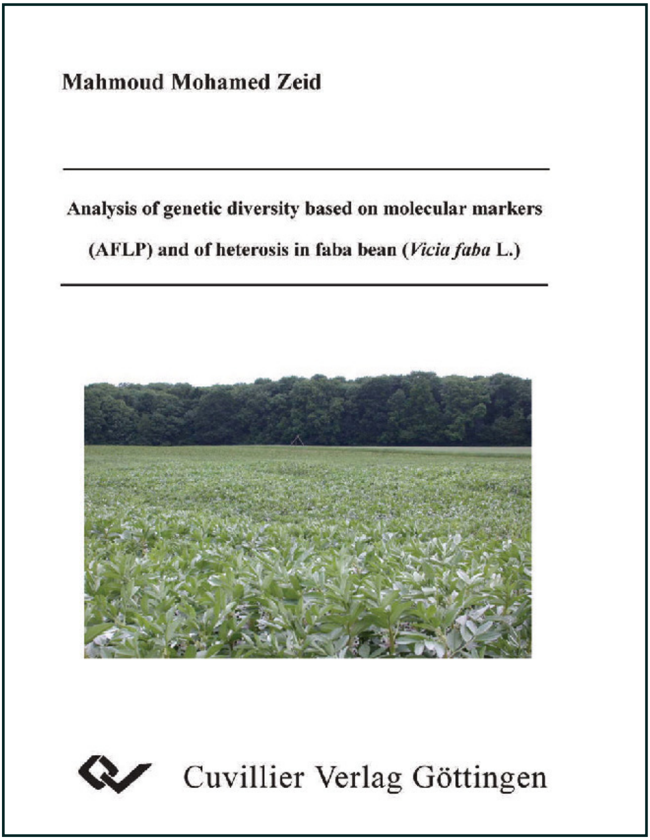
Analysis of genetic diversity based on molecular markers (AFLP) and of heterosis in faba bean (Vicia faba L.)



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1 Introduction

1.1 Importance and production of faba bean

Faba bean (*Vicia faba* L.) is a valuable protein-rich food that provides a large sector of the human populations in developing countries with a cheap protein source thus partly compensating for the large deficiency in animal protein sources. In developed countries faba bean provides an alternative to soybean meal for animal feed, this being particularly important in the more industrialized countries. The world area devoted to faba bean is continuously in decline, falling from 3.7 m ha in 1979-81 to 2.4 m ha in 2000-01. This reduction is mainly attributed to the unreliable yields and the poor returns from the crop.

After the harvest shortfall of soybean in the USA in 1973, the European Community realized the importance of domestic source of protein for animal feed. It is worth mentioning that the political decision to support protein crops production was set up 20 years after cereals and 12 years after oil seed regulations were made (Carrouee 1995). Under the current policy of the European Union it is expected that the area under protein crops will be at least maintained at around 1.3 m ha, thus ensuring a domestic production of 5-6 million tons of excellent quality protein (Hulot 1999). Today about 75% of protein-rich products (mainly soy meal and soybean) in Europe is imported, and is likely to increase with the ongoing ban on the use of meat and bone meal in the animal feed industry (Struan, 2002).

For decades, faba bean was the only grain legume crop which had been widely grown in Europe. During the eighties soybean and pea production suddenly rose and today faba bean shares sweet lupines in only about 19% of the 1.3 m ha planned for protein crops. Yield instability (Fig. 1.1) and low prices are behind the continuous drop in the area (Fig. 1.2) devoted to faba bean in Germany, France and the European Union as a whole. France has lately regained interest in the crop with a tremendous jump of 0.43 m ha in 2001 without any noticeable improvement in yield/ha (mean area being 0.13 m ha over the past 10 years). This could be explained by the fact that faba bean is replacing peas in areas infested with root rot *Aphanomyces euteiches* where reduction in pea yield amounts to 90% (Lacampagne 2001).

