<https://helda.helsinki.fi>

Rust resistance in faba bean (*Vicia faba* L.) : status and strategies for improvement

Ijaz, Usman

2018-01

Ijaz , U , Adhikari , K N , Stoddard , F L & Trethowan , R M 2018 , ' Rust resistance in faba bean (*Vicia faba* L.) : status and strategies for improvement ' , Australasian Plant Pathology , vol. 47 , no. 1 , pp. 71-81 . <https://doi.org/10.1007/s13313-017-0528-6>

<http://hdl.handle.net/10138/308203>

<https://doi.org/10.1007/s13313-017-0528-6>

unspecified

acceptedVersion

Downloaded from Helda, University of Helsinki institutional repository.

This is an electronic reprint of the original article.

This reprint may differ from the original in pagination and typographic detail.

Please cite the original version.

Rust resistance in faba bean (*Vicia faba* L.): Status and strategies for improvement

^{*1}Usman Ijaz, ²Kedar N. Adhikari, ³Frederick L. Stoddard and ²Richard Trethowan

¹Plant Breeding Institute, University of Sydney, Cobbitty, NSW, Australia.

²I.A. Watson Grain Research Centre, University of Sydney, Narrabri, NSW, Australia

³Department of Food and Environmental Sciences and Viikki Plant Science Centre, University of Helsinki, Finland.

*Corresponding author: usman.ijaz@sydney.edu.au

Published in *Australasian Plant Pathology* **47**: 71-81. Doi: 10.1007/s13313-017-0528-6

Abstract:

Faba bean (*Vicia faba* L.) is an important grain legume used as food and feed. Its production is threatened by abiotic stresses and diseases, of which rust (*Uromyces viciae-fabae*) is one of the major diseases in East and North Africa, China and the northern grain-growing region of Australia. Understanding the genetic and physiological mechanisms of rust resistance in faba bean is in an early phase. The presence of seedling and adult plant resistance genes has been observed. The resistance most frequently utilised in applied plant breeding is race-specific, where the interaction between resistance genes in the host and avirulence genes in the pathogen confers resistance. The main drawback of using race-specific resistance is lack of durability, when deployed singly. Slow rusting or partial resistance, controlled by multiple genes of small effect, is generally non-race specific, so it can be more durable. We present the current knowledge of host resistance and pathogen diversity and propose rational breeding approaches aided with molecular markers to breed durable rust resistance in faba bean.

Key words: Faba bean rust (*Uromyces viciae-fabae*), breeding, major resistance genes, gene pyramiding, molecular markers.

Introduction:

Faba bean (*Vicia faba* L.) is a protein-rich grain legume, grown on all inhabited continents for food and feed, and it is a key source of dietary protein in North and East Africa, the

Middle East and China. It is a diploid ($2x=12$) species having one of the largest described genomes (13 Gb) among crop legumes, with at least 85% repetitive DNA (Flavell et al. 1974). It is believed to have been domesticated in the Fertile Crescent during the Neolithic era 9000-10000 BP (Tanno and Willcox 2006). Its secondary centres of diversity include the Nile Valley, Central and Eastern Asia, and South America (Duc et al. 2010). Faba bean is the sixth most important grain legume with 4.3 million tonnes annual production worldwide in 2014 (FAOSTAT 2017). Australia is the fourth largest producer (after China, Ethiopia and the UK) and the largest exporter globally (Eurostat 2016; FAOSTAT 2017), with nearly 85% of its total produce going to Egypt (70%) and Saudi Arabia (15%) (ABSTAT 2015). Similar to other legumes, faba bean is a good source of renewable nitrogen through biological nitrogen fixation (Jensen et al. 2010). It has a mixed breeding system, averaging one-third outcrossing and two-thirds inbreeding, which adds complexity to populations in the field, their genetic and phenotypic analysis, and practical plant breeding (Stoddard and Bond 1987).

Faba bean area decreased worldwide from 1962 to 1991, and since then has more or less stabilised at 2.1-2.7 million hectares, mostly across Asia and the Mediterranean region (FAOSTAT 2017). Apart from the commodity price, one of the reasons for decline is the prevalence of pathogenic fungi and the absence of disease-resistant cultivars (Sillero et al. 2010). Measures to control diseases include chemicals, biological control agents and crop management or cultural practices (Sillero et al. 2010). Systemic fungicides provide rapid and effective control, but they are expensive and can be detrimental to the environment (Emeran et al. 2011). Pathogens can also develop insensitivity following prolonged exposure to chemicals (Crute 1992). Biological control agents for pathogenic fungi are not yet available commercially (Stoddard et al. 2010). Therefore, breeding cultivars for resistance is widely considered the most efficient, cost effective and environmentally safe method of disease control (Sillero et al. 2010).

Rust is a major disease of faba bean in North Africa and the northern parts of the Australian grain belt (northern New South Wales and southern Queensland). The disease is caused by the biotrophic fungus *Uromyces viciae-fabae* (Pers.) J. Schrot., a macrocyclic rust and true obligate parasite with a sexual phase that does not require an alternate host to complete its lifecycle (Mendgen 1997; Voegelé 2006). In addition to faba bean, it can parasitize common vetch (*Vicia sativa* L.), pea (*Pisum sativum* L.) and lentil (*Lens culinaris* Medik.) (Conner and Bernier 1982a; Cummins 1978). Rust usually occurs at the time of pod filling in Europe

and southern Australia, causing up to 20% yield reduction (Sillero et al. 2011), although early rust epidemics can cause up to 70% loss of the standing crop (Liang 1986; Rashid and Bernier 1991). Losses up to 30% were estimated in northern New South Wales where the disease occurs in the seedling stage. Rust resistance in general restricts fungal proliferation before sporulation. In contrast to other pathosystems such as soybean-rust (*Phakopsora pachyrhizi* Syd.), for which complete monogenic resistance exists (Silva et al. 2008), resistance in faba bean is generally described as incomplete (Garcia et al. 2008; Miklas et al. 2006). Incomplete resistance, a type of quantitative resistance either expressed as hypersensitive type (Adhikari et al. 2016a; Sillero et al. 2000) with visible necrosis/chlorosis or non-hypersensitive type (Herath et al. 2001; Polignano et al. 1990; Rashid and Bernier 1984; 1991; Sillero et al. 2000) typically fits into the definition of partial resistance or field resistance (Parlevliet 1983). The hypersensitive reaction type, causing significant restriction on fungal growth and reduction of an infection type, is still considered as incomplete in faba bean (Sillero et al. 2010).

In this review, we highlight the current state of knowledge of rust resistance in faba bean and explore options for extending the genetic diversity of resistance and enhancing the resistance of this crop with new and evolving genomic technologies.

A brief history of rust resistance

The genetic control of plant disease resistance was established soon after the rediscovery of Mendel's work, when Biffen (1905) reported that resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*) of common wheat (*Triticum aestivum* L. emend Thell.) was controlled by a major gene with partial dominance. Variation in a pathogen population, resulting in variability for virulence (Barrus 1911) and resistance (McRostie 1919), was noted soon thereafter. Consequently, the concept of physiological races was defined. Physiological races are morphologically indistinguishable, but they can be differentiated in their reaction against a set of host lines known as differentials.

Studies on the inheritance of host resistance and resistance breakdown based on hypersensitive responses culminated in Flor's (1956) gene-for-gene hypothesis, wherein genes for host resistance (*R*) and pathogen avirulence (*avr*) control the outcome of the host-pathogen interaction. Virulent mutated pathotypes do not elicit the hypersensitive response because the corresponding *R* gene is no longer effective. Mutation events resulting in a deletion or substitution of an essential amino acid responsible for protein function may cause

a complete loss of the *avr* gene (de Wit et al. 2009). Thus, genetic resistance in obligate parasites such as rust can be highly race-specific (Keller et al. 2000).

Single *R* genes have generally failed to provide long-lasting resistance to rust (Johnson and Law 1973), because new virulent pathotypes frequently evolve, forcing breeders to seek new resistance genes. Lack of durability of resistance is not common to all plant pathosystems, but it is common among air-borne pathogens that possess both sexual and asexual cycles of reproduction. In the latter system, breeding for disease resistance became locked in a ‘boom-bust’ cycle (Pink 2002), where resistant cultivars are sown on large areas, exerting considerable selection pressure on avirulence in the pathogen. This sequence led to the concept of multi genes and gene pyramiding, discussed later.

Genetic diversity of rust resistance in faba bean

The success of resistance breeding depends upon the presence of genetic variability and our ability to detect and utilise it. This variability is captured in the collection of germplasm. The largest collection of faba bean worldwide is maintained by ICARDA, Terbol, Lebanon, with over ten thousand accessions from 71 countries. The second largest reserve with 5,200 accessions is preserved at the Chinese Academy of Agriculture Research, Beijing, China followed by 2,445 collections in the Australian Grains Genebank, Victoria, Australia. The USA, Russia, Ethiopia, France, Spain, Germany and Italy also have large numbers of accessions.

