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Ijaz, Usman

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1 **Rust resistance in faba bean (*Vicia faba* L.): Status and** 2 **strategies for improvement**

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

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

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

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

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

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10

11 **Abstract:**

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

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

27 **Introduction:**

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

30 Middle East and China. It is a diploid ($2x=12$) species having one of the largest described
31 genomes (13 Gb) among crop legumes, with at least 85% repetitive DNA (Flavell et al.
32 1974). It is believed to have been domesticated in the Fertile Crescent during the Neolithic
33 era 9000-10000 BP (Tanno and Willcox 2006). Its secondary centres of diversity include the
34 Nile Valley, Central and Eastern Asia, and South America (Duc et al. 2010). Faba bean is
35 the sixth most important grain legume with 4.3 million tonnes annual production worldwide
36 in 2014 (FAOSTAT 2017). Australia is the fourth largest producer (after China, Ethiopia
37 and the UK) and the largest exporter globally (Eurostat 2016; FAOSTAT 2017), with nearly
38 85% of its total produce going to Egypt (70%) and Saudi Arabia (15%) (ABSTAT 2015).
39 Similar to other legumes, faba bean is a good source of renewable nitrogen through
40 biological nitrogen fixation (Jensen et al. 2010). It has a mixed breeding system, averaging
41 one-third outcrossing and two-thirds inbreeding, which adds complexity to populations in
42 the field, their genetic and phenotypic analysis, and practical plant breeding (Stoddard and
43 Bond 1987).

44 Faba bean area decreased worldwide from 1962 to 1991, and since then has more or less
45 stabilised at 2.1-2.7 million hectares, mostly across Asia and the Mediterranean region
46 FAOSTAT 2017). Apart from the commodity price, one of the reasons for decline is the
47 prevalence of pathogenic fungi and the absence of disease-resistant cultivars (Sillero et al.
48 2010). Measures to control diseases include chemicals, biological control agents and crop
49 management or cultural practices (Sillero et al. 2010). Systemic fungicides provide rapid
50 and effective control, but they are expensive and can be detrimental to the environment
51 (Emeran et al. 2011). Pathogens can also develop insensitivity following prolonged exposure
52 to chemicals (Crute 1992). Biological control agents for pathogenic fungi are not yet
53 available commercially (Stoddard et al. 2010). Therefore, breeding cultivars for resistance
54 is widely considered the most efficient, cost effective and environmentally safe method of
55 disease control (Sillero et al. 2010).

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

63 and southern Australia, causing up to 20% yield reduction (Sillero et al. 2011), although
64 early rust epidemics can cause up to 70% loss of the standing crop (Liang 1986; Rashid and
65 Bernier 1991). Losses up to 30% were estimated in northern New South Wales where the
66 disease occurs in the seedling stage. Rust resistance in general restricts fungal proliferation
67 before sporulation. In contrast to other pathosystems such as soybean-rust (*Phakopsora*
68 *pachyrhizi* Syd.), for which complete monogenic resistance exists (Silva et al. 2008),
69 resistance in faba bean is generally described as incomplete (Garcia et al. 2008; Miklas et
70 al. 2006). Incomplete resistance, a type of quantitative resistance either expressed as
71 hypersensitive type (Adhikari et al. 2016a; Sillero et al. 2000) with visible necrosis/chlorosis
72 or non-hypersensitive type (Herath et al. 2001; Polignano et al. 1990; Rashid and Bernier
73 1984; 1991; Sillero et al. 2000) typically fits into the definition of partial resistance or field
74 resistance (Parlevliet 1983). The hypersensitive reaction type, causing significant restriction
75 on fungal growth and reduction of an infection type, is still considered as incomplete in faba
76 bean (Sillero et al. 2010).

