Breeding and genomics status in faba bean (Vicia faba)

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Abstract
Faba bean is an important legume crop because of its high-yield potential and nutrition-dense grains. There have been significant achievements in faba bean improvement in the last four decades, which led to the doubling of the global yield average. This study reviews the genetic diversity, the breeding methodologies, major achievement on biotic and abiotic traits, and the recent molecular approaches. The high genetic diversity among faba bean accessions has been useful for increasing yield potential of the crop. Substantial increase in yield potential can be gained through the development of cultivars in open pollinated conditions. In the past, many faba bean varieties that are tolerant to abiotic and biotic stresses were released worldwide. The average yield gains varied from 1.65% per year in Syria to 4.17% per year in Ethiopia. The recent advances in molecular technologies will be used to develop more coherent genetic maps, which would also facilitate assembling and ordering genomic scaffolds in a future genome-sequencing effort and molecular-breeding approach.

KEYWORDS
abiotic stresses, biotic stresses, diversity, Faba bean, genetic maps, outcrossing

INTRODUCTION

According to FAOSTAT (2018), Vicia faba L. (faba bean, named also broad beans or horse bean) is the fourth most widely grown cool season legume after pea (Pisum sativum), chickpea (Cicer arietinum) and lentil (Lens culinaris). Its protein content is higher than other common food legumes (Griffiths & Lawes, 1978; Burstin, Gallardo, Mir, Varshney, & Duc, 2011). Moreover, the total grain yield of faba bean is positively correlated with high seed protein contents (El-Sherbeeny & Robertson, 1992). Faba bean has advantage over legumes such as soybean in cool season environments being adapted to grow under low temperatures. As such, it is well suited to sustainable farming practices in temperate to cool environments (Temesgen, Keneni, Sefera, & Jarso, 2015). However, faba bean yield remains unstable due to biotic and abiotic stresses, as is the case with many other major legumes (Cernay, Ben-Ari, Pelzer, Meynard, & Makowski, 2015).

The cultivation of faba bean traces back to the beginning of agriculture (Cubero, 1973), and it remains an important crop today because of its high-yield potential, nutrition-dense grains and its role as forage and cover crop. As other legumes crops, faba bean plays a critical role in improving cereal-based system and it can improve soil fertility (Jensen, Peoples, & Hauggaard-Nielsen, 2010). The global faba bean-harvested area was 2.4 million ha in 2016, covering wide latitudinal range from approximately 50°N to 40°S and from the sea level up to 3,000 m altitude (Gnanasambandam et al., 2012). The total production in 2016 was 4.46 million tons of dry grains (FAOSTAT, 2018).
The global area of faba bean cultivation has been declining, especially in China and countries in North Africa and West Asia. This reflects a general trend, observed since the 1960s, of increasing reliance by farmers on N fertilizers as a source of N input (Crews & Peoples, 2004). In addition, there is a weak seed system; biotic stresses (foliar diseases and orobanche) and abiotic stresses (heat, drought, acidic soils and waterlogging), the absence of efficient herbicides and poorly adapted mechanization by smallholder farmers make it very difficult to perform weed control. However, despite the problems affecting the production of faba bean, its global average yield has increased from 0.9 tons/ha between 1961 and 1964 to 1.86 tons/ha in 2016 (FAOSTAT, 2018), thanks to the significant achievements in genetic improvement. This study reviews and highlights progress made in understanding the origin of the cultivated crop, achievement in genomics, breeding and genetic diversity of faba bean, breeding methodologies, achievements on breeding for resistance/tolerance to major biotic and abiotic stresses and recent advances in genomics tools.

2 | ORIGIN AND GENETIC DIVERSITY

Vicia faba L. (2n = 12) is a close relative of Narbonensis (2N = 14) within subgenus Vicia, even though they have different chromosome numbers and nuclear DNA content (Kew Science’s data and resources, 2017). Several Narbonensis species possess useful agronomical characters such as winter hardiness, resistance to black aphids (Aphis fabae) and chocolate spot disease caused by Botrytis fabae (Birch, Tithecott, & Bisby, 1985). But no successful interspecific crosses between V. faba and other Vicia species have been made (Caracuta et al., 2016) as it was the case of other legume crops.

