Recent advances in faba bean genetic and genomic tools for crop improvement

Hamid Khazaei¹ | Donal M. O'Sullivan² | Frederick L. Stoddard³ | Kedar N. Adhikari⁴ | Jeffrey G. Paull⁵ | Alan H. Schulman⁶,⁷ | Stig U. Andersen⁸ | Albert Vandenberg¹

¹Department of Plant Sciences, University of Saskatchewan, Saskatoon, Saskatchewan, Canada
²School of Agriculture, Policy and Development, University of Reading, Reading, UK
³Department of Agricultural Sciences, Viikki Plant Science Centre, and Helsinki Sustainability Science Centre, University of Helsinki, Helsinki, Finland
⁴Plant Breeding Institute, Faculty of Science, The University of Sydney, Narrabri, New South Wales, Australia
⁵School of Agriculture, Food and Wine, The University of Adelaide, Adelaide, South Australia, Australia
⁶Production Systems, Natural Resources Institute Finland (Luke), Helsinki, Finland
⁷Institute of Biotechnology and Viikki Plant Science Centre, University of Helsinki, Helsinki, Finland
⁸Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark

Abstract
Faba bean (Vicia faba L.), a member of the Fabaceae family, is one of the important food legumes cultivated in cool temperate regions. It holds great importance for human consumption and livestock feed because of its high protein content, dietary fibre, and nutritional value. Major faba bean breeding challenges include its mixed breeding system, unknown wild progenitor, and genome size of ~13 Gb, which is the largest among diploid field crops. The key breeding objectives in faba bean include improved resistance to biotic and abiotic stress and enhanced seed quality traits. Regarding quality traits, major progress on reduction of vicine-convicine and seed coat tannins, the main anti-nutritional factors limiting faba bean seed usage, have been recently achieved through gene discovery. Genomic resources are relatively less advanced compared with other grain legume species, but significant improvements are underway due to a recent increase in research activities. A number of bi-parental populations have been constructed and mapped for targeted traits in the last decade. Faba bean now benefits from saturated synteny-based genetic maps, along with next-generation sequencing and high-throughput genotyping technologies that are paving the way for marker-assisted selection. Developing a reference genome, and ultimately a pan-genome, will provide a foundational resource for molecular breeding. In this review, we cover the recent development and deployment of genomic tools for faba bean breeding.
1 | INTRODUCTION

Faba bean (Vicia faba L.) is one of the first domesticated food legumes and has a long history of cultivation; seeds as old as 14,000 years were identified in the southern Levant (Caracuta et al., 2016). Faba beans are widely grown for food and feed as a generous source of high-quality protein, dietary fibre and other valuable nutrients (Duc, 1997; Khazaei & Vandenberg, 2020). The protein content of faba bean seeds is about 29% of the dry matter (Warsame et al., 2018), which makes it one of the main sources of affordable protein for people in the Middle East, Latin America and Africa, and for livestock feed in many developed countries. Faba bean, like most other legumes, forms a symbiosis with nodule-forming bacteria that have nitrogen fixing ability, which provides major benefits to cropping systems and the environment and contributes to agricultural sustainability by soil improvement. It is considered an excellent protein crop due to its ability to provide nitrogen inputs into temperate agricultural systems on account of its wide adaption (Rispail et al., 2010), as well as its high yield potential and nitrogen-fixing capacity even when nitrogen is present in the soil (Herridge et al., 2008), compared with other grain legumes (Cernay et al., 2015). These particular nitrogen-fixing traits in combination with yield potential mean that faba bean can be produced in a sustainable manner, making it particularly well-suited for providing the protein required for the globally expanding plant-based food chain. Faba bean delivers plant protein products suitable for consumption both by those with soybean (Glycine max (L.) Merr.) allergy or intolerance and by those wishing local products. According to Food and Agriculture Organization Corporate Statistical Database (2019), faba bean is the fourth most widely grown cool-season grain legume (pulse) globally after pea (Pisum sativum L.), chickpea (Cicer arietinum L.), and lentil (Lens culinaris Medik.), with annual production of around 4.5 million tonnes from nearly 2.5 Mha.