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

80 **A brief history of rust resistance**

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

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

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

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

105 **Genetic diversity of rust resistance in faba bean**

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

114 No wild species is sufficiently closely related to faba bean that fertile hybrids have been
115 derived, so genetic diversity is restricted to the primary gene pool of the species itself
116 (Cubero 2011). Accessions from the Mediterranean region are considered to have the best
117 potential to provide genetic variation for rust resistance (Maalouf et al. 2010). Genotypes
118 showing significant rust resistance with late necrosis originated from Central Europe (V-
119 300, V-313, V-1271, V-1272), East Africa (2N-34) and Spain (V-1335) (Sillero et al. 2000).
120 Most of these reported rust resistance germplasm lines were already imported in Australia
121 and named with Australian accession numbers located at Australian Grain Genebank,
122 Horsham, Victoria (Table 1). However, interaction of these resistance genes with prevailing
123 faba bean rust pathotypes is unknown. Interspecific sources of resistance cannot be
124 transferred to faba bean by conventional breeding methods, and the practical significance of
125 induced mutation is relatively low due to low success rates (van Harten 1998). The species
126 is generally intractable to tissue culture, but successful transformation by *Agrobacterium* has

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

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

139 The national faba bean breeding programme in Australia aims to provide cultivars for
140 southern regions (South Australia and Victoria) and the northern plains (New South Wales
141 and Queensland). Rust is a major disease in the northern plains, so breeding for its resistance
142 is one of the main objectives. A great deal of genetic diversity of rust resistance at the
143 seedling stage has been found in the Australian collection of faba bean germplasm (Fig. 1).
144 The first moderately rust resistant cultivar, Doza, was released for cultivation in the northern
145 plains in 2008, followed by PBA Warda and PBA Nasma during 2012 and 2016, respectively
146 (Pulse Australia 2017). Three rust resistance genes were reported in segregating populations
147 from crosses of exotic (Ac1227#14908 and Ac1655) accessions and local genotype
148 Doza#12034 (Ijaz and Adhikari 2016a) (Fig. 1). Two of these genes (from Ac1655 and
149 Doza#12034) were independently dominant, along with possible additive response
150 (Adhikari et al. 2016a), and were mapped onto two different chromosomes (Sudhesh et al.
151 2016). However, the resistance in Ac1227#14908 was governed by complimentary gene
152 action (Ijaz and Adhikari 2016b).

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

158 **Slow rusting: an insight into faba bean breeding**

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

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

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

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

186 **Understanding the pathogen: *Uromyces viciae-fabae***

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

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

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

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

223 Link et al. (2014) provided an early insight into the genomic architecture of *U. viciae-fabae*.
224 Sequencing was performed by using HiSeq2000 with a 500 nt library. Three different
225 estimates of genome size were made: with 15-mer analysis, genome size was estimated at
226 329 Mb, whereas 17-mer analysis predicted a genome size of 422 Mb and filtered assembly
227 estimated 216 Mb. Flow cytometry on the same isolate generated a size of 379 Mb (Tavares
228 et al. 2014). Thus, the estimate of the actual genome size is in the range of 330 and 422 Mb,
229 making it about 4 times the size of *P. graminis* f. sp. *tritici*, indicating a high significance of
230 transposable elements (TE) (Duplessis et al. 2011). Link et al. (2014) identified numerous
231 families of secreted proteins and candidate effectors, and reported 62 genes encoding
232 proteins secreted from haustoria that have presumed roles in infection and specificity of *U.*
233 *viciae-fabae*.

234 **Deployment of resistance genes with major effects**

235 Disease resistance is generally inherited as a dominant factor in natural populations (Barrett
236 1985), as natural selection acts on it favourably. Resistance based on major hypersensitive
237 genes is usually considered non-durable (Stuthman et al. 2007) according to the gene-for-
238 gene hypothesis. Hypersensitivity has been observed among faba bean genotypes, where
239 necrosis appears during the later stages of rust proliferation and restricts fungal growth by
240 controlled cell death (Sillero et al. 2000; Torres et al. 2006). Hypersensitivity is easily
241 detectable with specific pathotypes (Emeran et al. 2005; Rojas Molina et al. 2006). Avila et
242 al. (2003) and Adhikari et al. (2016b) showed that this type of hypersensitive response in
243 faba bean is under the control of major genes. Nevertheless, the longevity of major genes in
244 faba bean is less understood than in wheat as there is limited knowledge on pathogen
245 variability. If these resistance genes of major effect can be deployed in combination through
246 gene pyramiding, multiline cultivars or gene mixtures, the durability of resistance can be
247 increased.