The long history of cultivation, the wide distribution across diverse environments, the mating system and responses to human selection have led faba bean to become one of the most variable species, possessing a wide spectrum of variation in plant architecture, leaf size and shape, seed colour, seed shape and size (Maalouf et al., 2013). There is more than 10-fold range of seed sizes among the faba bean accessions (20–250 g/100 seeds). Trait analyses have separated them into three groups: (a) faba bean small-seeded forms in south-western Asia, (b) large-seeded forms in the west and (c) the third group, medium-seeded type very ancient and can be traced back to Neolithic agriculture. This group is found over a large area, from Spain to the Himalayas (Muratova, 1931). Archaeological findings at Tell El-Kerkh, north-west Syria, indicate a date of origin for faba bean domestication during the late 10th millennium BC (Tanno & Willcox, 2006). In addition, Caracuta et al. (2016) identified the 14,000-year-old specimens, discovered in the Mount Carmel region, as the lost ancestor of faba bean. After examining the seed size variation, Muratova (1931) proposed to divide V. faba into four subspecies based on seed size: major (large-seeded type), equine (medium-seeded type), minor (small-seeded type) and paucijuga (small-seeded type). However, as neither genetic nor sterility barriers are produced among these subspecies, Cubero (1973) considered them as one species with four different botanical groups. It is considered that the larger seed of faba bean is a result of human selection (Tanno & Willcox, 2006). The medium-sized type was found in the Iberian Peninsula—both in Portugal and in Spain—as well as Central Europe 5,000 before present (BP). The flattened larger types were not known before 1,500 BP (Ladizinsky, 1998).

The first scientific study on genetic variability in faba bean began in 1976 when the natural variability and selection in some local and exotic populations of faba beans were reported (Abdalla, 1976). Since then studies on genetic diversity were based on morphological characteristics, isozyme markers (Jaaska, 1997; Polignano, Alba, Uggenti, & Scippa, 1999) of the variation of selected traits in the context of cultivar diversification. The application of DNA-based markers since the mid-1990s offered great opportunity to assess the genetic diversity in faba bean germplasm (Torres, Weeden, & Martin, 1993; Zeid, Schön, & Link, 2003; Göl, Doğanlar, & Frary, 2017). Moreover, a global composite collection of 996 accessions established from ICARDA-Aleppo-Syria (499 accessions), INRA-Dijon-France (245 accessions) and CSIC-Cordoba-Spain (252 accessions) were evaluated at ICARDA in the spring of 2007 at Tel Hadya as part of the Generation Challenge Program (GCP). Thirty-five polymorphic SSR markers were identified, and 20 were used for diversity analysis. The cluster analysis (Figure 1) indicated two major clusters separating European and African genotypes in two different clusters. Mediterranean genotypes were divided between these two major clusters. ICARDA breeding lines clustered together close to North African, Asian and Mediterranean genotypes. According to the cluster analysis, a subset of 135 faba bean genotypes representing the diversity observed in the total set of 996 accessions was identified. Thus, the major outcomes of this research are the identification of this subset as high diverse reference set and refocusing the ICARDA breeding programme with the development of wider crosses using germplasm from different origins (ICARDA, unpublished results).

Similarly, other studies using molecular markers could discriminate that faba bean accessions originated from different geographical area. For example, amplified fragment length polymorphism (AFLP) genotype data could separate (a) the Asian accessions as distinct as a group from those of European and North African origin (Zeid et al., 2003) and (b) the Chinese germplasm from the germplasm collected outside of China and the winter types from the spring types (Zong et al., 2009). In addition, SNP markers are used to study genetic diversity within and between faba bean populations and could lead to differentiate Australian accessions on the basis of geographical origin (Kaur et al., 2014). However, molecular marker could not distinguish groups with different seed size groups (Göl et al., 2017).

3 | BREEDING

The Mendelian inheritance traits for faba bean were first characterized in the 1930s (Erith, 1930); however, faba bean genetics received only intermittent interest in the following years (O’Sullivan & Angra, 2016). In this section, major breeding methods used in faba
bean and also breeding for biotic and abiotic stresses are highlighted.