Faba bean improvement is currently impeded by development of rich genomic resources having not kept pace with those of other cool-season grain legumes. Faba bean is a partially allogamous diploid species with six pairs of remarkably large chromosomes. Its genome is one of the largest of any diploid field crop, about 13 Gbp in the haploid complement (Soltis et al., 2003) and contains more than 85% repetitive DNA (Novák et al., 2020). The large genome of faba bean is 2.9, 3.0 and 15.9 times larger than pea, lentil and chickpea, respectively. Assembly of the faba bean genome and map-based cloning was delayed both due to its genome complexity (e.g. abundance of transposable elements) and the lower investment in its study compared with, for example, soybean. In the absence of a reference genome assembly for this species, high-throughput approaches such as transcriptome analysis have been efficient tools for enrichment of genomic resources (e.g. Arun-Chinnappa & McCurdy, 2015; Braich et al., 2017; Khan et al., 2019; Ocaña et al., 2015; Ray et al., 2015). However, from these reported transcriptome datasets, only limited DNA sequence data are available in public databases (Mokhtar et al., 2020). Additionally, the development of high-density genetic maps derived from multiple populations and gene-based molecular markers, particularly those developed by Webb et al. (2016) and Carrillo-Perdomo et al. (2020), has paved the road to marker-assisted selection (MAS) and gene discovery. For example, the elucidation of the biosynthetic pathway for the pyrimidine glycosides vicine and convicine (v-c) (Björnsdotter et al., 2020), which have been the main factors limiting faba bean cultivation and usage in many warm regions, was not possible without the combination of transcriptome data (Ray et al., 2015) and gene-based comparative mapping approaches (Khazaei et al., 2015, 2017).

Two recent review papers on this topic cover the coming of age of faba bean genetics and genomics in some detail (see Maalouf et al., 2019; O’Sullivan & Angra, 2016), but, since then, major progress on the key seed anti-nutrients v-c (Björnsdotter et al., 2020), seed coat tannins (e.g. Gutiérrez et al., 2020; Gutiérrez & Torres, 2019), as well as improved mapping approaches (Carrillo-Perdomo et al., 2020) and transcriptome data (see Section 3), has been made (e.g. Gao et al., 2020; Wu et al., 2020; Yang et al., 2020). We provide here a comprehensive review on the mapping population and genomic resources in this species.

2 | GENOMIC RESOURCES

2.1 | Genetic maps

Genetic linkage maps have been developed in faba bean using different types of populations and molecular markers (Table 1). Sirks (1931) was the first to report a faba bean genetic map, identifying 19 genetic factors that formed four linkage groups. His genetic resources were lost during World War II. Four decades later, Sjödin (1971) used translocation lines for the assignment of different loci (for morphological observations, flower and seed coat colour) to their respective chromosomes. Genetic mapping studies were developed in the 1990s first with the aid of morphological markers, isozymes, seed protein genes and random amplified polymorphic DNA (RAPD) markers. Later, the development of expressed sequence tags (ESTs), microsatellites or single sequence repeats (SSRs), EST-SSRs and single nucleotide polymorphism (SNP) markers helped to enrich faba bean genetic studies and breeding. The first DNA-based linkage map in faba bean was constructed with only 17 markers, of which 10 were RFLPs (restriction fragment length polymorphism) (van de Ven et al., 1991). The first set of SSR markers were developed by Požárková et al. (2002) and then mapped by Román et al. (2004). A composite gene-based map, anchored with orthologous markers mapped in Medicago truncatula.

KEYWORDS
breeding, gene discovery, genomic resources, mapping population, Vicia faba