248 a. Pyramiding resistance genes

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

254 complex pyramid unless virulent pathotypes or specific DNA markers are available (Pink
255 2002).

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

274 b. Using multilines and crop mixtures

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

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

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

295 **Using systemically acquired resistance (SAR)**

296 Systemic acquired resistance (SAR) is a “whole plant” resistance response that is usually
297 triggered under biotic and abiotic stresses (Walters and Murray 1992). SAR is accompanied
298 by the up-regulation of large numbers of so-called pathogenesis-related (PR) genes
299 (Melchers and Stuiver 2000) that have an important role in defence against fungal infection
300 and genes encoding fungitoxic or fungistatic proteins provide resistance against invading
301 fungi (Cornelissen and Melchers 1993). Murray and Walters (1992) reported that inoculation
302 of lower leaves of faba bean with rust spores increased resistance in the upper leaves and
303 enhanced their photosynthesis, as shown by the significantly increased accumulation of ¹⁴C-
304 labelled assimilate after ¹⁴CO₂ was fed to the upper leaves. However, the virulence of the
305 inoculated rust pathotype was unknown. Systemic resistance in faba bean can be induced by
306 exposure of plants to certain chemicals. Application of salicylic acid or benzo-(1,2,3)-
307 thiadiazole-7-carbothionic acid (benzothiadiazole, BTH) increased the resistance of faba
308 bean leaves to rust, *Ascochyta* blight (*Ascochyta fabae* Speg.) and broomrape (*Orobancha*
309 *crenata* Forsk.) (Sillero et al. 2012). Similarly, foliar application of BTH and DL-β-
310 aminobutyric acid (BABA) to pea significantly reduced pea rust (*Uromyces pisi*) infection
311 levels locally and systemically (Barilli et al. 2010). Cheng et al. (2012) characterized the
312 resistance mechanism in faba bean against *Puccinia striiformis* f. sp. *tritici* (*Pst*) as non-host.
313 Although no symptoms were visible on inoculated leaves, microscopy showed that the
314 fungus successfully located the stomata, but failed to develop mature haustoria.
315 Transcriptomic expression revealed the up-regulation of seven defence-related genes during
316 *Pst* infection (Table 2). In the light of these observations, and the strong similarities in
317 mechanisms of resistance to adapted and unadapted pathogens, SAR in faba bean may be