No wild species is sufficiently closely related to faba bean that fertile hybrids have been derived, so genetic diversity is restricted to the primary gene pool of the species itself (Cubero 2011). Accessions from the Mediterranean region are considered to have the best potential to provide genetic variation for rust resistance (Maalouf et al. 2010). Genotypes showing significant rust resistance with late necrosis originated from Central Europe (V-300, V-313, V-1271, V-1272), East Africa (2N-34) and Spain (V-1335) (Sillero et al. 2000). Most of these reported rust resistance germplasm lines were already imported in Australia and named with Australian accession numbers located at Australian Grain Genebank, Horsham, Victoria (Table 1). However, interaction of these resistance genes with prevailing faba bean rust pathotypes is unknown. Interspecific sources of resistance cannot be transferred to faba bean by conventional breeding methods, and the practical significance of induced mutation is relatively low due to low success rates (van Harten 1998). The species is generally intractable to tissue culture, but successful transformation by *Agrobacterium* has

been reported (Hanafy et al. 2013), opening the door for eventual interspecific transfer of resistance or editing of endogenous genes.

Pioneering work on rust resistance in faba bean was done in Canada in the 1980s, followed by Spain and Australia in recent years (Marcellos et al. 1995; Rose and van Leur 2006), with the identification of new genes and preservation of pathogen isolates (Adhikari et al. 2016a). Three race-specific genes (*fr1*, *fr2* and *fr3*) were found for rust resistance in seven faba bean inbred lines in Canada (Conner and Bernier 1982b). Conner and Bernier (1982c) identified seven genes conferring resistance to the specific rust isolates of Manitoba, Canada. Four years later, the same group of scientists reported the presence of five genes conditioning resistance against rust isolates collected from Canada (Rashid and Bernier 1986a). The hypersensitive resistance response has been mapped to gene *Uvf-1* by a group based in Spain (Avila et al. 2003).

The national faba bean breeding programme in Australia aims to provide cultivars for southern regions (South Australia and Victoria) and the northern plains (New South Wales and Queensland). Rust is a major disease in the northern plains, so breeding for its resistance is one of the main objectives. A great deal of genetic diversity of rust resistance at the seedling stage has been found in the Australian collection of faba bean germplasm (Fig. 1). The first moderately rust resistant cultivar, Doza, was released for cultivation in the northern plains in 2008, followed by PBA Warda and PBA Nasma during 2012 and 2016, respectively (Pulse Australia 2017). Three rust resistance genes were reported in segregating populations from crosses of exotic (Ac1227#14908 and Ac1655) accessions and local genotype Doza#12034 (Ijaz and Adhikari 2016a) (Fig. 1). Two of these genes (from Ac1655 and Doza#12034) were independently dominant, along with possible additive response (Adhikari et al. 2016a), and were mapped onto two different chromosomes (Sudhesh et al. 2016). However, the resistance in Ac1227#14908 was governed by complimentary gene action (Ijaz and Adhikari 2016b).

The nomenclature for rust-resistance genes in faba bean is not uniform and coordinated. The Canadians named their genes *fr*, whereas the Spanish named theirs *Uvf*, so a universal system of naming the resistance genes and pathogen variants (pathotypes) is needed similar to that used for wheat (McIntosh et al. 1995). This will enable comparison and verification of different genes identified in different areas.

Slow rusting: an insight into faba bean breeding

Several sources of slow rusting in faba bean have been reported (Conner and Bernier 1982d, Torres et al. 2006). Slow rusting limits the development and spread of infection, thus allowing the plant to outgrow the disease with little impact on productivity. Slow rusting or partial rust resistance has been reported in many crops, such as maize and sweet corn (*Zea mays* L.) (Hooker 1969; Groth et al. 1983), wheat (Singh et al. 2000) and barley (*Hordeum vulgare* L.) (Wilcoxson 1981). Slow rusting against *U. viciae-fabae* has also been identified in pea (*Pisum sativum* L.) (Chand et al. 2006) and lentil (Gupta and Singh 2010).

Generally, slow rusting is considered as durable because of its stable expression and reduced selection pressure on the pathogen (Bond et al. 1994). Macroscopically, slow rusting in faba bean is characterized by reduced lesion size and infection frequency with a longer latent period (Sillero et al. 2000). Microscopy revealed that reduced hyphal growth inhibited haustorial maturation, thereby limiting the formation of sporulating colonies (Rubiales and Sillero 2003; Sillero and Rubiales 2002).

Reduced yield losses (1-2%) were reported in a slow-rusting population compared to a susceptible population (61%) (Rashid et al. 1991) in faba bean. The area under the disease progress curve (AUDPC) provides a way to estimate slow rusting, and significant diversity in this trait has been reported in faba bean (Rashid and Bernier 1986b), lentil (Negussie et al. 2005) and pea (Singh et al. 2015). Low AUDPC in some accessions may not be entirely due to slow rusting because other factors, including cell wall thickness, number of stomata (Heath et al. 1990), temperature, leaf wetness and light (Joseph and Hering 1997) influence the disease development.

Information on the genetic basis of slow rusting in faba bean is limited. Both cytoplasmic and nuclear genes have been implicated (Stoddard and Herath 2001). The non-hypersensitive reaction is believed to be polygenic with minor effects, but such minor genes have not been identified or characterized. Torres et al. (2006) proposed studying this type of resistance using mapping populations and quantitative analysis, but no results have yet come to light.

Understanding the pathogen: *Uromyces viciae-fabae*

Uromyces, with about 600 species, is the second most important genus after *Puccinia* in the order Pucciniales (Maier et al. 2003). *Puccinia* species mainly infect grasses while *Uromyces* infects legumes. Genome sequence information is available for *Puccinia striiformis* f. sp. *tritici* (*Pst*) and *Puccinia graminis* f. sp. *tritici* (*Pgt*) (Cantu et al. 2013;

Duplessis et al. 2011; Zheng et al. 2013). Research activities have been concentrated on a very limited number of the 7,000 known rust fungi. Despite their ecological and economic values, little is known about the phylogeny within and between these groups. On the basis of sequences of the beta-tubulin 1 gene, a phylogeny showed that neither *Puccinia* nor *Uromyces* had evolved as a monophyletic group, and species of both fell into two distinct clades (Van der Merwe et al. 2007). Moreover, it was speculated that the pathogen genera diversified in parallel with their respective hosts under the situation when unrelated hosts met randomly in close proximity due to similar ecological needs.

Faba bean rust is an autoicous and macrocyclic fungus exhibiting completes all five spores stages, telio-, pycnio- basidio-, aecio- and urediospores, in a single host (Mendgen and Deising 1993). Host-specialized isolates were characterized on the basis of distinct spore dimension and infection structure morphology (Emeran et al. 2005), suggesting that *U. viciae-fabae* may be divided into intraspecific groups with differential pathogenicity to these hosts (Rubiales et al. 2013). This intraspecific distribution was confirmed through RAPD DNA markers (Emeran et al. 2008). Therefore, *Uromyces viciae-fabae sensu lato* is apparently a species complex that cannot be divided into *formae speciales*.

In addition to the knowledge of host diversity, understanding the pathogen is also a crucial step to ensure longevity of resistance. As faba bean rust is an obligate parasite with a sexual phase, new and virulent pathotypes can evolve frequently, thus requiring continuous genetic improvement to ensure that new cultivars are resistant to prevailing pathotypes. There is little evidence of faba bean rust pathogen diversity in Australia. Herath (2000) found that some isolates were generally less virulent than others, and showed some isozyme variation among these isolates, but further advances have been held back by the lack of differential hosts to characterise the various isolates, despite the early progress in Canada in this regard. It is essential that pathogen diversity be properly understood, including the implications for virulence, but the differentials developed in Spain (Avila et al. 2003) and Canada (Conner and Bernier 1982b) have not been used elsewhere, making it difficult to judge the effectiveness of one gene in other areas. Nine faba bean rust races were characterised from twelve faba bean lines on the basis of differential pathogenicity in Australia (Ijaz et al. 2017). Moreover, a binary nomenclature system (Steadman et al. 2002) for naming pathotypes is under development and will be available soon. These pathotypes are cryopreserved at the University of Sydney's Plant Breeding Institute, Cobbitty, Australia.