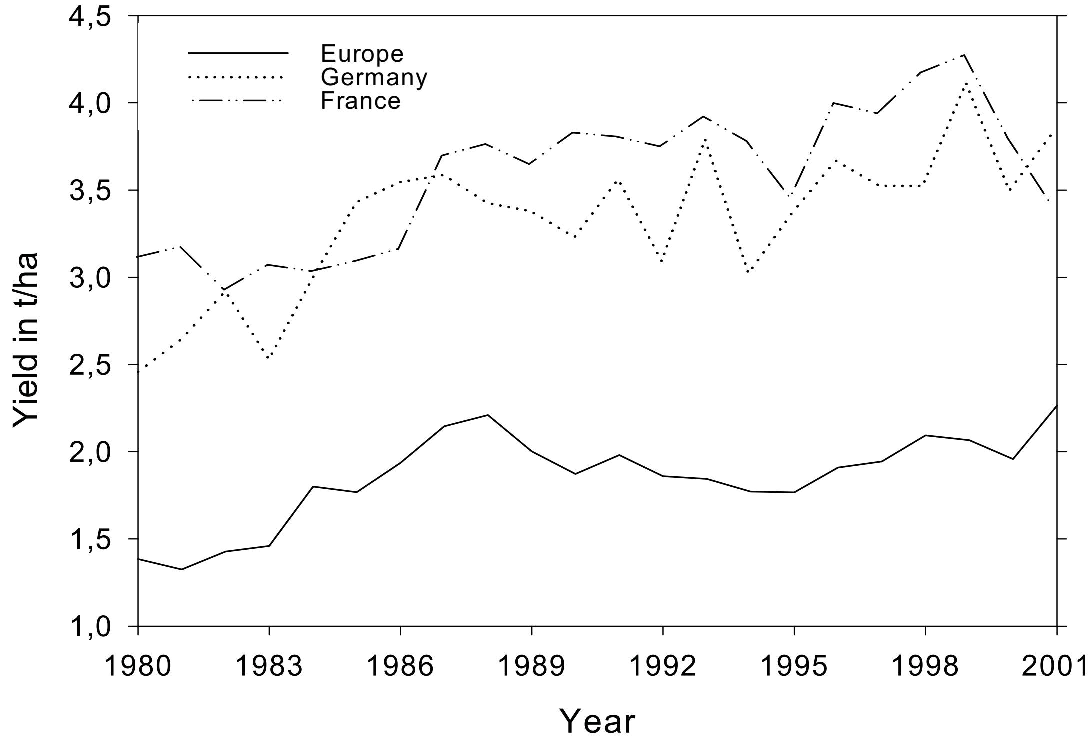

Fig. 1.1 Yiled of faba bean in the period 1980-2001
Source: FAO (2001)