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

320 **Molecular breeding technologies**

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

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

351 Sequence Tags (EST) from the early to mid-developing embryo of broad bean cultivar
352 'Windsor' (Ray and Georges 2010). Yang et al. (2012) constructed a library of 125,559
353 putative SSR sequences and determined a set of 28,503 primers, of which 15,094 primers
354 showed polymorphism among 32 diverse faba bean accessions. The observed heterozygosity
355 ranged from 0.0908 to 0.840. The validation of these markers based on Nei's genetic
356 distance showed their high quality and effectiveness. Kaur et al. (2014b) undertook 454
357 sequencing of the transcriptome to underpin SSR to significantly increase the volume of
358 transcriptome data in mixed genotypes. In addition, Kaur et al. (2014a) evaluated inter- and
359 intra-population diversity in 45 diverse accessions of faba bean by using a set of 768
360 genome-wide SNP markers, of which 657 were successfully amplified. The genetic structure
361 of these accessions was analysed through a neighbour-joining (NJ) dendrogram that
362 categorized them into two major groups and several sub-groups. Group I was comprised of
363 accessions from Ecuador, Australia and ICARDA, Group IIA consisted of African and
364 European materials, whereas Groups IIB and IIC were predominantly of Australian origin.
365 These results confirmed that a high level of heterozygosity is present, presumably due to the
366 partial allogamous nature of the species. Subsequently, transcriptome studies were focused
367 on multiple inbred lines (Ocaña et al. 2015) as well as multiple tissues (Ray et al. 2015). The
368 deepest transcriptome coverage yet produced through Illumina sequencing library of mixed
369 tissues from cultivar 'Fiord' (Arun-Chinnappa and McCurdy 2015; Zhang et al. 2015),
370 which was the first released cultivar in Australia and was highly susceptible to all known
371 variants of rust. These available transcript datasets can be used to compare with
372 transcriptomes of resistant lines to identify transcripts conferring resistance. Webb et al.
373 (2016) developed and validated 845 SNPs by using NGS. Each SNP was assigned by
374 BLAST analysis to a single *Medicago* orthologue and a set of 757 markers was used to
375 genotype six mapping populations. The resulting linkage maps were merged into a single
376 consensus map of 687 SNPs on six linkage groups, each corresponding to one chromosome.
377 Substantial collinearity was found with the fully sequenced genome of barrel medic
378 (*Medicago truncatula* L.), allowing relatively easy prediction of gene contents. Recently,
379 using Illumina paired end sequencing, genomic reads were assembled *sub de novo* in to
380 contigs of size 50-5000 bp, out of which, over 85% sequences did not align with known
381 gene, representing ~10% known repetitive genetic elements (Cooper et al. 2017).

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

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

388 **Conclusion**

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

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

411 Genomics and biotechnology have been little used in breeding faba bean for rust resistance
412 because of it being a minor crop with a large genome size, and the resistance is complex in
413 nature. Genetic and genomic knowledge has advanced far enough now to assist in the
414 identification of genes and their successful deployment in this crop, and we can expect soon
415 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.

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876 Table 1 Reported rust resistant germplasm resources in Australia.

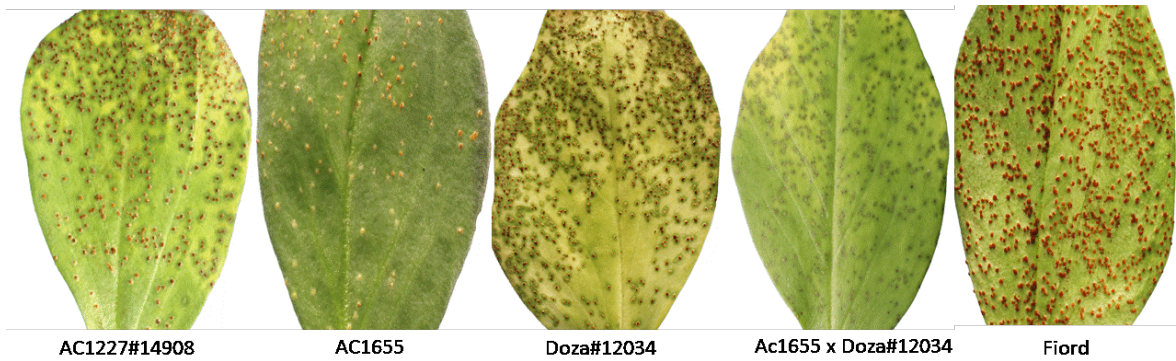
Australian accession No.	Donor Accession No.	Source	Country	Reference
AC341	ILB53	ICARDA	England	Rashid and Bernier (1984)
AC408	ILB159	ICARDA	Greece	Rashid and Bernier (1984)
AC466	BPL7	ICARDA	Jordan	Rashid and Bernier (1984)
AC472	ILB226	ICARDA	Turkey	Bond et al. (1994)
AC479	BPL8	ICARDA	Jordan	Rashid 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



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.