Link et al. (2014) provided an early insight into the genomic architecture of *U. viciae-fabae*. Sequencing was performed by using HiSeq2000 with a 500 nt library. Three different estimates of genome size were made: with 15-mer analysis, genome size was estimated at 329 Mb, whereas 17-mer analysis predicted a genome size of 422 Mb and filtered assembly estimated 216 Mb. Flow cytometry on the same isolate generated a size of 379 Mb (Tavares et al. 2014). Thus, the estimate of the actual genome size is in the range of 330 and 422 Mb, making it about 4 times the size of *P. graminis* f. sp. *tritici*, indicating a high significance of transposable elements (TE) (Duplessis et al. 2011). Link et al. (2014) identified numerous families of secreted proteins and candidate effectors, and reported 62 genes encoding proteins secreted from haustoria that have presumed roles in infection and specificity of *U. viciae-fabae*.

Deployment of resistance genes with major effects

Disease resistance is generally inherited as a dominant factor in natural populations (Barrett 1985), as natural selection acts on it favourably. Resistance based on major hypersensitive genes is usually considered non-durable (Stuthman et al. 2007) according to the gene-for-gene hypothesis. Hypersensitivity has been observed among faba bean genotypes, where necrosis appears during the later stages of rust proliferation and restricts fungal growth by controlled cell death (Sillero et al. 2000; Torres et al. 2006). Hypersensitivity is easily detectable with specific pathotypes (Emeran et al. 2005; Rojas Molina et al. 2006). Avila et al. (2003) and Adhikari et al. (2016b) showed that this type of hypersensitive response in faba bean is under the control of major genes. Nevertheless, the longevity of major genes in faba bean is less understood than in wheat as there is limited knowledge on pathogen variability. If these resistance genes of major effect can be deployed in combination through gene pyramiding, multiline cultivars or gene mixtures, the durability of resistance can be increased.

a. Pyramiding resistance genes

Gene pyramiding is the simultaneous deployment of more than one *R* gene in the same cultivar. Such *R* gene pyramids can provide long-lasting resistance because multiple simultaneous mutational events in *avr* genes are required to elicit susceptibility in the host. A particular gene combination (*Avr/R*) will remain effective as long as matching virulence is not widespread in the population, but it is difficult to identify individual *R* genes in a

complex pyramid unless virulent pathotypes or specific DNA markers are available (Pink 2002).

Gene pyramiding provides an opportunity to achieve a ‘clean crop’ and ensures crop uniformity because all genes are deployed in a uniform genetic background. Nevertheless, R gene pyramids are vulnerable to virulence if the individual components of resistance are deployed singly in other cultivars grown in the same region (Parlevliet 1997). The R gene in a pyramid exerts a strong unidirectional selection pressure against the matching virulence in a pathogen population. Therefore, pathogen monitoring to identify new virulent pathotypes is essential to minimise risk through early warning. The number of R genes in a pyramid should determine the spread of emerging virulent pathotypes in a pathogen population (Kolmer 1992; Mundt 1991), but conflicting results have been reported. For example, Mundt (1991) reported that an R gene pyramid failed to provide resistance against stem rust in wheat. This breakdown of resistance started a debate over the value of R-gene pyramids. Although there are some examples of cultivars where the R gene pyramid ensured durability of resistance, there is no clear association between the number of R genes in a pyramid and durability. The successful use of gene pyramiding against rust in faba bean has not been reported, but two recently reported (Adhikari et al. 2016a) seedling rust resistance genes provide a potential for pyramiding because the combination of both genes prevented fungal sporulation (Ijaz et al. Unpubl. data) (Fig.1). This kind of very strong resistant infection type has not been witnessed before in faba bean.

b. Using multilines and crop mixtures

In comparison to domestic crops, wild species are more heterogeneous for resistance genes and thus avoid disease epidemics (Bevan et al. 1993; Okamura and Ouchi 2007). The most appropriate breeding strategies that utilize crop diversity are multiline development (Browning and Frey 1969) and cultivar mixtures (Wolfe and Barrett 1980). A multiline is a composite of agronomically similar genotypes that differ for a few traits, such as resistance to different pathogen races (Jensen 1952). Wolfe (1985) defined a cultivar mixture as a “mixture of cultivars that vary for many characters including disease resistance, but have sufficient similarities to be grown together”.

Despite the documented benefits of growing cultivar mixtures over pure stands (Wolfe and Finckh 1997), their commercial impact has been low. Lack of uniformity can influence seed quality and certification, leading to legislative constraints on seed production and

distribution (Wolfe et al. 1992). Farmers often oppose growing mixtures because of uneven crop appearance. There are examples of large scale utilization of mixtures in barley (Wolfe and McDermott 1994), wheat (Garrett and Mundt 1999; Wolfe 1985) and rice (Skamnioti and Gurr 2009; Zhu et al. 2000), but their recent use for disease management is limited. Resistance to rust has never been deployed in mixtures or multilines in faba bean, largely because resistance is poorly characterized in this crop, but there is no reason to believe that multilines or mixtures will not work, as our understanding of genetic diversity for resistance increases. It is important to initiate investigation by testing a mixture of rust-susceptible and -resistant faba bean genotypes for disease suppression and yield stability.

Using systemically acquired resistance (SAR)

Systemic acquired resistance (SAR) is a “whole plant” resistance response that is usually triggered under biotic and abiotic stresses (Walters and Murray 1992). SAR is accompanied by the up-regulation of large numbers of so-called pathogenesis-related (PR) genes (Melchers and Stuiver 2000) that have an important role in defence against fungal infection and genes encoding fungitoxic or fungistatic proteins provide resistance against invading fungi (Cornelissen and Melchers 1993). Murray and Walters (1992) reported that inoculation of lower leaves of faba bean with rust spores increased resistance in the upper leaves and enhanced their photosynthesis, as shown by the significantly increased accumulation of ^{14}C -labelled assimilate after $^{14}\text{CO}_2$ was fed to the upper leaves. However, the virulence of the inoculated rust pathotype was unknown. Systemic resistance in faba bean can be induced by exposure of plants to certain chemicals. Application of salicylic acid or benzo-(1,2,3)-thiadiazole-7-carbothionic acid (benzothiadiazole, BTH) increased the resistance of faba bean leaves to rust, *Ascochyta* blight (*Ascochyta fabae* Speg.) and broomrape (*Orobancha crenata* Forsk.) (Sillero et al. 2012). Similarly, foliar application of BTH and DL- β -aminobutyric acid (BABA) to pea significantly reduced pea rust (*Uromyces pisi*) infection levels locally and systemically (Barilli et al. 2010). Cheng et al. (2012) characterized the resistance mechanism in faba bean against *Puccinia striiformis* f. sp. *tritici* (*Pst*) as non-host. Although no symptoms were visible on inoculated leaves, microscopy showed that the fungus successfully located the stomata, but failed to develop mature haustoria. Transcriptomic expression revealed the up-regulation of seven defence-related genes during *Pst* infection (Table 2). In the light of these observations, and the strong similarities in mechanisms of resistance to adapted and unadapted pathogens, SAR in faba bean may be

worth studying in greater detail. When the PR genes are validated, they can be used against rust.

Molecular breeding technologies

Early genetic studies in crops were based on morphological, cytological and biochemical evaluation (Xu 2010). A sequence of DNA markers, from Restriction Fragment Length Polymorphism (RFLP), through Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), and Simple Sequence Repeats (SSR) to Single Nucleotide Polymorphisms (SNP) has allowed increasingly high resolution and closer linkage to the traits of interest, which can assist gene tracking during selection and breeding (Collard et al. 2005). Markers that are closely linked (less than 5 cM of genetic distance) with the gene of interest reduce the probability of recombination and can provide reliable selection in crop improvement (Jiang 2013). Use of flanking markers increases the probability of success because of the low likelihood of double crossovers in a short length of chromosome. MAS is a practical and effective way to pyramid rust resistance genes if the genes and closely linked markers are available (Servin et al. 2004) and is already in use for other traits such as vicine-convicine content (Khazaei et al. 2015). Association mapping and linkage mapping are powerful tools used for identifying QTLs and genes responsible for a particular phenotype (Collard and Mackill 2008). To date, only one of the successful mapping efforts in faba bean is relevant to rust resistance (Avila et al. 2003), where bulk segregant analysis (BSA) was done to tag RAPD markers linked to the hypersensitive response in faba bean line 2N-52 against rust race 1 in Spain. Three RAPD markers, namely OPD13₇₃₆, OPL18₁₀₃₂ and OPI20₉₀₀, were mapped in coupling phase to the resistance gene *Uvf-1*. Two additional markers were linked to the gene in repulsion at distances of 9.9 cM (OPP02₁₁₇₁) and 11.5 cM (OPR07₉₃₀). The line 2N-52 used in this study was resistant to seven other rust isolates (2, 4, 5, 8, 9, 10 and 13) (Emeran et al. 2001), but this broad resistance was not solely attributable to gene *Uvf-1*.