INTRODUCTION

KHAZAEI ET AL.
| Population      | Marker type                                           | Population type | Population size | Map length (cM) | Ave. inter-marker distance (cM) | Mapped traits                                           | References                                    |
|-----------------|------------------------------------------------------|-----------------|-----------------|-----------------|-------------------------------|---------------------------------------------------------|----------------------------------------------|
| 172 × Optica    | 7 RFLPs, 4 morphologicals, 3 isozymes, 3 RAPDs      | BC              | 231             |                 | 300–350                      | Biochemical and morphological traits                    | van de Ven et al. (1991)                    |
| Vf6 × (Vf173, Vf35) | 43 RAPDs, 7 isozymes, 1 RFLP                     | 2 F2s           | 20 + 44         |                 |                               |                                                          | Torres et al. (1993)                         |
| 172 × Optica    | 8 morphologicals, 7 RFLPs, 4 isozymes, 4 RAPDs      | BCF2            | 300             |                 |                               |                                                          | Ramsay et al. (1995)                         |
| Vf6 × (Vf12 T5.6;Vf133 T3.4; Vf159 T4.5.6) | 147 RAPDs, 9 isozymes, 1 morphological | 7 F2s           | 813 (total)     |                 | 850                           |                                                          | Satovic et al. (1996)                        |
| Vf6 × (Vf17, Vf27, Vf46) | 105 RAPDs, 7 isozymes, 3 seed protein genes, 1 morphological | 3 F2s           | 175             |                 | 1200                          | Seed weight                                              | Vaz Patto et al. (1999)                      |
| 34 Morocco × Kristall 25 | 77 RAPDs                                      | F2              | 57              |                 | 973                           | Broomrape and ascochyta blight resistance               | Surahman (2001)                              |
| Vf6 × Vf136     | 117 RAPDs, 2 isozymes, 2 seed protein genes         | F2              | 196             |                 | 1445                          | Broomrape and ascochyta blight resistance               | Román et al. (2002; 2003)                    |
| Vf6 × (Vf12 T5.6; Vf133 T3.4; Vf27; Vf27 T4.6; Vf136; Vf159 T4.5.6) | 176 RAPDs, 6 isozymes, 4 SSRs, 3 seed protein genes, 2 morphological | 11 F2s           | 654 (total)     |                 | 1559                          | Rust, broomrape and ascochyta blight resistance       | Román et al. (2004)                          |
| 29H × Vf136     | 94 RAPDs, 4 isozymes, 3 SSRs, 2 seed protein genes | F2              | 159             |                 | 1308                          | Rust and ascochyta blight resistance, and agronomic traits | Avila et al. (2003; 2004; 2005)               |
| Vf6 × Vf27      | 151 ITAPs                                           | F6              | 94              |                 | 1686                          | Frost tolerance and physiologically related traits      | Ellwood et al. (2008)                        |
| Côte D’Or/1 × BPL 4628 | 131 RAPDs, 1 morphological                          | F6              | 101             |                 | 1635                          | Frost tolerance and physiologically related traits      | Arbaoui et al. (2008)                        |
| Vf6 × Vf136     | 238 RAPDs, 21 ISMs, 6 SSRs, 5 EST-derived markers, 4 isozymes, 2 STSs, 1 SCAR | F6              | 165             |                 | 2857                          | Ascochyta blight and broomrape resistance               | Díaz-Ruiz, Satovic, et al. (2009), Díaz-Ruiz, Torres, et al. (2009) and Díaz-Ruiz et al. (2010) |
| Vf6 × Vf27      | 167 EST-derived markers, 71 RAPDs, 11 SSRs, 3 RGAs, 3 seed protein genes, 2 isozymes, 1 morphological | F6              | 124             |                 | 1875                          | Flowering, yield-related traits, plant architecture and yield | Cruz-Izquierdo et al. (2012) and Avila et al. (2017) |
| 91825 × K1563   | 128 SSRs                                            | F2              | 129             |                 | 1587                          |                                                          | Ma et al. (2013)                             |

(Continues)
| Population          | Marker type                                                                 | Population type | Population size | Map length (cM) | Ave. inter-marker distance (cM) | Mapped traits                                      | References                                      |
|---------------------|------------------------------------------------------------------------------|-----------------|-----------------|-----------------|-------------------------------|--------------------------------------------------|------------------------------------------------|
| 29H × Vf136         | 121 RAPDs, 38 EST-derived markers, 6 SSRs, 5 RGAs, 1 defense-related gene, 1 seed protein gene | F7,8            | 119             | 1402            | 9.87                          | Broomrape resistance                              | Gutiérrez et al. (2013)                         |
| Vf6 × Vf27, Vf6 × Vf136, 29H × Vf136 | 729 markers in total                                             | 3 RILs          | 124 + 165 + 119 | 4613            | 6                             | Consensus map                                     | Satovic et al. (2013)                           |
| Icarus × Ascot      | 465 SNP markers, 57 EST-SSRs                                                 | F5,6            | 95              | 1217            | 2.3                           | Ascochyta blight resistance and flowering time    | Kaur, Kimber, et al. (2014) and Catt et al. (2017) |
| Mélodie/2 × ILB 938/2 | 188 SNP markers, 1 morphological                                           | F5              | 211             | 928             | 5.8                           | Drought adaptation-related and morphological traits, and vicine-convicine | Khazaei et al. (2014a, 2014b, 2015)             |
| Nubaria 2 × Misr 3  | 552 EST-SSRs                                                                | F2              | 109             | 688             | 1.25                          | Consensus map, flower color (zt1)                 | El-Rodeny et al. (2014)                         |
| Albus × BPL 10, Albus × 29H, Hedlin × CGN07715 cf-3, NV644-1 × IG 12658, Mélodie/2 × ILB 938/2, Côte D'Or/1 × BPL 4628/1521 | 687 SNP markers                                      | 4 F2,5, 2 RILs  | 136 + 165 + 52 + 192 + 200 + 101 | 1404            | 2.6                           | Consensus map, flower color (zt1)                 | Webb et al. (2016)                              |
| Fiord × Doza#12034 | 2784 SNP markers                                                            | F6              | 104             | 1027            | 0.37                          | Rust resistance                                  | Ijaz (2018)                                    |
| 91825 × K1563       | 465 SSRs                                                                    | F2              | 129             | 4517            | 9.71                          | Ascochyta blight resistance                       | Yang et al. (2019)                             |
| Nura × Farah        | 1152 SNP markers                                                            | F4              | 145             | 1022            | 1.45                          | Flower color (zt2)                               | Sudheesh et al. (2019)                          |
| Disco/2 × ILB 938/2 | 257 SNP markers, 2 morphologicals                                           | F6              | 176             | 918             | 5.4                           | Flower color (zt2)                               | Zanotto et al. (2020)                           |
| (Nova Gradiska, Silian & Quasar) × Hiverna  | 1728 SNP markers                                                              | 3 F3,5           | 102 + 147 + 96  | 1548            | 0.89                          | Consensus map                                    | Carrillo-Perdomo et al. (2020)                  |
| Vf6 × Vf27          | Cruz-Izquierdo et al. (2012) + 44 KASPs and 37 dehiscence-related markers | F8,9            | 124             | 4421            | Pod dehiscence                 | Aguilar-Benitez et al. (2020)                    |