3.1 | Breeding methods

_Vicia faba_ L. is a partial allogamous species with outcrossing rates varying from 4% to 84% (Suso, Moreno, & Melchinger, 1999). The level of cross-fertilization potentially determines the amount of realized heterosis, which increase yield and yield stability and resistance to abiotic stresses (Gasim & Link, 2007). The elevated level of cross-fertilization in faba bean (>0.5) is essential to developing synthetic varieties and improved open population (Metz, Buiel, Van Norel, & Helsper, 1994). The suitable option is to develop synthetic varieties using autofertile lines to ensure minimum yield in the absence of insect pollinators and to take advantage of their presence by exploring heterosis to increase yield and yield stability (Cubero & Moreno, 1984).

Some authors have proposed transforming the mating system of faba beans (partial allogamy) towards autogamy and developing inbred lines especially for organic agriculture uniformity and specific adaptability (Ghaouti, Vogt-Kaute, & Link, 2008). Selection for high self-fertility degree might represent an important advantage for simplifying the breeding and facilitating the seed production technology. However, it has been difficult to fix a low level of allogamy in faba bean as single plant selection with lower outcrossing rate (t) than the average of original population tended to express a higher t in the offspring, or vice versa (Suso & Maalouf, 2010).

Faba bean pure lines obtained by pedigree methodology, bulk lines by recurrent selection and synthetic varieties were evaluated at ICARDA from 2013 to 2017 to assess the performance of the different breeding methodologies. Genetic gains per year (%) for grain yield and biological yield were higher in the synthetics (9.3) and recurrent lines (4.3) than the inbred lines (0.5; Figure 2). The yield increase in open pollinated population is mainly due to the exploitation of heterosis (Link, Stelling, & Ebmeyer, 1994). The maximum heterosis is achievable in faba bean hybrids but this is not yet possible at commercial level as stable Cytoplasmic Male Sterility (CMS) systems have not been yet identified (Palmer, Gai, Dalvi, & Suso, 2011) as in the case of other legume crops such as mung bean (Mallickarjuna & Saxena, 2005). Hybrid-enabled line profiling (HELP) described by van Ginkel and Ortiz (2018) for self-pollinated crops would help to exploit maximum heterosis and reduce breeding cycles in faba bean.

3.2 | Breeding for biotic stresses

The major biotic constraints of faba bean are foliar diseases, insects’ pests, viruses and parasitic weeds. Foliar diseases are caused mainly by ascochyta blight (*Ascochyta fabae*), chocolate spot (*B. fabae*), rust (*Uromyces viciae-fabae*) and gall disease (*Olpidium viciae* Gusano). Chocolate spot can affect up to 61% the faba bean productivity increase in open pollinated population is mainly due to the exploitation of heterosis (Link, Stelling, & Ebmeyer, 1994). The maximum heterosis is achievable in faba bean hybrids but this is not yet possible at commercial level as stable Cytoplasmic Male Sterility (CMS) systems have not been yet identified (Palmer, Gai, Dalvi, & Suso, 2011) as in the case of other legume crops such as mung bean (Mallickarjuna & Saxena, 2005). Hybrid-enabled line profiling (HELP) described by van Ginkel and Ortiz (2018) for self-pollinated crops would help to exploit maximum heterosis and reduce breeding cycles in faba bean.
levels of resistance and a high yield in Ethiopia and in Spain, respectively, were also found resistant to Orobanche in Tel Hadya Syria. Due to the release and the availability of Orobanche resistant cultivars in Egypt (‘Misr3’, ‘Giza843’), faba bean has regained some farming areas in Egypt.

In addition to above biotic stresses, a number of insect pests such as Sitona weevil (Sitona lineatus L.), cowpea aphid (Aphis craccivora Koch) and black bean aphid (A. fabae Scopoli) cause damage by direct feeding as well as by transmission of viruses (Mwanauta, Mtei, & Ndakidemi, 2015). There are currently integrated pest management options to control these insect pests as described by Redden et al. (2018). In addition, borer weevil (Lixus algirus L.) causes serious damage in faba bean in North Africa. Recently, new sources for resistance to this insect were identified and would be utilized to develop resistant cultivars (Ait taadaouit et al., 2018).