Recent advances in the next generation sequencing (NGS) technologies provide a platform to sequence a large volume of individuals efficiently and cost-effectively (Morozova and Marra 2008). This advancement opens a new era in agricultural research including the identification of key genes correlated with breeding traits through SNPs and Genome-Wide Association Analysis Studies (GWAS) (Lam et al. 2010). Another utility of sequence data is to identify flanking SSR regions to develop locus-specific markers for downstream genotyping. In faba bean, the first major contribution was the release of 5000 Expressed

Sequence Tags (EST) from the early to mid-developing embryo of broad bean cultivar ‘Windsor’ (Ray and Georges 2010). Yang et al. (2012) constructed a library of 125,559 putative SSR sequences and determined a set of 28,503 primers, of which 15,094 primers showed polymorphism among 32 diverse faba bean accessions. The observed heterozygosity ranged from 0.0908 to 0.840. The validation of these markers based on Nei’s genetic distance showed their high quality and effectiveness. Kaur et al. (2014b) undertook sequencing of the transcriptome to underpin SSR to significantly increase the volume of transcriptome data in mixed genotypes. In addition, Kaur et al. (2014a) evaluated inter- and intra-population diversity in 45 diverse accessions of faba bean by using a set of 768 genome-wide SNP markers, of which 657 were successfully amplified. The genetic structure of these accessions was analysed through a neighbour-joining (NJ) dendrogram that categorized them into two major groups and several sub-groups. Group I was comprised of accessions from Ecuador, Australia and ICARDA, Group IIA consisted of African and European materials, whereas Groups IIB and IIC were predominantly of Australian origin. These results confirmed that a high level of heterozygosity is present, presumably due to the partial allogamous nature of the species. Subsequently, transcriptome studies were focused on multiple inbred lines (Ocaña et al. 2015) as well as multiple tissues (Ray et al. 2015). The deepest transcriptome coverage yet produced through Illumina sequencing library of mixed tissues from cultivar ‘Fiord’ (Arun-Chinnappa and McCurdy 2015; Zhang et al. 2015), which was the first released cultivar in Australia and was highly susceptible to all known variants of rust. These available transcript datasets can be used to compare with transcriptomes of resistant lines to identify transcripts conferring resistance. Webb et al. (2016) developed and validated 845 SNPs by using NGS. Each SNP was assigned by BLAST analysis to a single *Medicago* orthologue and a set of 757 markers was used to genotype six mapping populations. The resulting linkage maps were merged into a single consensus map of 687 SNPs on six linkage groups, each corresponding to one chromosome. Substantial collinearity was found with the fully sequenced genome of barrel medic (*Medicago truncatula* L.), allowing relatively easy prediction of gene contents. Recently, using Illumina paired end sequencing, genomic reads were assembled *sub de novo* in to contigs of size 50-5000 bp, out of which, over 85% sequences did not align with known gene, representing ~10% known repetitive genetic elements (Cooper et al. 2017).

At present, significant transcriptome datasets are available in the arsenal of the faba bean breeders. These recent advances in faba bean genomics provide background for successful

manipulation of genomics and proteomics tools. When rust response phenotypes are linked with genotypes at a high level of resolution, resistance breeding will accelerate through the high selection efficiency that can then be used in pyramiding genes and constructing multilines or mixtures.

Conclusion

Knowledge is growing about host-pathogen interactions, the diversity of host resistance genes and their genetic bases, and pathogen variation of faba bean rust. The resistance mechanisms in faba bean germplasm are well established as either slow-rusting or hypersensitive responses. Closely linked molecular markers for both types of resistance are needed for the successful development of durable cultivars. Although the progenitor of faba bean has not yet been identified, enough variation for rust resistance seems to be present in the cultivated species. Although *Uromyces* is the second most important rust genus after *Puccinia*, its physiology and genetics have received little attention.

Gene pyramiding is a possible avenue for deploying resistance genes, but needs to be underpinned by an extensive search for new rust resistance sources and the concurrent identification of genes and linked molecular markers. With current mapping efforts on known seedling genes for rust resistance, marker-assisted gene pyramiding can be considered as a viable strategy in resistance breeding. However, continuous monitoring will be needed to keep an eye on the evolution of virulent pathotypes against these effective resistance genes. As the virulence evolves, the pyramid will have to be reconstructed with new resistance sources to avoid epidemics. Although mixtures or multilines can provide some measure of resistance, their successful commercialisation requires that they should provide more benefits to farmers than provided by conventional cultivars. Until now, whole plant resistance or antifungal gene strategy is unable to provide a strong successful reference in applied breeding, but with growing understanding, it has potential in the future. Moreover, genes of systemically acquired resistance against a non-host pathogen have been well studied in faba bean, and have potential for use against host pathogens such as rust.

Genomics and biotechnology have been little used in breeding faba bean for rust resistance because of it being a minor crop with a large genome size, and the resistance is complex in nature. Genetic and genomic knowledge has advanced far enough now to assist in the identification of genes and their successful deployment in this crop, and we can expect soon to have plenty of genome-wide SNP variants for rust resistance across diverse backgrounds.

416 In parallel, these datasets can also be used for gene mapping, gene cloning and successful
417 gene transformation. We can anticipate that plant breeders will be able to use genomics to
418 identify the resistance with high accuracy, to ensure a rust-free crop in the future.

419

420

References:

- ABSTAT (2015) Australian Bean Export, Pulse Australia Limited. Australia
- Adhikari KN, Zhang P, Sadeque A, Hoxha S, Trethowan R (2016a) Single independent genes confer resistance to faba bean rust (*Uromyces viciae-fabae*) in the current Australian cultivar Doza and a central European line Ac1655 Crop and Pasture Science 67:649-654 doi:<http://dx.doi.org/10.1071/CP15333>
- Adhikari KN, Zhang P, Sadeque A, Hoxha S, Trethowan R (2016b) Single independent genes confer resistance to faba bean rust (*Uromyces viciae-fabae*) in the current Australian cultivar Doza and a central European line Ac1655 Crop and Pasture Science
- Arbaoui M, Link W, Satovic Z, Torres A-M (2008) Quantitative trait loci of frost tolerance and physiologically related trait in faba bean (*Vicia faba* L.) Euphytica 164:93-104
- Arun-Chinnappa KS, McCurdy DW (2015) *De novo* assembly of a genome-wide transcriptome map of *Vicia faba* (L.) for transfer cell research Frontiers in plant science 6:217
- Ausemus E (1943) Breeding for disease resistance in wheat, oats, barley and flax The Botanical Review 9:207-260
- Avila C, Sillero J, Rubiales D, Moreno M, Torres A (2003) Identification of RAPD markers linked to the *Uvf-1* gene conferring hypersensitive resistance against rust (*Uromyces viciae-fabae*) in *Vicia faba* L Theoretical and Applied Genetics 107:353-358
- Avila CM, Satovic Z, Sillero JC, Rubiales D, Moreno MT, Torres AM (2004) Isolate and organ-specific QTLs for ascochyta blight resistance in faba bean (*Vicia faba* L) Theoretical and applied genetics 108:1071-1078
- Bailey-Serres J, Mittler R (2006) The roles of reactive oxygen species in plant cells. Am Soc Plant Biol,
- Barilli E, Sillero JC, Rubiales D (2010) Induction of systemic acquired resistance in pea against rust (*Uromyces pisi*) by exogenous application of biotic and abiotic inducers Journal of phytopathology 158:30-34
- Barrett J The gene-for-gene hypothesis: parable or paradigm. In: LINN. SOC. SYMP. SER. 1985., 1985.
- Barrus MF (1911) Variation of varieties of beans in their susceptibility to anthracnose Phytopathology 1:190-195
- Bevan J, Clarke D, Crute I (1993) Resistance to Erysiphe fischeri in two populations of Senecio vulgaris Plant Pathology 42:636-646
- Biffen RH (1905) Mendel's laws of inheritance and wheat breeding The Journal of Agricultural Science 1:4-48
- Bolwell GP, Wojtaszek P (1997) Mechanisms for the generation of reactive oxygen species in plant defence—a broad perspective Physiological and Molecular Plant Pathology 51:347-366
- Bond DA, Jellis GJ, Rowland GG, Le Guen J, Robertson LD, Khalil SA, Li-Juan L (1994) Present status and future strategy in breeding faba beans (*Vicia faba* L.) for resistance to biotic and abiotic stresses. In: Muehlbauer FJ, Kaiser WJ (eds) Expanding the Production and Use of Cool Season Food Legumes, vol 19. Current Plant Science and Biotechnology in Agriculture. Springer Netherlands, pp 592-616. doi:10.1007/978-94-011-0798-3_36
- Bowles DJ (1990) Defense-related proteins in higher plants Annual review of biochemistry 59:873-907
- Brogue K et al. (1991) Transgenic plants with enhanced resistance to the fungal pathogen *Rhizoctonia solani* Science 254:1194-1197