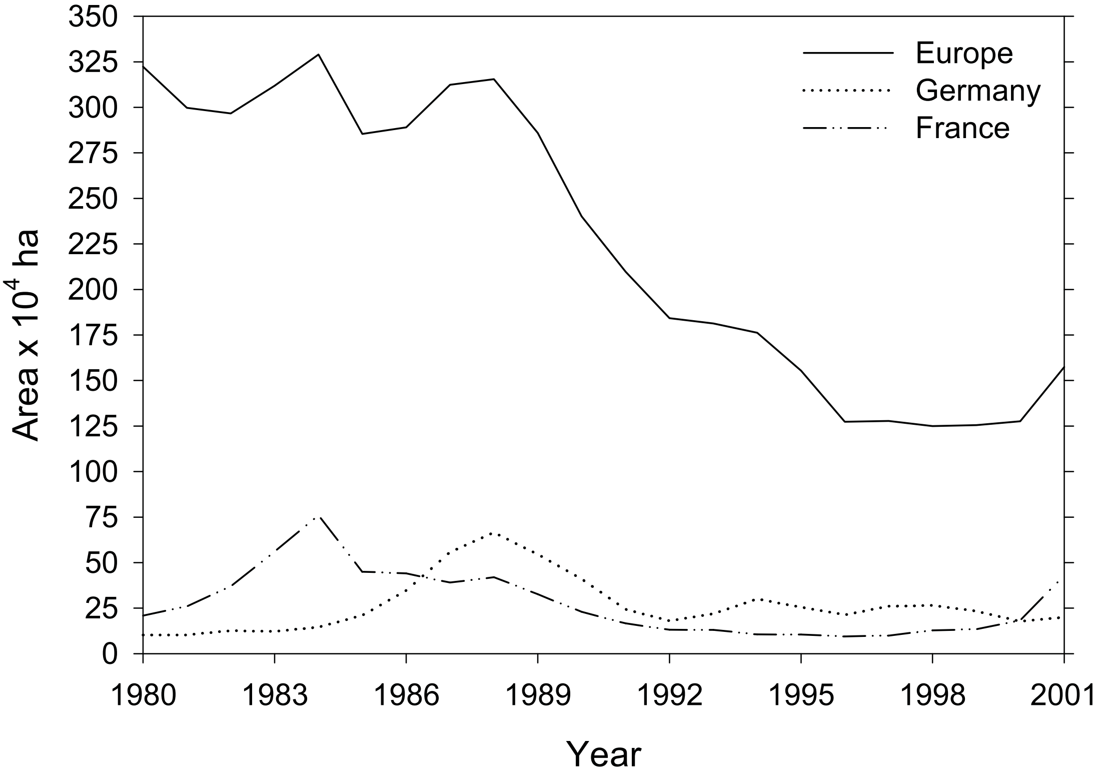

Fig. 1.2 Area devoted to faba bean in the period 1980-2001
Source: FAO (2001)

Faba bean plays a significant role in improving the productivity of the soil in the cereal-based rotations where it serves as a break crop; yields of cereal crops following faba bean are improved and needs for nitrogen fertilizer applications are reduced. Studies on the fixation of atmospheric nitrogen through symbiosis in organic farming (Schmidtke and Rauber 2000) have shown that faba bean surpasses peas in the amount of nitrogen fixed. In addition, an effective management of faba bean in crop rotations by reducing plant-available soil nitrogen before, during and after growing faba bean could achieve maximum symbiotic activity, low levels of nitrogen leaching and high yield of the succeeding non-legume crops. Schmidtke and Rauber (2000) reported that grain legumes play a more important role in organic farming than in conventional farming systems. In Germany, the area of arable land grown with faba bean and pea was 1.2% on conventional farms but 4.9% on organic farms. This demonstrates the need for supply of home grown legumes for animal feed and at the same time the ability of legumes to meet their own demand of nitrogen through symbiosis and the positive nitrogen effect of grain legumes on non-legume crops following in the crop rotation.

1.2 Distribution

Faba bean is widely believed to have originated in the Mediterranean-West Asia region probably in the Neolithic period (Cubero 1974). Throughout their long history as a cultivated crop, faba bean has been subjected to both natural selection and selection by farmers in the different environments where the crop has been grown. Although no successful crossing between faba bean and any of the other *Vicia* species has been reported, a wide range of genetic variation in the species still exists (Lawes et al., 1983). In spite of centuries of such selection, *Vicia faba* retains vestiges of its wild past and, in certain aspects, can be regarded as an incompletely domesticated species. The indeterminate nature of the growth habit and the existence of dehiscent pods in many populations can be cited as examples (Hanelt 1972). The mating system of the species, which stands between full autogamy and full allogamy, may be another (Hawtin 1982).

Distinct groups within faba bean based mainly on seed size, ranging from small seeded *minor* beans (0.2-0.5 g per seed) to medium seeded *equina* beans and the large seeded *major* beans (single seed weight of more than 2.0 g) have been recognized by Muratova (1931). These groups can still be recognized in the major areas of production where the *minor* and *major* groups are grown in Central and Northwest Europe, both groups in addition to the *equina* group in South Europe, North Africa and up to West Asia and the *major* group in South America.