### 3.3 Breeding for abiotic stresses

Heat, drought, waterlogging and frost are the major abiotic stresses affecting food legume crops (Anjum, 2016; Stoddard et al., 2006) and particularly faba bean. Heat stress is particularly harmful at the reproductive stage of faba bean plants where high sensitivity is caused by gametophyte damage and consequent failure of fertilization (Bishop, Potts, & Jones, 2016). Additionally, terminal heat stress can significantly reduce yield and the yield components of faba bean genotypes (Abdelmula & Abuanja, 2007). Adverse effect leads to the development of a small, short-stemmed crop with few branches and pods as in case of other legume crops (Sita et al., 2017). Traits such as number of pods and number of seeds that exhibited strong and positive association with yield could be used as selection criteria for improving faba bean sensitivity under heat stress conditions. At ICARDA, seven accessions with higher rate of pollen viability under 35°C, higher values of pod number, and seed number per plant were identified (Maalouf, Ahmed, & Somanagouda, 2018).

Terminal drought is an important constraint to faba bean production in semi-arid areas under rainfed conditions. Identification of early maturing genotypes adapted to drylands with a short rainfed period and supplemental irrigation would have a significant impact on final yield. Genotypic variation in the response of faba bean to drought has been documented (Abdelmula, Link, Kittlitz, & Stelling, 1999). Physiological traits associated with drought, such as carbon isotope discrimination, leaf temperature and stomatal conductance (Khan et al. 2010), and spectral indices structure-insensitive pigment index and normalized phohtynization index (Maalouf, Nachit, Ghanem, & Singh, 2015) could be used for selection for grain yield under drought-prone environments. Maalouf et al. (2015) reported significant differences among faba bean genotypes under different water regimes without any significant interactions. FLIP06-010FB was the highest yielding and most stable among the tested genotypes across different environments.

Frost tolerance is an important breeding focus to increase yield stability in North Europe and North America. And winter hardiness expands fall sowing area, which usually has higher yield than those...
sown in spring (Landry, Coyne, McGee, & Hu, 2016). A significant progress in breeding for frost resistance and winter hardiness was made in northern Europe (Link, Balko, & Stoddard, 2010; Arbaoui et al., 2008). Several cultivars and improved lines were identified to be tolerant to winter hardiness in Germany, Bulgaria, and other areas with a similar winter climate (Ali et al., 2016; Landry, Lafferty, Coyne, Pan, & Hu, 2015).

Soil acidity and waterlogging are among the common problems that limit the production of faba bean in Ethiopia (Keneni, Assefa, & Prabu, 2010). The variety ‘Walki’ was developed for waterlogged and vertisols areas and is gaining popularity in the central highlands of Ethiopia.

4 | GENOMICS

The first major contribution to faba bean transcriptome knowledge was the release of approximately 5,000 Expressed Sequence Tags (EST) from developing embryos of a broad bean variety ‘Windsor’ (Ray & Georges, 2010). This study provided a useful snapshot of the functional classification and relative expression level of more abundant transcripts from the embryo transcriptome in the early to middle stages of its development. Subsequently, studies began to encompass multiple, separate inbred genotypes (Webb et al., 2016), as well as multiple tissues and genotypes (Ray, Bock, & Georges, 2015). The deepest transcriptome coverage produced has come from Illumina sequencing of a library of mixed tissues enriched with embryo transfer cells (Zhang et al., 2015). The burgeoning transcriptome and genomic sequence data sets have permitted the development of greater numbers of molecular markers, leading to a corresponding increase in map coverage and marker density. Also, an increasing number of powerful mapping population designs are now being explored, meaning genetic resolution is improving in tandem.