Abbreviations: EST, expressed sequence tags; ISM, intron-spanning marker; ITAP, intron targeted amplified polymorphism; KASP, kompetitive allele specific PCR; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; RGA, resistant gene analogs; RIL, recombinant inbred lines; SCAR, sequence characterized amplified region; SNP, single nucleotide polymorphism; SSR, simple sequence repeat; STS, sequence tagged sites.

*T refers to the assignment of linkage groups to chromosomes by trisomic segregation.
Gaertn., was developed by Ellwood et al. (2008); synteny and genic collinearity among the legumes make the data applicable to V. faba and other legumes (Lee et al., 2017). Kaur, Kimber, et al. (2014) reported the first exclusively SNP-based generic map of faba bean. Satovic et al. (2013) reported the first reference consensus genetic map, which covered 4062 cM (centiMorgan) in six main linkage groups, corresponding to the six chromosomes of faba bean. Table 1 shows that with the development of faba bean sequences and marker datasets, there was a correspondingly encouraging increase in the density and utility of gene-based genetic maps. In the last few years, the significant advancements in genotyping and sequencing technologies have led to two new SNP-based highly dense consensus maps. An international effort resulted in the first consensus map for six mapping populations, based on SNP markers derived from M. truncatula (Webb et al., 2016). It contained 687 SNP markers on six linkage groups, each presumed to correspond to one of the faba bean chromosomes. Carrillo-Perdomo et al. (2020) recently reported the most saturated consensus genetic map to date: it was constructed using three mapping populations and encompassed 1728 SNP markers distributed in six linkage groups. Solid proof of macro-synteny was also observed between this map and the most closely related legume species that have been sequenced. Recently, a database of ESTs, EST-SSRs, mtSSRs (mitochondrial-simple sequence repeats) and microRNA-target markers in faba bean has been launched (Mokhtar et al., 2020). Now that most pulse genomes are available, it is important to implement comparative genomic approaches, which will ultimately assist in the identification of candidate genes, quantitative trait loci (QTL) mapping, and assembly of the genome in faba bean.

2.2 Mapping populations

Published studies in faba bean to date have mostly involved bi-parental populations, derived from crosses between two inbred lines. Several types of bi-parental mapping populations, such as F₂, backcrosses and recombinant inbred lines (RILs), have been employed for genetic map construction and trait mapping. The relatively large set of interconnected bi-parental populations that segregate for diverse important traits in this species will help advance faba bean breeding (Table 1). These types of populations are easy to construct and represent a powerful tool for QTL detection. Their optimal allele frequency and low rate of linkage disequilibrium decay within chromosomes means that only a few hundred RILs/markers are needed to map a QTL (Scott et al., 2020). Despite the advantages of bi-parental populations, their mapping precision is low due to the low total amount of genetic recombination, as only two alleles are present at any locus, and to the low amount of genetic diversity that can be created by only two founders. These factors may limit the number of QTLs captured. Multi-parent populations have been developed to cope with the limitations of bi-parental populations (Scott et al., 2020). In faba bean, a multi-parent population derived from 11 European winter bean founders was created and employed to identify genomic regions controlling frost adaptation (Sallam & Martsch, 2015). A multi-parent population from four founders (ILB 938/2, Disco/2, IG 114476 and IG 132238) was developed for preliminary characterization of important morphological and biochemical traits (Khazaei, Stoddard, et al., 2018). A genetic map with 11 K loci is being developed using a 50 K Axiom SNP genotyping array (O’ Sullivan et al., 2019). This population segregates for a number of traits including v-c, seed coat tannin (white-flowered parent carrying the zt2 gene), seed size and colour, and branching. A MAGIC (multi-parent advanced-generation intercross) population comprising over 2000 F₄ individuals is currently under development at ICARDA (International Center for Agricultural Research in Dry Areas), combining eight diverse parents with sources for heat, drought, ascochyta blight, chocolate spot, rust and broomrape resistance (Maalouf et al., 2019). Because in multi-parental populations there can be as many alleles per locus as founders, quantifying genetic interactions between loci requires the large numbers of individuals (>1000), found in typical MAGIC populations.