1.3 Breeding

Progress in breeding faba beans for resistance to biotic and abiotic stresses has been slow and with only few breakthroughs to be considered. Bond et al. (1994) reviewed the contributions of plant breeding facing these problems showing resistance to Chocolate spot (*Botrytis fabae*) has been identified in ICARDA lines coming from Ecuador, resistance to *Orobanche crenata* by breeding the cultivar 'Giza 402' in Egypt and breeding of the frost hardy cultivars Côte d'Or in France and Hiverna and Webo in Germany as the main breakthroughs. The problem of yield instability (mainly due to biotic and abiotic stresses) in faba bean however dominates over all achieved progress as indicated by the drastic reduction in the area devoted to the crop.

Although improvement in seed yield and yield stability are the primary objectives of most faba bean breeding programmes, in Germany, an annual increase of only 0.6% in yield could be achieved as compared to a yield improvement of 2% per year in case of wheat (Schön 1997). The number of approved faba bean cultivars is low and pedigree information regarding available cultivars is scarce and not well documented, especially since cultivars are not the outcome of a specific cross but from natural crosses or are populations improved through recurrent selection (Stelling et al., 1994).

The challenge in breeding faba bean resides mainly in its reproductive system being partial allogamous. Both cross and self-pollination may occur on the same plant as assisted by insects. Natural outcrossing also varies greatly, reported to be 35% (Bond and Poulsen 1983) and 60% on the average (Suso and Moreno 1999), depending on the genotype and environmental conditions. Results of Link et al. (1994b) showed that outcrossing in faba bean inbred lines ranged from 38 to 73% with an average of 54%. Ebmeyer (1988) has already pointed out that in open pollinated populations of crossbred faba bean plants tend to self-pollinate, whereas inbred plants tend to cross-pollinate.

Heterosis has been realized by Bond (1966) in diallel crosses of winter bean types, with an average yield improvement of almost 23% above the best parent. Other studies showed 50% heterosis and more above the midparent (Von Kittlitz 1986; Link and Ruckenbauer 1988). Link et al. (1994a) stressed on the importance of utilizing the heterosis still available in faba bean, if yield stability is to be achieved. The genetic variability within open-pollinated faba bean varieties and the proportion of heterosis occurring under partial allogamy was studied by Ebmeyer and Stelling (1994). Their results showed that 70% heterosis for grain yield was achieved for crosses between inbred lines of different varieties and that only two thirds of this heterosis could be utilized in open pollination.

Two ways appear to be available to utilize heterosis in faba bean, namely: the production of hybrids and formulation of synthetics. Both methods appear to promise higher yield and yield stability than open-pollinated populations.

Results presented by Bond (1966) and Ebmeyer (1988) showed a large variation in the general combining ability and the low levels of specific combining ability in different faba bean crosses. These results are generally in favour of breeding synthetic varieties; however, after considering the high degree of self-fertilization occurring in the crop, hybrids appear to be more appropriate. Faba bean hybrids have also shown better adaptation to a wide range of abiotic conditions as compared to open pollinated or inbred cultivars, expressed in improved fertilization at high temperatures (Bond et al., 1994), and tolerance to lack of pollinating insects owing to their heterotic autofertility (Link, 1990), reduced winter damage (Bond et al., 1986) and better tolerance to drought stress (Abdelmula et al., 1999). Three–way hybrids, double hybrids, or single hybrids grown in blends were suggested as the key to yield improvement and stability in faba bean (Stelling et al., 1994).

Cytoplasmic male sterility (CMS) is a prerequisite for commercial hybrid variety production. More than 35 years after Bond et al. (1964) have discovered the first CMS system in faba bean, no hybrid cultivar is available. The latest work done by

Link et al. (1997) resulted in a more stable system (CMS199). Although studies regarding this system are still in progress (Martsch et al., 2001) hybrid varieties today are unfeasible mainly due to the instability of the available CMS systems.

Until hybrid varieties are ready for commercial production, synthetics offer a means of exploiting an intermediate level of heterosis from highly selected and characterised parents. A synthetic variety in faba bean was defined by Bond (1982) as any population, which has been constituted from a limited number of distinct and well-evaluated components (usually inbred lines). Four or five are common; this provides the best balance between the danger of too high sibbing and the inclusion of more lowly ranked components if the number is greater (Bond 1982).