The first genetic maps in V. faba were largely based on random amplified polymorphic DNA (RAPD) markers (Torres et al., 1993), which are relatively difficult to score and reproduce (Penner et al., 1993). The main route for exploitation of these early maps was through conversion of RAPD to sequence amplified characterized region markers (Gutierrez et al., 2006). A key breakthrough was the creation of a genetic map composed entirely of sequence-based markers (Ellwood et al., 2008), which allowed patterns of collinearity with related taxa (e.g., Medicago) to be easily traced for the first time. The association of each marker with a low copy gene-encoding sequence meant that it was possible to efficiently convert and reproduce the markers on other marker platforms, for example, the conversion of cleaved amplified polymorphic sequences (CAPS) markers to “kompetitive allele-specific PCR” (KASP) assays by Cottage et al. (2012). Following this, molecular marker development for mapping purposes went down to two main routes. First, EST-SSRs began to be systematically mined and offered both high information content per assay of SSR repeats and the ability to study synteny based on the orthologies between the ESTs in which these SSRs were embedded, and orthologous genes in other taxa (Kaur et al., 2012) showed a high validation rate for SSRs mined from 454 sequencing derived EST sequences and subsequently mapped 71 of these (Kaur et al., 2014). Second, in parallel with efforts targeting EST-SSRs, mining of SNPs from transcriptome data began in earnest in 2014. Kaur et al. (2014) produced an Illumina OPA—“oligonucleotide pool assay” bead array with 768 SNP markers mined from Icarus and Ascot transcriptomes, of which 465 were mapped. Webb et al. (2016) reported 757 new, validated KASP assays, of which 653 newly mined SNPs and 34 legacy SNPs (Cottage et al., 2012) were placed in a consensus map.

The majority of published V. faba genetic studies have used biparental F2 and recombinant inbred line (RIL) populations with less than 200 RILs. Therefore, there were inherent limitations both in terms of the allelic diversity that could be embodied in two parental inbred lines and the modest numbers of progeny lines examined. Limited opportunities for meiosis meant that for a time, all faba bean genetic maps were fragmentary (i.e., more linkage groups than chromosomes), and relatively low resolution. A major step towards alignment of genetic linkage groups was the consensus map by Satovic et al. (2013), who achieved a high number of mapped loci by merging genetic maps of three inter-related biparental RIL populations encompassing four diverse parents. Of the 23 linkage groups found, 13 of the largest could be tentatively assigned to parts of the six chromosomes. However, aside from the fragmentation of chromosomes, the backbone of the Satovic et al. (2013) consensus map was still RAPD markers, limiting the degree to which the map could be synthetically aligned with other genomes and the ease of reproducing the same markers across other populations. The first V. faba genetic map where all markers fell into just six linkage groups corresponding to the six haploid chromosomes was developed by Webb et al. (2016). The key achievement of this study was genotyping six F2 and RIL populations with the same set of gene-based SNP assays in the KASP format, such that most polymorphic markers were present in two or more component maps. The product of this study was a map consisting of just 687 markers in six linkage groups spanning 1,403.8 cM with each linkage group assigned to a physical chromosome. It made possible for all gaps in marker coverage to be quantified, and users of the consensus map could pick an individual trait-linked marker and reproduce it cheaply with little effort, or take a subset of spaced markers to sample the whole genome (e.g., in a genetic diversity study), or simply use the whole set. Importantly, the 687 mapped markers contained 34 converted CAPS markers from previous studies, which meant that this new consensus map was backwards-compatible (Webb et al., 2016).

While Webb et al. (2016) took the SNP mining and mapping approach on a genomewide scale, it is possible now to mine and deploy subsets of SNPs targeted to a region of interest. This is what has recently been reported by Ocaña-Moral, Gutiérrez, Torres, and Madrid (2017) and Khazaeei et al. (2017), whereby SNPs were added to a series of Orobanche and Ascochyta resistance.