Table 2 lists the faba bean genotypes and parental lines that have been used for genetic map construction or transcriptome analysis. Over 70% of the germplasm used for mapping purposes belongs to the Mediterranean adaptation zones (Australia, southern Europe and North Africa). The global collection of faba bean germplasm across 37 genebanks exceeds 43,000 accessions. The ICARDA collection comprises more than 8500 accessions held in Lebanon and Morocco by April 2020 (20% of the global collection, Westengen et al., 2020). Despite the wealth of faba bean germplasm, characterization and preliminary evaluation remain a challenge. Faba bean is represented in the collections by only the cultivated forms, and a wide range of variation in plant and seed phenotypic characteristics have been reported (Khazaei, 2014; Maalouf et al., 2019). The development of a reference genome, gene functional analyses and genotype-phenotype association, together with the development of high-throughput genotyping platforms, will facilitate characterization of the genetic diversity within the germplasm collections as well as understanding of its potential. It will aid exploitation of the diversity as a key resource for breeding.

2.3 Trait mapping

The first faba bean QTL mapping study was reported by Ramsay et al. (1995), who detected several loci for morphological and biochemical traits including v-c. QTL mapping in faba bean for biotic stresses, such as resistance to pathogenic fungi or parasitic plants, has been attempted (Table 1). Two of the major constraints in Mediterranean climates, namely, ascochyta blight (caused by Ascochyta fabae Speg...) and broomrape (Orobanche crenata Forsk. and O. foetida Poir.), have been widely subjected to QTL studies using F₂ and RIL populations (Table 1). The QTLs accounting for significant proportions of ascochyta blight resistance have been validated in multi-environment trials (Atienza et al., 2016). In addition, some attention has been given to rust resistance (Uromyces viciae-fabae (Pers.) J. Shört.) (Avila et al., 2003; Ijaz, 2018). Recently, two mapping populations (Fiord x Doza#12034 and Fiord x Ac1655) have been
| Line          | Origin/donor                | Trait(s) of interest                | Description                                                                                                                                 |
|--------------|-----------------------------|------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| **Mapping population**                                                                                                                             |
| Ac1655       | Australia                   | Rust resistance                    | European line (V-300) introduced from Spain (Adhikari et al., 2016)                                                                        |
| Albus         | Poland                      | Low tannin                         | White-flowered (zt1). Albus (Latin) means white                                                                                             |
| Ascot         | Australia                   | Resistant to ascochyta blight      | Selection from cv. Fiord. Original source of germplasm is Greece (Kaur, Cogan, et al., 2014)                                             |
| BPL 10        | Jordan                      | Nematode resistant                 | Pure line selection from accession IG 101769 (ILB 6)                                                                                       |
| BPL 228       | Morocco                     | Frost tolerant                     | Pure line selection from IG 11335 (ILB 141)                                                                                               |
| BPL 4628      | China                       | Frost tolerant                     | Pure line selection from IG 106387 (ILB 3009) from Anhui, China                                                                         |
| CGN07715      | GAUG, Germany               | Closed flower                      | From CGN grain legumes collection, Wageningen, Netherlands                                                                                |
| Côte d’Or     | INRA, France                | Frost tolerant                     | Old French winter bean from Côte d’Or region of Burgundy (Picard et al., 1985). Yellow (buff) seed coat (Yg)                                 |
| Disco         | INRA, France                | Low tannin                         | Low v-c, white-flowered (zt2)                                                                                                             |
| Doza          | Australia                   | Rust resistance                    | Pedigree: Ac383 × triple White. Original sources of germplasm are Ethiopia and Sudan, respectively                                            |
| Farah         | Australia                   | Resistant to ascochyta blight      | Selection from cv. Fiesta (selection from BPL 1196 from Spain) (Kaur, Cogan, et al., 2014)                                              |
| Fiord         | Australia                   |                                     | The first faba bean cultivar released in Australia. Selection from Ac59 from the island of Naxos, Greece (Kaur, Cogan, et al., 2014)       |
| Hedin         | GAUG, Germany               | Highly inbred and autofertile, small seed size, and high seed number | It has already been adopted in a number of genomics projects as a reference genotype. Released in 1986 and has “Herz Freya” in its background |
| Hiverna       | Germany                     | Frost tolerant                     | Large-seeded winter bean, from NPZ released in 1986 (Link et al., 2010)                                                                   |
| ILB 938       | Andean region of Colombia and Ecuador | Drought adaptation, chocolate spot and rust resistance | ILB 938 (BPL 1179) is the result of mass selection from ILB 438 (BPL 710) based on seed size (Khazaei, Link, 2018). It carries a rare allele (ssp1) that decouples pigmentation in flowers from that in stipules (Khazaei et al., 2014b) |
| Icarus        | Australia                   | Resistant to chocolate spot and rust | Icarus was derived from BPL 710 (see above) (Kaur, Cogan, et al., 2014)                                                                   |
| IG 12658      | Ethiopia                    | Dwarf                              | A dwarf accession carrying gibberellic acid deficiency gene (Hughes et al., 2020)                                                            |
| K1563         | China                       | Winter bean                        | Small-seeded                                                                                                                             |
| Kasztelan     | Poland                      | Low tannin                         | White-flowered (zt1), the NIAB accession code is NV644                                                                                   |
| Kristall 25   | Germany                     |                                     | Developed in Lochow Petkus in 1973                                                                                                         |
| Mélodie       | INRA, France                | Low v-c                            | High water use efficiency (Khazaei et al., 2014a)                                                                                        |