- Browning JA, Frey KJ (1969) Multiline cultivars as a means of disease control Annual Review of Phytopathology 7:355-382
- Cantu D et al. (2013) Genome analyses of the wheat yellow (stripe) rust pathogen *Puccinia striiformis* f. sp. *tritici* reveal polymorphic and haustorial expressed secreted proteins as candidate effectors BMC genomics 14:270
- Castro AJ et al. (2003) Mapping and pyramiding of qualitative and quantitative resistance to stripe rust in barley Theoretical and Applied Genetics 107:922-930
- Chand R, Srivastava C, Singh B, Sarode S (2006) Identification and characterization of slow rusting components in pea (*Pisum sativum* L.) Genetic Resources and Crop Evolution 53:219-224
- Cheng Y et al. (2012) Characterization of non-host resistance in broad bean to the wheat stripe rust pathogen BMC plant biology 12:1
- Chhuneja P et al. (2011) Marker-assisted pyramiding of leaf rust resistance genes *Lr24* and *Lr28* in wheat (*Triticum aestivum*) Indian Journal of Agricultural Sciences 81:214
- Collard B, Jahufer M, Brouwer J, Pang E (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts Euphytica 142:169-196
- Collard BC, Mackill DJ (2008) Marker-assisted selection: an approach for precision plant breeding in the twenty-first century Philosophical Transactions of the Royal Society B: Biological Sciences 363:557-572
- Conner R, Bernier C (1982a) Host range of *Uromyces viciae-fabae* Phytopathology 72:687-689
- Conner R, Bernier C (1982b) Inheritance of rust resistance in inbred lines of *Vicia faba* Phytopathology 72:1555-1557
- Conner R, Bernier C (1982c) Race identification in *Uromyces viciae-fabae* Canadian Journal of Plant Pathology 4:157-160
- Conner R, Bernier C (1982d) Slow rusting resistance in *Vicia faba* Canadian Journal of Plant Pathology 4:263-265
- Cooper JW, Wilson MH, Derks MF, Smit S, Kunert KJ, Cullis C, Foyer CH (2017) Enhancing faba bean (*Vicia faba* L.) genome resources Journal of Experimental Botany 68:1941-1953
- Cornelissen BJ, Melchers LS (1993) Strategies for Control of Fungal Diseases with Transgenic Plants Plant physiology 101:709
- Cox T, Raupp W, Gill B (1994) Leaf rust-resistance genes *Lr41*, *Lr42*, and *Lr43* transferred from *Triticum tauschii* to common wheat Crop science 34:339-343
- Crute IR (1992) The role of resistance breeding in the integrated control of downy mildew (*Bremia lactucae*) in protected lettuce Euphytica 63:95-102
- Cruz-Izquierdo S et al. (2012) Comparative genomics to bridge *Vicia faba* with model and closely-related legume species: stability of QTLs for flowering and yield-related traits Theoretical and applied genetics 125:1767-1782
- Cubero JI (2011) The faba beans: a historic perspective Grain legumes 56:5-7
- Cummins GB (1978) Rust fungi on legumes and composites in North America. Univ. Arizona Press.,
- de Wit PJ, Mehrabi R, van den Burg HA, Stergiopoulos I (2009) Fungal effector proteins: past, present and future Molecular Plant Pathology 10:735-747
- Díaz-Ruiz R, Satovic Z, Avila C, Alfaro C, Gutierrez M, Torres A, Román B (2009a) Confirmation of QTLs controlling *Ascochyta fabae* resistance in different generations of faba bean (*Vicia faba* L.) Crop and Pasture Science 60:353-361
- Díaz-Ruiz R et al. (2009b) Mapping of quantitative trait loci controlling *Orobanche foetida* Poir. resistance in faba bean (*Vicia faba* L.) African journal of biotechnology 8

- Duc G et al. (2010) Diversity maintenance and use of *Vicia faba* L. genetic resources Field Crops Research 115:270-278
- Duplessis S et al. (2011) Obligate biotrophy features unraveled by the genomic analysis of rust fungi Proceedings of the National Academy of Sciences 108:9166-9171
- Ellwood SR et al. (2008) Construction of a comparative genetic map in faba bean (*Vicia faba* L.); conservation of genome structure with *Lens culinaris* BMC genomics 9:380
- Emeran A, Roman B, Sillero JC, Satovic Z, Rubiales D (2008) Genetic variation among and within *Uromyces* species infecting legumes Journal of phytopathology 156:419-424
- Emeran A, Sillero J, Niks R, Rubiales D (2005) Infection structures of host-specialized isolates of *Uromyces viciae-fabae* and of other species of *Uromyces* infecting leguminous crops Plant disease 89:17-22
- Emeran A, Sillero J, Rubiales D Physiological specialisation of *Uromyces viciae-fabae*. In: Proceedings, 4th European Conference on Grain Legumes, Cracow, Poland, 2001.
- Emeran AA, Sillero JC, Fernández-Aparicio M, Rubiales D (2011) Chemical control of faba bean rust (*Uromyces viciae-fabae*) Crop Protection 30:907-912
- Epple P, Apel K, Bohlmann H (1997) Overexpression of an endogenous thionin enhances resistance of Arabidopsis against *Fusarium oxysporum* The Plant Cell Online 9:509-520
- Eurostat (2017) Crop statistics. European Commission. <http://ec.europa.eu/eurostat/data/database>.
- FAOSTAT (2017) World statistics on faba bean. Food and Agriculture Organization of the United Nations. Available at <http://faostat.fao.org/>, Rome, Italy.
- Flavell R, Bennett M, Smith J, Smith D (1974) Genome size and the proportion of repeated nucleotide sequence DNA in plants Biochemical genetics 12:257-269
- Flor H (1956) The complementary genic systems in flax and flax rust Advances in genetics 8:29-54
- Friedrich L et al. (1996) A benzothiadiazole derivative induces systemic acquired resistance in tobacco The Plant Journal 10:61-70
- Garcia A, Calvo ÉS, de Souza Kiihl RA, Harada A, Hiromoto DM, Vieira LGE (2008) Molecular mapping of soybean rust (*Phakopsora pachyrhizi*) resistance genes: discovery of a novel locus and alleles Theoretical and Applied Genetics 117:545-553
- Garrett K, Mundt C (1999) Epidemiology in mixed host populations Phytopathology 89:984-990
- Goudriaan J, Zadoks J (1995) Global climate change: modelling the potential responses of agro-ecosystems with special reference to crop protection Environmental Pollution 87:215-224
- Grisson R, Grezes-Besset B, Schneider M, Lucante N, Olsen L, Leguay J-J, Toppan A (1996) Field tolerance to fungal pathogens of *Brassica napus* constitutively expressing a chimeric chitinase gene Nature Biotechnology 14:643-646
- Groth J, Davis D, Zeyen R, Mogen B (1983) Ranking of partial resistance to common rust (*Puccinia sorghi* Schr.) in 30 sweet corn (*Zea mays*) hybrids Crop Protection 2:219-223
- Gupta R, Singh SK (2010) Slow rusting in lentil infected with *Uromyces fabae* Indian Phytopathology 63:350-351
- Gutiérrez N et al. (2013) QTLs for *Orobanche* spp. resistance in faba bean: identification and validation across different environments Molecular breeding 32:909-922
- Hanafy MS, El-Banna A, Schumacher HM, Jacobsen H-J, Hassan FS (2013) Enhanced tolerance to drought and salt stresses in transgenic faba bean (*Vicia faba* L.) plants

by heterologous expression of the PR10a gene from potato Plant cell reports 32:663-674

Heath MC, Valent B, Howard RJ, Chumley FG (1990) Interactions of two strains of *Magnaporthe grisea* with rice, goosegrass, and weeping lovegrass Canadian Journal of Botany 68:1627-1637

Herath I, Stoddard F, Marshall D (2001) Evaluating faba beans for rust resistance using detached leaves Euphytica 117:47-57

Herath IHMB (2000) Genetic studies on faba bean rust and rust resistance in faba bean. M.Sc. Thesis, The University of Sydney, Australia.