The SNP sets referred to above represent only a tiny fraction of mined polymorphisms. Therefore, SNP genotyping products with one or two orders of magnitude higher density are now possible. In fact, a high-density genotyping array with >50 K features has been recently developed (O’Sullivan, pers. comm.). The trend towards
Development of multiparent populations and association panels across many plant species is being fulfilled in V. faba. In recent years, markers associated with frost tolerance were discovered using the Goettingen Winter Bean MAGIC panel (Sallam & Martsch, 2015). The ICARDA faba bean MAGIC population comprising >2,200 F₄ lines is currently under development, combining eight diverse parents with sources for heat, drought, ascochytta blight, chocolate spot, rust and Orobanche resistance.

Genetic modification represents both a research tool, permitting testing of hypotheses on gene function by over- and expression or knockdown/v/knockout studies and an outlet for genetic research in generation of targeted phenotypic modifications based on knowledge of gene function. In faba bean, Böttinger, Steinmetz, Schieder, and Pickardt (2001) and Hanafy, Pickardt, Kiesecker, and Jacobsen (2005) were the first reports on transgenic approaches by generating stable germline transformation using in vitro regeneration. Hanafy, El-Banna, Schumacher, Jacobsen, and Hassan (2013) reported faba bean transgenic lines overexpressing potato PR10a using their previous methods. This is the sole successful demonstration to date of the feasibility of a biotechnological approach to faba bean improvement.

The prospects afforded by new insights into the phenotypic effects of allelic variation and the more refined biotechnological possibilities afforded by rapidly maturing genome-editing technologies (Gaj, Gersbach, & Barbas, 2013) could potentially stimulate renewed interest in genetic transformation.

5 | CONCLUSIONS

Faba bean remains one of the most important staple food and feed legume crops in many countries, improving the livelihood of smallholder farmers. There is a high level of genetic diversity within a large number of accessions, especially within the primitive forms and landraces. The genetic relationship among faba bean accessions disclosed by DNA markers is associated with their geographical origin and ecological habitat suggesting gradual diversification and differentiation in response to natural and artificial selections. This genetic diversity is very useful for increasing yield potential of the faba bean crop through development of cultivar in open pollinated conditions to increase genetic gains.

6 | FUTURE RESEARCH

To enhance farm profitability through adoption of improved faba bean technologies, there is to continue developing high-yielding nutritious varieties resistant/tolerant to multiple pests (stem borer in North Africa, Faba bean necrotic yellow virus in Egypt and new Gall disease in Ethiopia). There is also a need to continue developing climate-smart varieties, which can tolerate stress of drought, heat, waterlogged and acid soils, and suitable to different cropping systems.

To accelerate breeding cycles, there is a need to use emerging biotechnological tools, such as marker-assisted selection, which have not been widely adopted despite significant achievements of quantitative trait loci studies (Ocaña-Moral et al., 2017; Webb et al., 2016). Using new population design and genotyping technologies, we can expect in the near future high-density multiparent maps, revealing finally a fine-scale gene order for V. faba and providing a genotyping product, which can be readily accessed, and a unified framework for systematically reading across populations and maps. These maps would also greatly facilitate assembling and ordering genomic scaffolds in a future genome-sequencing effort.

Unravelling the nuclear and organelar genomes of faba bean, their plasticity and regulation in response to environmental challenges will not only increase understanding of how stress tolerance is regulated but will also accelerate breeding progress of faba bean. This could pave the way for future realization of its considerable potential for improvement. Faba research community needs to look ahead to pooling and constituting one well-funded programme to guarantee exploitation of these novel advances in Genomics (Molecular markers, Molecular Diversity, exploitation of syntheny, mutagenesis) for a near future full genome sequencing, which would offer foundation for future faba bean genomewide association studies. Current efforts are isolated and need to be centralized.

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CONFLICT OF INTEREST

Authors confirmed that there is no conflict of interest to declare.

AUTHOR CONTRIBUTION

 Fouad Maalouf wrote the manuscript with contribution from Jinguo Hu in the origin and genetic diversity and breeding for abiotic stresses sections; from Donal O’Sullivan in the Genomics section. Aladdin Hamwieh and Michael Baum contributed to faba bean genetic diversity research at ICARDA and on assembling faba bean GCP reference set. Xuxiao Zong contributed to the Genomics section. Shiv Kumar led the food legume breeding programme at ICARDA. All authors provided critical feedback to the manuscript.

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