(Continues)
| Line       | Origin/donor | Trait(s) of interest | Description                                                                 |
|-----------|--------------|----------------------|-----------------------------------------------------------------------------|
| Misr 3    | Egypt        | Resistance to broomrape | Early flowering, small-seeded. Pedigree: ((Giza 3 x ILB 938) x Cairo 2411) x (Giza 3 x 23A/45/76) (Attia et al., 2013) |
| Nova Gradiska  | Croatia     | Resistance to seed weevils (Bruchus spp.) | Small-seeded                                                                |
| Nubaria 2 | Egypt        | Drought adaptation    | Adapted to the Nubaria region in Egypt. Late flowering, large-seeded. Pedigree: ILB 1550 x Radiation 2095/76 |
| Nura      | Australia    | Resistant to ascochyta blight and moderate resistant to chocolate spot | Pedigree: Icarus x Ascot. Original sources of germplasm are Ecuador and Greece, respectively (Kaur, Cogan, et al., 2014) |
| Optica    | Netherlands  | Resistant to freezing, low tannin | Large-seeded, white-flowered (zt1)                                           |
| Quasar    | UK           | Resistance to seed weevils (Carrillo-Perdomo et al., 2019) | Winter bean adapted to oceanic climate                                        |
| Silian    | Northern Sudan |                                | Small-seeded                                                                |
| Vf6       | IFAPA, Spain | Resistant to ascochyta blight | Asynaptic breeding line program from Córdoba                                |
| Vf27      | IFAPA, Spain | Pod dehiscent            | Paucljugo type                                                              |
| Vf136*    | IFAPA, Spain | Moderate level of resistance to broomrape | From the progeny selection of Vf1071 x alameda. Vf1071 is a broomrape resistant line selected from cv. Giza 402. Alameda is a commercial variety well adapted to southern Spain |
| 172       | Afghanistan  | High levels of post-harvest seed dormancy | Paucljugo type                                                              |
| 91825     | China        | Winter bean             | Large-seeded                                                                |
| 29H*      | INRA, France | Resistant to ascochyta blight | Small-seeded breeding line developed at INRA                                  |

**Transcriptome**

| Line       | Origin/donor | Trait(s) of interest | Description                                                                 |
|-----------|--------------|----------------------|-----------------------------------------------------------------------------|
| AO 1155   | INRA, France | Low v-c              | Small-seeded, white-flowered (zt1)                                          |
| CDC Fatima | Canada       |                      | An established cultivar developed for use in the prairie provinces of Canada. Selection from a landrace known as Chinese broad bean (Graf & Rowland, 1987) |
| Hassawi-2 | Saudi Arabia | Drought adaptation   | Local landrace                                                              |
| SSNS-1    | Canada       | Small-seeded         | Bulk selection from cv. Ackerperle from Germany                             |
| Tongxian-2 | China       | Winter bean          | Vegetable type                                                              |
| Windsor   | UK           |                      | Large-seeded, long pods                                                     |
| Wizard    | UK           | High-yielding with large attractive seeds, ascochyta blight resistance | Large-seeded winter bean from Wherry & Sons, UK, released in 2002           |
| Y078      | China        | Salt sensitive       |                                                                             |
| Y134      | China        | Salt tolerant        |                                                                             |

**Note:** ICARDA maintains faba bean germplasm in two classes, international legume bean (ILB) accessions from different countries, and bean pure line (BPL) accessions that are derived through selfing from accessions drawn from the ILB collection. Abbreviations: CDC, Crop Development Centre; CGN, Centre for Genetic Resources, the Netherlands; GAUG, Georg-August-University, Göttingen; ICARDA, International Center for Agricultural Research in Dry Areas; IFAPA, Instituto de Investigación y Formación Agroalimentaria; INRA, Institut National de la Recherche Agronomique; NAIB, National Institute of Agricultural Botany; v-c, vicine-convicine.