Hooker A (1969) Widely based resistance to rust in corn Iowa State Univ Ext Spec Rep

Huang L, Brooks SA, Li W, Fellers JP, Trick HN, Gill BS (2003) Map-based cloning of leaf rust resistance gene Lr21 from the large and polyploid genome of bread wheat Genetics 164:655-664

Ijaz U, Adhikari K (2016a) Understanding the genetics of seedling rust resistance in faba bean. Pulse Breeding Australia, PBA - Summer Newsletter

Ijaz U, Adhikari K, Bansal U, Bariana H, Trethowan R P179– S18 Development of differential sets to identify *Uromyces viciaefabae* pathotypes in Australia. In: Second International Legume Society Conference, Troia, Portugal, 2016a. p 328

Ijaz U, Adhikari KN (2016b) Understanding genetics of seedling rust resistance in faba bean., Summer -16 edn., Australia

Ijaz U, Adhikari KN, Bariana HS, Bansal UK Identifying pathogen variability and virulence of *Uromyces viciae-fabae* on common cultivated legumes in Australia. In: ICLGG 2017 - Book of abstracts, Siofok, Hungary, 2017. p 70

Ijaz U, Kedar A, Urmil B, Harbans B, Richard T Virulence status of *Uromyces viciae-fabae* pathotypes in Australia. In: Australian Pulse Conference, Tamworth, Australia., 2016b. p 66

Jensen ES, Peoples MB, Hauggaard-Nielsen H (2010) Faba bean in cropping systems Field Crops Research 115:203-216

Jensen NF (1952) Intra-varietal diversification in oat breeding Agron J 44:30-34

Jiang G-L (2013) Molecular markers and marker-assisted breeding in plants

Johnson R, Law C (1973) Cytogenetic studies in the resistance of the wheat variety Bersée to *Puccinia striiformis* Cereal Rusts Bull 1:38-43

Jongedijk E et al. (1995) Synergistic activity of chitinases and β -1, 3-glucanases enhances fungal resistance in transgenic tomato plants Euphytica 85:173-180

Joseph M, Hering T (1997) Effects of environment on spore germination and infection by broad bean rust (*Uromyces viciae-fabae*) The Journal of Agricultural Science 128:73-78

Kaur S, Cogan NO, Forster JW, Paull JG (2014a) Assessment of genetic diversity in faba bean based on single nucleotide polymorphism Diversity 6:88-101

Kaur S, Kimber RB, Cogan NO, Materne M, Forster JW, Paull JG (2014b) SNP discovery and high-density genetic mapping in faba bean (*Vicia faba* L.) permits identification of QTLs for ascochyta blight resistance Plant Science 217:47-55

Keller B, Feuillet C, Messmer M (2000) Genetics of disease resistance. In: Mechanisms of resistance to plant diseases. Springer, pp 101-160

Khazaei H, O'Sullivan DM, Sillanpää MJ, Stoddard FL (2014) Use of synteny to identify candidate genes underlying QTL controlling stomatal traits in faba bean (*Vicia faba* L.) Theoretical and Applied Genetics 127:2371-2385

Khazaei, H., O'Sullivan, D.M., Jones, H., Pitts, N., Sillanpää, M.J., Pärssinen, P., Manninen, O. & **Stoddard, F.L.** 2015. Flanking SNP markers for vicine-convicine concentration in faba bean (*Vicia faba* L.). *Molecular Breeding* **35**: 38.

- Kolmer J (1992) Enhanced leaf rust resistance in wheat conditioned by resistance gene pairs with Lr13 *Euphytica* 61:123-130
- Kolmer J, Broers B (1995) Characterization of Lr34, a major gene conferring nonhypersensitive resistance to wheat leaf rust *Plant disease* 79
- Kuc J (1995) Phytoalexins, stress metabolism, and disease resistance in plants *Annual review of phytopathology* 33:275-297
- Kuźniak E, Urbanek H (2000) The involvement of hydrogen peroxide in plant responses to stresses *Acta Physiologiae Plantarum* 22:195-203 doi:10.1007/s11738-000-0076-4
- Lam H-M et al. (2010) Resequencing of 31 wild and cultivated soybean genomes identifies patterns of genetic diversity and selection *Nature genetics* 42:1053-1059
- Liang J, Wu Y, Rosenberger C, Hakimi S, Castro S, Berg J (1998) AFP genes confer disease resistance to transgenic potato and wheat plants. In: Abstract no. L-49. In 5th International workshop on pathogenesis-related proteins in plants, 1998.
- Liang X (1986) Faba bean diseases in China *FABIS Newsletter, Faba Bean Information Service, ICARDA*:49-51
- Lin W, Anuratha C, Datta K, Potrykus I, Muthukrishnan S, Datta SK (1995) Genetic engineering of rice for resistance to sheath blight *Nature Biotechnology* 13:686-691
- Link T, Seibel C, Voegelé RT (2014) Early insights into the genome sequence of *Uromyces fabae* *Frontiers in plant science* 5: 587
- Liu J et al. (2000) Molecular marker-facilitated pyramiding of different genes for powdery mildew resistance in wheat *Plant Breeding* 119:21-24
- Luo Y, Tebeest D, Teng P, Fabellar N (1995) Simulation studies on risk analysis of rice leaf blast epidemics associated with global climate change in several Asian countries *Journal of Biogeography*:673-678
- Ma Y et al. (2013) Genetic linkage map of Chinese native variety faba bean (*Vicia faba* L.) based on simple sequence repeat markers *Plant Breeding* 132:397-400
- Maalouf F, Ahmed S, Kabakebbi M, Khalil S, Abang M, Kabbabeh M, Street S (2010) Sources of multiple resistances for key foliar diseases of faba bean. Paper presented at the 5th International Food Legumes Research Conference and 7th European Conference on Grain Legumes., Antalya, Turkey.,
- Maier W, Begerow D, Weiß M, Oberwinkler F (2003) Phylogeny of the rust fungi: an approach using nuclear large subunit ribosomal DNA sequences *Canadian Journal of Botany* 81:12-23
- Marcellos H, Moore K, Nikandrow A (1995) Influence of foliar-applied fungicides on seed yield of faba bean (*Vicia faba* L.) in northern New South Wales *Australian Journal of Experimental Agriculture* 35:97-102 doi:<http://dx.doi.org/10.1071/EA9950097>
- Maxted N, Callimassia M, Bennett M (1991) Cytotaxonomic studies of Eastern mediterranean *Vicia* species (Leguminosae) *Plant Systematics and Evolution* 177:221-234
- McIntosh RA, Welling CR, Park RF (1995) Wheat rusts: an atlas of resistance genes. CSIRO, Melbourne, Australia
- McRostie G (1919) Inheritance of anthracnose resistance as indicated by a cross between a resistant and a susceptible bean *Phytopathology* 9:141-148
- Mehdy MC (1994) Active oxygen species in plant defense against pathogens *Plant physiology* 105:467
- Melchers LS, Stuijver MH (2000) Novel genes for disease-resistance breeding *Current opinion in plant biology* 3:147-152
- Mendgen K (1997) The uredinales. Springer,

- Mendgen K, Deising H (1993) Infection structures of fungal plant pathogens—a cytological and physiological evaluation *New Phytologist* 124:193-213
- Miklas PN, Kelly JD, Beebe SE, Blair MW (2006) Common bean breeding for resistance against biotic and abiotic stresses: from classical to MAS breeding *Euphytica* 147:105-131
- Morozova O, Marra MA (2008) Applications of next-generation sequencing technologies in functional genomics *Genomics* 92:255-264
- Moullet O, Fossati D, Mascher F, Guadagnolo R, Schori A Use of marker-assisted selection (MAS) for pyramiding two leaf rust resistance genes, (Lr9 and Lr24) in wheat. In: Int. Conf. “Conventional and Molecular Breeding of Field and Vegetable Crops, 2009. pp 24-27
- Moullet O, Fossati D, Mascher F, Guadagnolo R, Schori A (2010) Use of marker-assisted selection (MAS) for pyramiding leaf rust resistance genes (Lr9, Lr24, Lr22a) in wheat *Tagungsband der 60 Jahrestagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs* 24-26 November 2009, Raumberg-Gumpenstein:143
- Mundt C (1991) Probability of mutation of multiple virulence and durability of resistance gene pyramids: Further comments *Phytopathology* 81:240-242
- Murray D, Walters D (1992) Increased photosynthesis and resistance to rust infection in upper, uninfected leaves of rusted broad bean (*Vicia faba* L.) *New Phytologist* 120:235-242
- Negussie T, Pretorius Z, Bender C (2005) Components of rust resistance in lentil *Euphytica* 142:55-64
- Niks RE, Rubiales D (2002) Potentially durable resistance mechanisms in plants to specialised fungal pathogens *Euphytica* 124:201-216
- Ocaña S, Seoane P, Bautista R, Palomino C, Claros GM, Torres AM, Madrid E (2015) Large-scale transcriptome analysis in faba bean (*Vicia faba* L.) under *Ascochyta fabae* infection *PloS one* 10:e0135143
- Okamura H, Ouchi M (2007) resistance to *erysiphe fischeri* in two populations of *senecio vulgaris* *Plant Pathology*
- Parlevliet J (1983) Can horizontal resistance be recognized in the presence of vertical resistance in plants exposed to a mixture of pathogen races?[Examples with the rice blast fungus, *Pyricularia oryzae*, *Oryza sativa*] *Phytopathology (USA)*
- Parlevliet JE (1997) Durable resistance. In: Hartleb HH, Heitefuss R, Hoppe HH (eds) *Resistance of crop plants against fungi*. Gustav Fischer, Jena, Germany, pp 238-253
- Patto MV, Torres A, Koblikova A, Macas J, Cubero J (1999) Development of a genetic composite map of *Vicia faba* using F2 populations derived from trisomic plants *Theoretical and applied genetics* 98:736-743
- Pink DA (2002) Strategies using genes for non-durable disease resistance *Euphytica* 124:227-236
- Polignano G, Casulli F, Ugenti P (1990) Resistance to *Uromyces viciae-fabae* in Ethiopian and Afghan faba bean entries *Phytopathologia Mediterranea* 29:135-142
- Pulse-Australia (2017) Faba bean and broad bean - Variety selection <http://www.pulseauscomau/growing-pulses/bmp/faba-and-broad-bean#variety-selection>
- Ramsay G, Van de Ven W, Waugh R, Griffiths D, Powell W, AEP Mapping quantitative trait loci in faba beans. In: *Proceedings of the 2nd European conference on grain legumes*, Copenhagen, Denmark, 1995. pp 444-445
- Rashid K, Bernier C (1984) Evaluation of resistance in *Vicia faba* to two isolates of the rust fungus *Uromyces viciae-fabae* from Manitoba [Accessions and cultivars, comparisons] *Plant Diseases*

