

Response of *Vicia* Species to *Ascochyta fabae* and *Uromyces viciae-fabae*

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Abstract

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A collection of 267 accessions belonging to 61 *Vicia* species other than *V. faba* was screened under growth chamber conditions for response to *Ascochyta fabae* and *Uromyces viciae-fabae*, causal agents of ascochyta blight and faba bean rust, respectively. High resistance to both diseases was very frequently detected in *Vicia* spp. in contrast to the high susceptibility previously reported in most *V. faba* accessions. Most of the *Vicia* spp. accessions studied here were very resistant or even immune to *A. fabae* with only one per cent of the accessions allowing development of small lesions bearing pycnidia. High resistance or immunity to *U. viciae-fabae* was also frequently found in the collection, with only ten per cent of the accessions showing compatible interaction although with reduced disease severity. These findings reinforce the specificity of *A. fabae* and *U. viciae-fabae* and clarify the potential role of cultivated and wild *Vicia* spp. in the epidemiology of these faba bean diseases.

Keywords: ascochyta blight; faba bean rust; germplasm collection; *Vicia* spp.

Legumes supply an important added value for agriculture by fixing atmospheric nitrogen in symbiosis with soil bacteria. Several species of the genus *Vicia* are cultivated both as grain and forage crops. Faba bean (*Vicia faba* L.) is the most important one, but other species such as *V. sativa* L., *V. villosa* Roth, *V. ervilia* Willd., *V. narbonensis* L., *V. benghalensis* L. and *V. articulata* Hornem are also cultivated for green manure, hay, fodder or grain legume for animal feed (HANELT & METTIN 1989).

Exhaustive studies have been performed concerning the morphological, physiological, qualitative or agronomical behaviour of *Vicia* species (BERGER *et al.* 2002a, b; VAN DE WOUW *et al.* 2003; MIRALI *et al.* 2007; DE LA ROSA & GONZÁLEZ 2010). However, limited research has been published about foliar diseases of these species, and there is insufficient information on their potential as a green bridge for dissemination of faba bean diseases.

Ascochyta blight, caused by the fungus *Ascochyta fabae* Speg. (teleomorph *Didymella fabae* G.J. Jellis & Punith.), is a common disease on faba bean and is distributed worldwide (TIVOLI *et al.* 2006),

particularly under wet and cool weather conditions. Conidia are dispersed by rain splash to a short distance and are responsible for new disease cycles during the growing season. Damage caused by *A. fabae* includes reduction in photosynthetic area, lodging following stem girdling, and pod and seed abortion. Seed infection also occurs, and is the main source of inoculum; although the fungus is borne in the testa only (PRITCHARD *et al.* 1989), the disease can be transmitted from seed to seedling. Disease control through crop rotation, clean seed and chemical treatment is not completely effective (STODDARD *et al.* 2010) and the development of resistant cultivars is widely recognized as the most efficient method of control. Several sources of resistance to *A. fabae* have been identified and used in faba bean breeding programs (SILLERO *et al.* 2001, 2010; RUBIALES *et al.* 2012) although none resulted in complete resistance. Other *Ascochyta* species infect a number of legumes, including *Ascochyta rabiei* (Pass.) Lab., *A. pisi* Lib., *A. lentis* Vassiljevsky, and *A. viciae-villosae* Ondrej, pathogens of chickpea (*Cicer arietinum* L.), pea (*Pisum sativum* L.), lentil (*Lens culinaris* Medik.),

and hairy vetch (*V. villosa* Roth), respectively. Although these species are host specific (KAISER *et al.* 1997; HERNANDEZ-BELLO *et al.* 2006) under certain conditions, they can infect other species. Little is known of the degree of susceptibility of *Vicia* spp. to *A. fabae*. Several *Ascochyta* isolates have been sampled from various *Vicia* species (PEEVER *et al.* 2007), but little is understood about their host specialization and all of them had identical cultural morphology, being indistinguishable from *A. lentis*, *A. fabae* and *A. pisi* isolates.