*Used for both mapping and transcriptome research.
developed at the University of Sydney, in which KASP (Kompetitive Allele Specific PCR) markers for rust resistance genes Uvf-2 and Uvf-3 have been identified (Ijaz, 2018). However, until now, there has been no attempt to map QTLs or genes governing chocolate spot (caused by Botrytis fabae) resistance, in spite of the importance and widespread nature of this disease globally. A few RIL populations suitable for chocolate spot genetic studies have been developed using ILB 938, an accession with proven resistance to chocolate spot (reviewed by Khazaei, Link, et al., 2018). Two mapping populations (Mélodie/2 × ILB 938/2 and Disco/2 × ILB 938/2) have been phenotyped at the University of Saskatchewan and QTL mapping is underway. In addition, a list of faba bean accessions with resistance to chocolate spot is available (Maalouf et al., 2016).

Some progress has been made in identifying QTLs for abiotic stresses such as frost tolerance (Arbaoui et al., 2008; Sallam et al., 2016; Sallam & Martsch, 2015), traits related to drought adaptation (Ali et al., 2016; Khazaei et al., 2014a), and yield (Ávila et al., 2017; Cruz-Izquierdo et al., 2012). QTLs controlling abiotic stress responses in faba bean, detected by either QTL mapping or association mapping approaches, have been discussed by Sallam and Ul-Allah (2019). Given the few QTLs reported in faba bean compared with other pulses, saturation of the genomic regions associated with target regions and QTL validation in multiple environments and genetic backgrounds are needed to uncover reliable marker-trait associations such as those reported by Aguilar-Benitez et al. (2020) for pod dehiscence. The marker density in faba bean has recently been significantly increased (Carrillo-Perdomo et al., 2020; O’Sullivan et al., 2019); this development will facilitate fine QTL mapping and gene identification.

### Successful gene discoveries in the absence of a faba bean reference genome

Despite the relatively limited discovery of genes and QTLs for disease resistance and abiotic stress tolerance, the discovery of genes for the seed anti-nutritional factors v-c and tannins, which place major limitations on faba bean usage, has progressed considerably very recently. Vicine and convicine are stored in cotyledons of most faba beans at about 1% of dry matter (Khazaei et al., 2019). They are toxic in people who have a hereditary recessive mutation affecting the enzyme glucose-6-phosphate dehydrogenase (G6PD, Luzzatto & Arese, 2018). A high proportion of transcripts (about 96%) from Webb et al. (2016) was captured by the transcriptome data of Braich et al. (2017). The sequence length data were enriched (Braich et al., 2017; Cooper et al., 2017). A high proportion of transcripts (about 96%) from Webb et al. (2016) was captured by the transcriptome data of Braich et al. (2017). The sequence length data were increased at 461 chromosomal loci and provided increased accuracy by Cooper et al. (2017) compared with transcriptome data in Webb et al. (2016). The transcriptome data of Braich et al. (2017) revealed that faba bean, despite its large complex genome, compared similarly with other legume species in expressed gene content.

Next-generation sequencing (NGS) platforms, especially high-throughput RNA sequencing (RNA-Seq) technology, one of the most powerful tools currently available for transcriptome profiling, has enhanced the efficiency and speed of gene discovery in faba bean (Table 3). For example, the identification and characterization of differential gene expression from tissues subjected to drought (Alghamdi et al., 2018; Wu et al., 2020), vernalization (Gao et al., 2020), and salinity stress (Yang et al., 2020) have benefited greatly. These findings will help in understanding the stress tolerance mechanisms in the plant.

### Transcriptomes

A number of transcriptomes have been reported for faba bean (Table 3), albeit in the absence of a reference genome. These datasets were generated from a selection of different genotypes and tissues at various development stages or treatments. Recent reviews of this topic (Maalouf et al., 2019; O’Sullivan & Angra, 2016) described the faba bean transcriptome contributions up to 2016 that were used for the development of molecular markers for genetic mapping (listed in Table 3). Since then, the transcriptome data coverage has been further enriched (Braich et al., 2017; Cooper et al., 2017). A high proportion of transcripts (about 96%) from Webb et al. (2016) was captured by the transcriptome data of Braich et al. (2017). The sequence length data were increased at 461 chromosomal loci and provided increased accuracy by Cooper et al. (2017) compared with transcriptome data in Webb et al. (2016). The transcriptome data of Braich et al. (2017) revealed that faba bean, despite its large complex genome, compared similarly with other legume species in expressed gene content.
crop and will provide resources for functional genomics. Coupled with allelic data and trait mapping, the data will be invaluable in the development of more resilient faba bean varieties. A high-quality reference transcriptome has been completed (Björnsdotter et al., 2020) and is being expanded to a pan-transcriptome using data from four different genotypes (Hedin, Hiverna, 153b and 2378), including data from both shoot and root tissues (Escobar-Herrera et al., 2020). This effort has provided a comprehensive faba bean reference gene set that will be a valuable new resource for differential gene expression analyses and genome annotation.