- Rashid K, Bernier C (1986a) The genetics of resistance in *Vicia faba* to two races of *Uromyces viciae-fabae* from Manitoba Canadian Journal of Plant Pathology 8:317-322
- Rashid K, Bernier C (1986b) Selection for slow rusting in faba bean (*Vicia faba* L.) to *Uromyces viciae-fabae* Crop Protection 5:218-224
- Rashid K, Bernier C, Conner R (1991) Genetics of resistance in faba bean inbred lines to five isolates of *Ascochyta fabae* Canadian Journal of Plant Pathology 13:218-225
- Rashid KY, Bernier CC (1991) The effect of rust on yield of faba bean cultivars and slow-rusting populations Canadian Journal of Plant Science 71:967-972 doi:10.4141/cjps91-139
- Ray H, Bock C, Georges F (2015) Faba Bean: Transcriptome Analysis from Etiolated Seedling and Developing Seed Coat of Key Cultivars for Synthesis of Proanthocyanidins, Phytate, Raffinose Family Oligosaccharides, Vicine, and Convicine The Plant Genome 8
- Ray H, Georges F (2010) A genomic approach to nutritional, pharmacological and genetic issues of faba bean (*Vicia faba*): prospects for genetic modifications GM crops 1:99-106
- Rojas Molina M, Rubiales D, Sillero J Pathogenic specialization of *Uromyces viciae-fabae* in Spain and Portugal. In: Congresos y Jornadas. Serie Cultivos Herbáceos-Junta de Andalucía (España), 2006.
- Román B, Satovic Z, Avila CM, Rubiales D, Moreno MT, Torres AM (2003) Locating genes associated with *Ascochyta fabae* resistance in *Vicia faba* Crop and Pasture Science 54:85-90
- Román B, Satovic Z, Pozarkova D, Macas J, Dolezel J, Cubero JI, Torres AM (2004) Development of a composite map in *Vicia faba*, breeding applications and future prospects Theoretical and applied genetics 108:1079-1088
- Román B, Torres AM, Rubiales D, Cubero JI, Satovic Z (2002) Mapping of quantitative trait loci controlling broomrape (*Orobanche crenata* Forsk.) resistance in faba bean (*Vicia faba* L.) Genome 45:1057-1063
- Rose I, van Leur J Breeding faba beans (*Vicia faba*) for adaptation to short season environments in Australia. In: Mercer CF (ed) Breeding for Success: Diversity in Action. Proceedings of the 13th Australasian Plant Breeding Conference. Christchurch, New Zealand 18-21 April 2006. pp 34-38
2006. pp 34-38.
- Ross AF (1961) Systemic acquired resistance induced by localized virus infections in plants Virology 14:340-358
- Rubiales D, Rojas-Molina MM, Sillero JC (2013) Identification of pre- and posthaustorial resistance to rust (*Uromyces viciae-fabae*) in lentil (*Lens culinaris*) germplasm Plant Breeding 132:676-680 doi:10.1111/pbr.12096
- Rubiales D, Sillero J (2003) *Uromyces viciae-fabae* haustorium formation in susceptible and resistant faba bean lines European journal of plant pathology 109:71-73
- Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner H-Y, Hunt MD (1996) Systemic acquired resistance The Plant Cell 8:1809
- Satovic Z et al. (2013) A reference consensus genetic map for molecular markers and economically important traits in faba bean (*Vicia faba* L.) BMC genomics 14:932
- Satovic Z, Torres AM, Cubero JI (1996) Genetic mapping of new morphological, isozyme and RAPD markers in *Vicia faba* L. using trisomics Theoretical and applied genetics 93:1130-1138

- Servin B, Martin OC, Mézard M (2004) Toward a theory of marker-assisted gene pyramiding *Genetics* 168:513-523
- Sillero J, Moreno M, Rubiales D (2000) Characterization of new sources of resistance to *Uromyces viciae-fabae* in a germplasm collection of *Vicia faba* *Plant Pathology* 49:389-395
- Sillero J, Rubiales D (2002) Histological characterization of resistance to *Uromyces viciae-fabae* in faba bean *Phytopathology* 92:294-299
- Sillero JC, Rojas-Molina MM, Ávila CM, Rubiales D (2012) Induction of systemic acquired resistance against rust, ascochyta blight and broomrape in faba bean by exogenous application of salicylic acid and benzothiadiazole *Crop Protection* 34:65-69
- Sillero JC, Rojas-Molina MM, Emeran AA, Rubiales D (2011) Rust resistance in faba beans. *Grain legumes* 56:27-28
- Sillero JC, Villegas-Fernández AM, Thomas J, Rojas-Molina MM, Emeran AA, Fernández-Aparicio M, Rubiales D (2010) Faba bean breeding for disease resistance *Field Crops Research* 115:297-307 doi:<http://dx.doi.org/10.1016/j.fcr.2009.09.012>
- Silva DC et al. (2008) Molecular mapping of two loci that confer resistance to Asian rust in soybean *Theoretical and Applied Genetics* 117:57-63
- Singh A, Singh V, Chand R, Kushwaha C, Srivastava C (2015) Evaluation of slow rusting components in pea *Journal of Plant Pathology* 97:87-92
- Singh R, Huerta-Espino J, Rajaram S, Barna B, Kiraly Z (2000) Achieving near-immunity to leaf and stripe rusts in wheat by combining slow rusting resistance genes *Acta phytopathologica et entomologica hungarica* 35:133-139
- Skamnioti P, Gurr SJ (2009) Against the grain: safeguarding rice from rice blast disease *Trends in biotechnology* 27:141-150
- Stakman E (1918) Can biologic forms of stemrust on wheat change rapidly enough to interfere with *Journal of Agricultural Research* 14:111
- Stakman EC, Levine MN (1922) The determination of biological forms of *Puccinia graminis* on *Triticum* spp. *Min Agric Exp Stn Bull*:8-10
- Steadman J, Pastor-Corrales M, Beaver J (2002) An overview of the 3rd bean rust and 2nd bean common bacterial blight international workshops, March 4-8, 2002, Pietermaritzburg, South Africa *ANNUAL REPORT-BEAN IMPROVEMENT COOPERATIVE* 45:120-124
- Stoddard F, Herath I (2001) Genetic analysis of partial rust resistance in faba beans *Crop and Pasture Science* 52:73-84
- Stoddard F, Nicholas A, Rubiales D, Thomas J, Villegas-Fernández A (2010) Integrated pest management in faba bean *Field Crops Research* 115:308-318
- Stoddard FL, Bond DA (1987) The pollination requirements of the faba bean *Bee World* 68:144-152
- Stuthman D, Leonard K, Miller-Garvin J (2007) Breeding crops for durable resistance to disease *Advances in agronomy* 95:319-367
- Sudhesh S, Braich S, Adhikari K, Kaur S Development of gene based linkage maps and identification of quantitative trait loci for rust (*Uromyces viciae-fabae*) resistance in faba bean (*Vicia faba* L.). In: Australian Pulse Conference., Tamworth, Australia., 12-14 September, 2016. 2016. p 101
- Tanno K-i, Willcox G (2006) The origins of cultivation of *Cicer arietinum* L. and *Vicia faba* L.: early finds from Tell el-Kerkh, north-west Syria, late 10th millennium BP *Vegetation History and Archaeobotany* 15:197-204
- Tavares S et al. (2014) Genome size analyses of Pucciniales reveal the largest fungal genomes *Frontiers in plant science* 5