Uromyces viciae-fabae (Pers.) J. Schröt. is the causal agent of the faba bean rust. It is a disease present in almost each area of the world where faba bean is grown. Several methods of rust control have been developed, but the use of genetic resistance is a more desirable and efficient strategy for the rust control (SILLERO *et al.* 2010). *U. viciae-fabae* also infects species of the genera *Vicia*, *Pisum*, *Lathyrus* and *Lens* (HIRATSUKA 1933; KAPOORIA & SINHA 1971; CONNER & BERNIER 1982; KUSHWAHA *et al.* 2006). *U. viciae-fabae sensu lato* is in fact a species complex with host specialized isolates (CUMMINS 1987; EMERAN *et al.* 2005)

The main objective of this research was to study the reactions to *A. fabae* and *U. viciae-fabae* isolates, collected on faba bean, in a *Vicia* species germplasm collection. This would allow us to compare the host range of both faba bean diseases in order to better understand their epidemiology and the potential of other *Vicia* spp. to act as a green bridge or source of inoculum of these faba bean diseases. In addition, this would allow the detection of new sources and different mechanisms of resistance available to a potential transfer to the cultivated species faba bean once the actual barriers for crossability have been overcome by new methodologies.

MATERIAL AND METHODS

A collection of *Vicia* accessions belonging to 61 species (Table 1) of the genus *Vicia* other than *V. faba* were screened under growth chamber conditions. These accessions, of different origins from all around the world, were kindly provided by IFAPA (Córdoba, Spain), USDA-ARS (Pullman, USA), IPK (Gatersleben, Germany) and CGN (Wageningen, the Netherlands) germplasm banks. A total of 267 accessions were screened for response to *A. fabae* and 244 accessions for response to *U. viciae-fabae*. Three consecutive replications were carried out, each with five plants per accession. Seeds were sown in 200 ml plastic pots

Table 1. Response to *Ascochyta fabae* and to *Uromyces viciae-fabae* in a germplasm collection of *Vicia* species; ascochyta disease development was assessed using the 0–9 scale described by SILLERO *et al.* (2001); rust infection type was recorded using the 0–4 scale defined by STAKMAN *et al.* (1962)

Species	No. of accession with scale values											
	<i>Ascochyta fabae</i>					<i>Uromyces viciae-fabae</i>						
	0	1	2	3	4	≥5	0	1	2	3	4	
<i>V. americana</i>	1						1					
<i>V. amoena</i>	1							2				
<i>V. amphicarpa</i>	2						1					
<i>V. anatolica</i>			1					1				
<i>V. angustifolia</i>	5		2				3	4				
<i>V. articulata</i>	9						3	7				
<i>V. benghalensis</i>	2	2	6				7	1	1			1
<i>V. biennis</i>			1	1							2	
<i>V. bithynica</i>	1		3				1					2
<i>V. cassubica</i>	3						1	2				
<i>V. cordata</i>	1		4				1	4				
<i>V. cracca</i>	2		5				1	4		1		
<i>V. cuspidata</i>	4		1									
<i>V. dalmatica</i>	2						2					
<i>V. disperma</i>	1		1						1	1		
<i>V. dumeforum</i>	2							1				
<i>V. elegans</i>	1							1				
<i>V. ervilia</i>	5		2	1			5	3	1			
<i>V. fulgens</i>			1				1					
<i>V. galilaea</i>	2		1								1	1
<i>V. graminea</i>	1						1					
<i>V. grandiflora</i>	4	1	1	1			2	5				
<i>V. hirsuta</i>	7	1	2				8	2				
<i>V. hybrida</i>	2	2	2				7					
<i>V. hyrcanica</i>	1		1				1		1			
<i>V. incana</i>	1											1
<i>V. incisaeformis</i>		1	1					1	1			
<i>V. johannis</i>	2	2	1							3	1	2
<i>V. lathyroides</i>	6		2				4		1			1
<i>V. lutea</i>	4	1	3				5	1			1	
<i>V. macrocarpa</i>		1						1				
<i>V. megalotropis</i>	2						2					
<i>V. melanops</i>	1	1	1					2				1
<i>V. meyeri</i>	2						1	1				

Table 1 to be continued

Species	No. of accession with scale values													
	<i>Ascochyta fabae</i>						<i>Uromyces viciae-fabae</i>							
	0	1	2	3	4	≥5	0	;	1	2	3	4	5	6
<i>V. michauxii</i>	3						3							
<i>V. monantha</i>	2	2	1				2	1					2	
<i>V. narbonensis</i>	11	4	3					1	6	5	2			
<i>V. neglecta</i>			1				1							
<i>V. ochroleuca</i>			1									1		
<i>V. onbrychioides</i>	1		1				1						1	
<i>V. orobus</i>	3						2	1						
<i>V. palaestina</i>	2		1				1					1		
<i>V. pannonica</i>	7		6				5	1				1		
<i>V. parviflora</i>	1						1	1						
<i>V. peregrina</i>	9						1	4				1		
<i>V. pilosa</i>	1							1						
<i>V. pisiformis</i>	1		1										1	
<i>V. pubescens</i>	1						1							
<i>V. pyrenaica</i>			1					2						
<i>V. sativa</i>	4	8	19	1	2		19	11	1				1	
<i>V. segetalis</i>	1						1							
<i>V. semiglabra</i>				1				1						
<i>V. sepium</i>			2				1	1						
<i>V. sericocarpa</i>	1						1							
<i>V. serratifolia</i>	1				1			1	1					
<i>V. striata</i>			1				1							
<i>V. sylvatica</i>	1						1							
<i>V. tennifolia</i>	4						1	1					1	
<i>V. tetrasperma</i>	3	1	1				5							
<i>V. vicioides</i>	1							1						
<i>V. villosa</i>	4	1	11				14	2	1					
Total <i>Vicia</i> spp.	139	28	92	5	3		119	74	15	10	12	14		
Faba bean ^a		2	2	6	268	474				7	1	640		