A synthetic variety usually equals or exceeds the yield of the highest yielding component whichever one it is. Synthetics are more stable compared to their more inbred components due to the degree of hybridity they possess. The optimum number of components entering a synthetic (Link and Ederer 1993) and the suitable synthetic generation for maximising yield (Stelling et al., 1994) have already been defined and the amount of expected performance from the highest yielding synthetic is estimated to be at least 15% higher than the highest yielding inbred line.

Reviewing the pedigree information of some German cultivars, Stelling et al. (1994) reported that both the cultivar Minica (from the *major* group) and its offspring Alfred were frequently used as parents for many cultivars. Similarly, the cultivar Kleinberger Kleinkörniger was shared in the pedigree of almost all cultivars in Austria.

With the introduction of molecular marker techniques, new reliable tools that are neither affected by the surrounding environment, nor by growth stage of the plant (as in case of morphological characters) became available for the breeder. These can be applied for organizing germplasm, identification of cultivars, assisting in the selection of parents for hybridization and reducing the number of accessions needed to ensure sampling a broad range of genetic variability. Restriction fragment length polymorphism (RFLP) has been first employed by Van de Ven et al. (1990) in faba bean as a first step to create a linkage map. With the introduction of the PCR (polymerase chain reaction) more cheaper and less labour intensive marker techniques than the RFLP became available. The random polymorphic DNA (RAPD) technique is one of those PCR-based methods that have become widely used in estimating the genetic relationships among genotypes. Link et al. (1995) have employed the RAPD technique to study the genetic diversity in European (*minor* and *major)* and Mediterranean faba bean germplasm. Their results were very promising, showing that within the European *minor* pool itself genetic diversity estimates were larger than that between the minor and major groups, implying the possibility of establishing genetically divergent heterotic groups even within the European minor germplasm.

Amplified fragment length polymorphism (AFLP), is another PCR based marker that exhibits several advantages over other markers available for this type of research, including: (i) the generation of a large number of markers in a single PCR reaction, (ii) a high level of polymorphism and (iii) a high reproducibility and reliability due to stringent PCR conditions (Vos et al. 1995).

The hypothesis of an association between molecular marker data and heterosis and/or hybrid performance is obvious. Experience taught breeders that there is a nearly linear relationship between pedigree distance of parents, i.e. heterozygosity and heterosis in the corresponding hybrid. Molecular markers allow inspection of an unselected sample of chromosomal loci for parental distance; hence, it is very tempting to forecast heterosis from this data. Prediction of heterosis has been investigated in different crops including maize (Lanza et al., 1997; Ajmone Marsan et al., 1998), soybean (Crena et al., 1997) and sunflower (Cheres et al., 2000). The aim of those studies were the prediction of the performance of single cross hybrids from marker information collected on their parental lines, thus predicting the performance of a large number of F1-hybrids beyond available capacity of any breeding program and at the same time saving the expenditure and time devoted for field evaluation. Contradicting results were reported ranging from suitability of the marker information for prediction purposes (Lanza et al., 1997) to lack of correlation between marker information and F1-hybrid performance or heterosis (Crena et al., 1997). The reasoning for the inconsistency was demonstrated by Frei et al. (1986), who showed that the usefulness of molecular markers for predicting hybrid performance depends on whether crosses were produced including related lines or were a result of unrelated lines only.

2 The objectives of this work were to:

1- Study the structure of the genetic diversity within a selected sample of elite cultivars representing the actual faba bean material used for breeding in Asia, Europe and North Africa based on AFLP markers

2- Analyze both the reliability of the AFLP technique and consistency of the results obtained

3- Determine whether selection of genetically independent faba bean inbred lines within the elite European gene pool, (i.e. leading to non-inbred hybrids) is possible, if selection is solely based on available pedigree information. To achieve this objective:

   (I) Available pedigree data on a sample of 18 inbred faba bean lines from the European gene pool were studied and independent parents were defined for hybrid production.

   (II) The pattern of genetic diversity within this set of faba bean lines was investigated through high input assessment of AFLP markers.

   (III) Associations between AFLP based genetic similarities of these inbred lines with agronomic performance and heterosis of their single cross hybrids were assessed.