### TABLE 3  Summary of published transcriptome data in faba bean

| References                  | Aim of study                                                                 | Tissue                                                                 | Output                                                                 | NGS platforms                  |
|-----------------------------|------------------------------------------------------------------------------|------------------------------------------------------------------------|------------------------------------------------------------------------|--------------------------------|
| Ray and Georges (2010)      | Development of EST sequences                                                 | Early to mid-developed embryo                                           | 5000 ESTs                                                             | 454 sequencing technology     |
| Kaur et al. (2012)          | Design and evaluation of EST-SSRs                                            | Young and mature leaf, stem, flower, immature pod, mature pod and immature seed | 802 SSRs                                                              | 454 Roche GS FLX titanium     |
| Kaur, Kimber, et al. (2014) | Development of SNP markers                                                   | Leaf                                                                   | 768 SNP markers                                                       | Illumina OPA-bead array        |
| Ray et al. (2015)           | Development of NGS libraries to elucidate the v-c pathway and other genes    | 5- to 6-days-old root and etiolated shoot and developing seed coat     | 8 libraries containing 1.2 million ESTs                               | 454 sequencing array           |
| Arun-Chinnappa and McCurdy (2015) | Generating a genome-wide transcriptome map of faba bean                   | Expanding and fully expanded leave, elongating and fully elongated stem, and closed and open flower, whole roots including root hairs, and cotyledon | 17,160 unigenes                                                        | Illumina HiSeq-2000            |
| Ocaña et al. (2015)         | Transcriptome analysis under ascochyta blight infection                      | Leaf tissue at 4, 8 and 12 h after inoculation                         | 21,243 transcripts, 39,060 SNPs and 3669 InDels                       | Illumina                       |
| Webb et al. (2016)          | SNP discovery                                                                 | 7-day-old seedling                                                      | 653 new mined SNP markers                                             | GS FLX/454 reads               |
| Braich et al. (2017)        | Development of reference unigene sets                                        | Immature pod and fully-open flower                                     | 26,295 new transcripts                                               | RNA-Seq, Illumina HiSeq 2000   |
| Cooper et al. (2017)        | Enhancement of faba bean genome resources                                   | Embryos                                                                | 16,300 unigenes                                                       | RNA-Seq, Illumina HiSeq 2500   |
| Alghamdi et al. (2018)      | Identify drought stress differentially expressed genes                      | Root at vegetative and flowering stages                                | 18,327 SSRs                                                           | RNA-seq, Illumina HiSeq 4000   |
| Gao et al. (2020)           | Identify response to vernalization genes                                     | Seed                                                                   | 6852 SSRs in 6552 transcripts                                         | RNA-seq, Illumina HiSeq 2500   |
| Yang et al. (2020)          | Identify salinity stress differentially expressed genes                     | Seed                                                                   | 4486 differentially expressed genes                                   | RNA-seq, Illumina HiSeq 4000   |
| Carrillo-Perdomo et al. (2020)| SNP discovery                                                                | Leaf                                                                   | 39,423 transcripts and 105,828 gene-based SNPs                       | RNA-seq, Illumina MiSeq        |
| Björnsdotter et al. (2020)  | Uncovering genes associated with the biosynthesis of vicine-convicine        | Young and mature leaf, flower, pod and whole seed at early seed-filling stage, embryo and pod at mid maturation, and stem | 49,277 transcripts                                                    | Illumina HiSeq PE150           |

### 4  CONCLUSIONS AND PERSPECTIVES

Recent technological advances now allow sequencing of the large genome of faba bean. A collaborative reference genome assembly effort is currently underway, coordinated by the NORFAB consortium (Protein for the Northern Hemisphere, https://bit.ly/37QxeUM), and a pan-genome initiative is being launched by the University of Helsinki and Luke (Natural Resources Institute Finland). The NORFAB project has developed an annotated reference transcriptome for faba bean (Escobar-Herrera et al., 2020), which will aid the development of gene
models for the reference assembly. The transcriptome work has also led to production of a high density faba bean genotyping array, which is now available from the University of Reading, UK. The array (known as ‘Vfaba_v2’), built on Life Technologies Axiom platform, contains 24,929 polymorphic high resolution SNP markers located in 15,846 different genes. Faba bean now benefits from saturated synteny-based genetic maps, NGS, and high-throughput genotyping technologies, which together will greatly aid genome assembly. Release of the reference genome will further advance the faba bean genomics and breeding revolution.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

AUTHOR CONTRIBUTION

H. K. did the writing of the original draft. All authors did the writing, review and editing of the manuscript. All authors read and agreed to the published version of the manuscript.

ETHICS STATEMENT

This manuscript does not contain any studies with human or animal subjects.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

ORCID

Hamid Khazaei https://orcid.org/0000-0002-5202-8764
Kedar N. Adhikari https://orcid.org/0000-0003-4662-2211

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