^aReaction of a screened *Vicia faba* collection to ascochyta blight (SILLERO *et al.* 2001) and to faba bean rust (SILLERO *et al.* 2000)

filled with a 1:1 mixture of sand and peat, one pot per genotype. For ascochyta blight testing, 14-days-old plants were inoculated by atomising with a conidial suspension (1×10^6 conidia/ml) of the *A. fabae* isolate Af-CO-01. This isolate was derived from a population collected at Córdoba, Spain that is highly aggressive to faba bean. The conidial suspension was prepared with

tap water, to which Tween-20 was added (0.03%, v/v) (SILLERO *et al.* 2001).

For rust testing, 14-days-old plants were inoculated by dusting freshly collected faba bean rust spores (0.2 mg spores/plant) diluted in pure talc (1:10) of the single spore isolate 96-Cord-2 of *U. viciae-fabae*. This isolate was derived from a rust population collected on faba bean at Córdoba, Spain that is highly aggressive to faba bean (SILLERO *et al.* 2000).

Inoculated plants were incubated for 24 h in an incubation chamber at 20°C in complete darkness at 100% relative humidity, and subsequently maintained in a growth chamber at 20°C with a 14 h light:10 h dark photoperiod and light intensity of 145 $\mu\text{mol}/\text{m}^2/\text{s}$ at the canopy level. These conditions were maintained for both pathogens.

Resistance to ascochyta blight was assessed 14 days after inoculation using the 0–9 disease scoring recommended by ICARDA (BERNIER *et al.* 1984), with slight modifications (SILLERO *et al.* 2001). This scale is a combination of lesion type, lesion frequency and extent of damage, where 0 = no lesions; 1 = very small non-sporulating flecks < 0.5 mm in diameter on leaves, no symptoms on stems; 2 = some lesions of 0.5–2 mm in diameter on leaves, without pycnidia; 3 = few small (2–3 mm in diameter), discrete, dark lesions on leaves without pycnidia, no symptoms on stems; 4 = slightly larger discrete lesions (4–5 mm in diameter) on leaves with pycnidia, no symptoms on stems; 5 = circular lesions, sometimes coalescing, with pycnidia on leaves and little defoliation, no symptoms on stems; 6 = circular lesions, frequently coalescing, with pycnidia on leaves, some defoliation and symptoms on stems appearing; 7 = many large, coalescing, irregular lesions with many pycnidia on leaves, pods and stems, with defoliation; 9 = extensive, large, coalescing, sporulating lesions on leaves, stems and pods, severe defoliation, stem constriction and girdling and many dead plants. Values lower than 4 were considered indicative of resistance. Each seedling was scored separately and the individual values averaged.

Response to rust was also assessed 14 days after inoculation, using the 0–4 infection type (IT) scale described by STAKMAN *et al.* (1962), where 0 = no symptoms, ; = necrotic flecks, 1 = minute pustules barely sporulating, 2 = necrotic halo surrounding small pustules, 3 = chlorotic halo, 4 = well-formed pustules with no associated chlorosis or necrosis. Each seedling was scored separately and the individual IT scores averaged. Lines were considered resistant to *U. viciae-fabae* infection when they displayed IT scores lower than 3.

Results were compared with previously reported responses of *V. faba* germplasm to the same *A. fabae* (SILLERO *et al.* 2001) and *U. viciae-fabae* (SILLERO *et al.* 2000) isolates.