- Tiwari R, Kumar Y, Saharan M, Mishra B (2008) Marker assisted approach for incorporating durable rust resistance in popular Indian wheat cultivars
- Torres A, Weeden N, Martin A (1993) Linkage among isozyme, RFLP and RAPD markers in *Vicia faba* Theoretical and Applied Genetics 85:937-945
- Torres AM et al. (2006) Faba bean breeding for resistance against biotic stresses: towards application of marker technology Euphytica 147:67-80
- Van de Ven W, Waugh R, Duncan N, Ramsay G, Dow N, Powell W (1991) Development of a genetic linkage map in *Vicia faba* using molecular and biochemical techniques Aspects of applied biology
- van de Wouw M, Enneking D, Robertson LD, Maxted N (2001) Vetches (*Vicia* L.). In: Plant Genetic Resources of Legumes in the Mediterranean. Springer, pp 134-158
- Van der Merwe M, Ericson L, Walker J, Thrall PH, Burdon JJ (2007) Evolutionary relationships among species of *Puccinia* and *Uromyces* (Pucciniaceae, Uredinales) inferred from partial protein coding gene phylogenies Mycological research 111:163-175
- van Harten AM (1998) Mutation breeding: theory and practical applications. Cambridge University Press,
- Vance C, Kirk T, Sherwood R (1980) Lignification as a mechanism of disease resistance Annual review of phytopathology 18:259-288
- Voegelé RT (2006) *Uromyces fabae*: development, metabolism, and interactions with its host *Vicia faba* FEMS microbiology letters 259:165-173
- Walters D, Murray D (1992) Induction of systemic resistance to rust in *Vicia faba* by phosphate and EDTA: effects of calcium Plant Pathology 41:444-448
- Waterhouse W Some observations on cereal rust problems in Australia. In: Proceedings of the Linnean Society of New South Wales, 1936. pp v-xxxviii
- Waterhouse W Australian rust studies. IX. Physiologic race determinations and surveys of cereal rusts. In: Proc. Linn. Soc. NSW, 1952. pp 209-258
- Watson IA (1958) The present status of breeding disease resistant wheats in Australia. NSW Department of Agriculture,
- Webb A et al. (2016) A SNP-based consensus genetic map for synteny-based trait targeting in faba bean (*Vicia faba* L.) Plant biotechnology journal 14:177-185
- Wei G, Kloepper JW, Tuzun S (1991) Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by select strains of plant growth-promoting rhizobacteria Phytopathology 81:1508-1512
- Wilcoxson R (1981) Genetics of slow rusting in cereals Phytopathology 71:989-993
- Wolfe M (1985) The current status and prospects of multiline cultivars and variety mixtures for disease resistance Annual Review of Phytopathology 23:251-273
- Wolfe M, Brändle U, Koller B, Limpert E, McDermott J, Müller K, Schaffner D (1992) Barley mildew in Europe: population biology and host resistance Euphytica 63:125-139
- Wolfe M, Finckh M (1997) Diversity of host resistance within the crop: effects on host, pathogen and disease Resistance of crop plants against fungi
- Wolfe M, McDermott J (1994) Population genetics of plant pathogen interactions: the example of the Erysiphe graminis-Hordeum vulgare pathosystem Annual Review of Phytopathology 32:89-113
- Wolfe MS, Barrett J (1980) Can we lead the pathogen astray? Plant Disease 64
- Wu G, Shortt BJ, Lawrence EB, Levine EB, Fitzsimmons KC, Shah DM (1995) Disease resistance conferred by expression of a gene encoding H₂O₂-generating glucose oxidase in transgenic potato plants The Plant Cell Online 7:1357-1368
- Xu Y (2010) Molecular plant breeding. CABI,

866 Yang T et al. (2012) High-throughput novel microsatellite marker of faba bean via next
867 generation sequencing BMC genomics 13:602
868 Zhang H-M, Wheeler S, Xia X, Radchuk R, Weber H, Offler CE, Patrick JW (2015)
869 Differential transcriptional networks associated with key phases of ingrowth wall
870 construction in trans-differentiating epidermal transfer cells of Vicia faba cotyledons
871 BMC plant biology 15:1
872 Zheng W et al. (2013) High genome heterozygosity and endemic genetic recombination in
873 the wheat stripe rust fungus Nature communications 4
874 Zhu Y et al. (2000) Genetic diversity and disease control in rice Nature 406:718-722
875

876 Table 1 Reported rust resistant germplasm resources in Australia.

Australian accession No.	Donor Accession No.	Source	Country	Reference
AC341	ILB53	ICARDA	England	Rashid and Bernier (1984)
AC408	ILB159	ICARDA	Greece	Rashid and Bernier (1984)
AC466	BPL7	ICARDA	Jordan	Rashid and Bernier (1984)
AC472	ILB226	ICARDA	Turkey	Bond et al. (1994)
AC479	BPL8	ICARDA	Jordan	Rashid and Bernier (1984)
AC481	BPL 260	ICARDA	Greece	Bond et al. (1994)
AC547	ILB318	ICARDA	England	Bond et al. (1994)
AC628	BPL8	ICARDA	Jordan	Rashid and Bernier (1984)
AC630	ILB403	ICARDA	Tunisia	Bond et al. (1994)
AC631	BPL260	ICARDA	Greece	Rashid and Bernier (1984)
AC638	ILB411	ICARDA	England	Bond et al. (1994)
AC647	ILB420	ICARDA	Iran	Rashid and Bernier (1984)
AC658	ILB431	ICARDA	Algeria	Rashid and Bernier (1984)
AC832	BPL 309	ICARDA	Turkey	Bond et al. (1994)
AC848	BPL406	ICARDA	Spain	Bond et al. (1994)
AC849	BPL417	ICARDA	Spain	Bond et al. (1994)
AC858	BPL484	ICARDA	Uruguay	Bond et al. (1994)
AC859	BPL427	ICARDA	Spain	Bond et al. (1994)
AC860	BPL533	ICARDA	USA	Bond et al. (1994)
AC983	BPL490	ICARDA	Spain	Bond et al. (1994)
AC984	BPL524	ICARDA	Japan	Bond et al. (1994)
AC999	BPL539	ICARDA	China	Bond et al. (1994)
AC1044	BPL261	ICARDA	Greece	Bond et al. (1994)
AC1045	BPL263	ICARDA	Greece	Bond et al. (1994)
AC1269	BPL 710	ICARDA	Ecuador	Bond et al. (1994)
AC1272	BPL 1179	ICARDA	Ecuador	Bond et al. (1994)

AC1443	BPL 627	ICARDA	Spain	Bond et al. (1994)
AC1652	2N-34	Spanish germplasm	Spain	Bernier and Conner (1982)
AC1653	2N-52	Spanish germplasm	Spain	Bernier and Conner (1982)
AC1654	VF-176	Spanish germplasm	Spain	Sillero et al. (2000)
AC1655	V-300	Spanish germplasm	Spain	Sillero et al. (2000)
AC1656	V-1271	Spanish germplasm	Spain	Sillero et al. (2000)
AC1657	V-1272	Spanish germplasm	Spain	Sillero et al. (2000)
AC1658	V-1273	Spanish germplasm	Spain	Sillero et al. (2000)

878 Table 2 Reported defence-related candidate genes in faba bean (Cheng et al. 2012)

Gene	Description	Related species	Arabidopsis homolog (TAIR BLAST)
Basal resistance			
<i>PR1</i>	Pathogenesis-related gene 1	<i>Pisum sativum</i>	AT2G14610.1
<i>PR2</i>	Beta-1,3 endoglucanase	<i>Pisum sativum</i>	AT3G57260.1
<i>PR5</i>	Thaumatococcus-like protein	<i>Medicago truncatula</i>	-
<i>PR10</i>	Pathogenesis-related gene 10	<i>Pisum sativum</i>	No sequence
Oxidative stress responses			
<i>SOS</i>	Superoxide dismutase	<i>Pisum sativum</i>	AT5G18100.1
<i>CAT</i>	Catalase	<i>Pisum sativum</i>	AT1G20620.1
Papillary callose formation			
<i>GSL5</i>	Glucan synthase-like 5	<i>Medicago truncatula</i>	AT4G03550.1

879

880

881

882 Fig. 1 Characterisation of three seedling resistance types in faba bean germplasm.

883 AC1227#14908 gave higher reaction type (complementary gene), Ac1655 produced small

884 pustules with necrotic lesions, Doza#12034 is showing aggressive chlorosis leading to leaf

885 death, Ac1655 × Doza#12034 hybrid (F₁) completely inhibited fungal sporulation and Fiord

886 showed large sporulating pustules without necrosis or chlorosis against isolate Uvf-8.