RESULTS

Most of the *Vicia* spp. accessions were resistant to the *A. fabae* and *U. viciae-fabae* isolates used. Disease scores to *A. fabae* were lower than 4 in 264 accessions out of the 267 studied, including 139 accessions that were immune, as they did not show any symptoms (disease score = 0) (Table 1; Figure 1). Moderate susceptibility was shown in only three accessions and these exhibited discrete lesions bearing pycnidia (disease score = 4). This contrasts with the high susceptibility reported in *V. faba*, where only 10 accessions out of 752 showed resistance preventing pycnidia development (disease score 1–3) (SILLERO *et al.* 2001). High resistance to *U. viciae-fabae* was also very common in accessions of the *Vicia* species (Table 1, Figure 2). About half of the *Vicia* spp. accessions (119 accessions out of 244) displayed immunity to faba bean rust (IT = 0), and 99 accessions displayed a resistant response (IT from ; to 2) with the presence of host cell necrosis associated with the infection sites. Only ten per cent of the entries (26 accessions) displayed a compatible interaction with sporulating pustules (IT \geq 3), although disease severity was reduced. These results contrasted with those previously found in the *V. faba* germplasm (SILLERO *et al.* 2000) where most of the faba bean

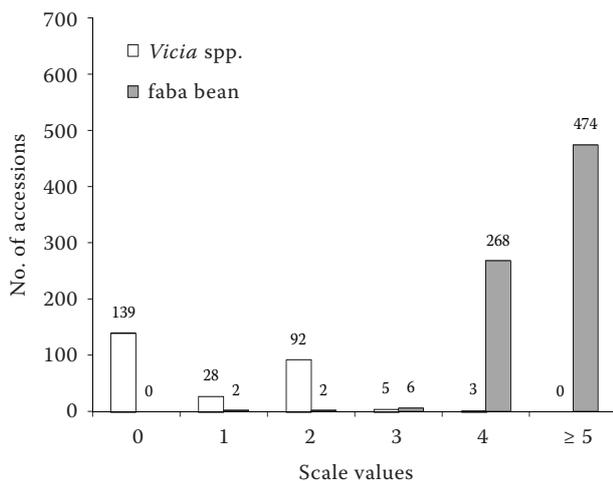


Figure 1. Reaction to the infection with *Ascochyta fabae* in a *Vicia* spp. collection (other than *V. faba*) and in a *V. faba* collection previously studied (SILLERO *et al.* 2001); disease development was assessed using the 0–9 scale described by SILLERO *et al.* (2001)

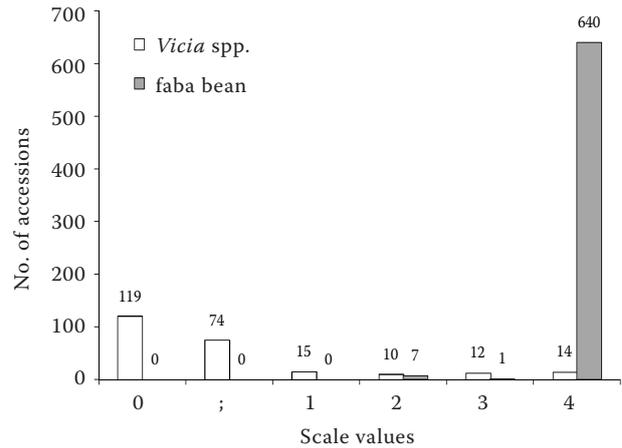


Figure 2. Reaction to the infection with *Uromyces viciae-fabae* in a *Vicia* spp. collection (other than *V. faba*) and in a *V. faba* collection previously studied (SILLERO *et al.* 2000); disease development was assessed using the infection type defined by STAKMAN *et al.* (1962)

accessions were susceptible to faba bean rust and only 1% of the accessions studied displaying IT < 3.

Common vetch (*V. sativa*) is one of the most important species cultivated in the genus *Vicia*. In the present study most of the *V. sativa* accessions studied were resistant to both faba bean diseases, although two accessions displayed susceptibility to ascochyta blight and one accession to faba bean rust. Another important species is hairy vetch (*V. villosa*) and all accessions of this species studied were resistant to both pathogens. Many other vetch species of the genus *Vicia*, such as *V. ervilia*, *V. narbonensis*, *V. benghalensis* and *V. articulata*, are cultivated although to a lesser extent. Of these species, all studied accessions were resistant to the *A. fabae* isolate used in this study and only one accession of *V. benghalensis* and two of *V. narbonensis* displayed a susceptible reaction to the faba bean rust isolate.

DISCUSSION

A number of *Ascochyta* and rust species are known to infect legume crops in a host-specific manner (LEATH *et al.* 1994; EMERAN *et al.* 2005; HERNANDEZ-BELLO *et al.* 2006; PEEVER *et al.* 2007; BARILLI *et al.* 2012; KHAN *et al.* 2013; RUBIALES *et al.* 2013). However, little is known of the host range of *A. fabae* and *Uromyces viciae-fabae* within the genus *Vicia*. We show here that faba bean isolates of *A. fabae* and of *Uromyces viciae-fabae* are very specific to *V. faba* only, infecting poorly other *Vicia* species. Our results are in agreement with previous studies

that suggested a coevolutionary history between ascochyta (PEEVER *et al.* 2007) and rust (VAN DER MERWE *et al.* 2008; RUBIALES *et al.* 2013) species and their legume hosts.

In the present research high levels of resistance to *A. fabae* and to *U. viciae-fabae* in the *Vicia* spp. germplasm were identified. Resistance to *A. fabae* had been previously described in wild species of the genus *Vicia*. For example, *V. narbonensis* was unaffected by *A. fabae* inoculations, and only a few atypical symptoms were observed at the site of the inoculum (TIVOLI *et al.* 1987). The eighteen *V. narbonensis* accessions included in the present study showed resistant reactions (Table 1). Only three accessions, belonging to *V. sativa* and *V. serratifolia*, displayed moderate susceptibility with 3–4 mm lesions bearing pycnidia. The rest of the accessions did not allow the fungus to develop. In contrast, susceptibility was very common in *V. faba* (SILLERO *et al.* 2001), although some accessions resistant to *A. fabae* have also been described in the cultivated crop (SILLERO *et al.* 2010; RUBIALES *et al.* 2012).

Most of the *Vicia* spp. accessions studied in the present work displayed resistant response to *U. viciae-fabae* as immunity or strong hypersensitive resistance were commonly detected. Similar results have already been described (HIRATSUKA 1933; RUBIALES *et al.* 2013). In contrast, in *V. faba* hypersensitive resistance has been described only recently (SILLERO *et al.* 2000) whereas resistance is generally of quantitative nature with a fully susceptible infection type most commonly reported (RASHID *et al.* 1991; SILLERO *et al.* 2000). We found susceptibility to faba bean rust in species such as *V. benghalensis*, *V. bithynica*, *V. biennis*, *V. monantha*, *V. narbonensis*, *V. palaestina* and *V. pisiformis*, in which susceptible accessions were already reported (KAPOORIA & SINHA 1966; CONNER & BERNIER 1982; RUBIALES *et al.* 2013). These authors also reported susceptibility to *U. viciae-fabae* in other *Vicia* species studied in the present work including *V. articulata*, *V. cracca*, *V. disperma*, *V. ervilia*, *V. hirsuta*, *V. pyrenaica*, *V. tetrasperma* and *V. villosa*, in which we found only resistant response. We also recorded high IT in some accessions of twelve other *Vicia* species. These differences between studies could be due to the use of different accessions of the same *Vicia* species, or could be an indication of the existence of different *U. viciae-fabae* races, as has been reported by other authors using different legume species (HIRATSUKA 1933; SINGH & SOKHI 1980; CONNER & BERNIER 1982). The present study confirms the host specialization of *U. viciae-fabae* ex *V. faba* recently reported (RUBIALES *et al.* 2013).

The role of minor crops in soil conservation, rescue of marginal areas and environmental improvement has been increasing in the framework of sustainable agriculture and new wild germplasm sources have been collected (AHMED *et al.* 2000; LAGHETTI *et al.* 2000). As well, several species of the genus *Vicia* are traditionally grown as grain and forage legume crops, or could appear as weeds in the crop. All of them could be the green bridge to enable ascochyta blight and faba bean rust to survive in crops of the cultivated species *V. faba*. The present study provides an insight into the role of several *Vicia* species in the epidemiology of these two faba bean diseases, and the knowledge of new potential co-hosts to both diseases has been clarified.

It would be desirable to transfer the resistance to both ascochyta blight and faba bean rust, found in wild species of the genus *Vicia*, to the cultivated species faba bean. So far, all attempts to obtain interspecific hybrids between faba bean and related *Vicia* species have been unsuccessful (BOND *et al.* 1985; RAMSAY & PICKERSGILL 1986). Those crossability barriers have hampered gene transfer, but modern technology might help to achieve this transference in the future. The need to diversify cropping in Mediterranean areas in order to capture benefits such as improved soil nutrition, disease and weed breaks, has led to a renewed interest in grain and forage legume alternatives. As a result many species with a low profile in modern agriculture are coming under considerable scrutiny in breeding and germplasm evaluation programs around the world. The new sources of resistance described to ascochyta blight and faba bean rust could be incorporated into the forage legume breeding programs.

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