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Evaluation of faba beans for resistance to sclerotinia stem rot caused by *Sclerotinia trifoliorum*
Évaluation de la résistance de la féverole à la pourriture à sclérotes causée par le *Sclerotinia trifoliorum*

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Article abstract

Sclerotinia stem rot, a fungal disease caused by *Sclerotinia trifoliorum*, is often a serious problem in faba beans (*Vicia faba*). The levels of resistance to sclerotinia stem rot were evaluated in 23 faba bean cultivars originating from various European countries, 18 cultivars from ICARDA, five cultivars from Canada, 58 populations originating from various provinces of Greece, and five *V. narbonensis* populations. Evaluation was done under controlled conditions following artificial inoculation with carrot root pieces colonized by the fungus. Faba bean plants were scored for resistance on a 0 to 3 severity scale. There was significant variability for resistance to *S. trifoliorum* among faba bean cultivars and among populations. Seven cultivars and 15 Greek faba bean populations showed a satisfactory level of resistance (⩽ 1.50), whereas 22 cultivars and 25 populations were susceptible (> 2.00). However, all *V. narbonensis* populations were resistant to *S. trifoliorum* (disease severity from 1.11 to 1.44) without significant variability within populations. Variability in the resistance of faba beans to *S. trifoliorum* would allow selection within appropriate genotypes for breeding in an effort to provide an effective alternative for sclerotinia stem rot management in this crop.

Evaluation of faba beans for resistance to sclerotinia stem rot caused by *Sclerotinia trifoliorum*

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Sclerotinia stem rot, a fungal disease caused by *Sclerotinia trifoliorum*, is often a serious problem in faba beans (*Vicia faba*). The levels of resistance to sclerotinia stem rot were evaluated in 23 faba bean cultivars originating from various European countries, 18 cultivars from ICARDA, five cultivars from Canada, 58 populations originating from various provinces of Greece, and five *V. narbonensis* populations. Evaluation was done under controlled conditions following artificial inoculation with carrot root pieces colonized by the fungus. Faba bean plants were scored for resistance on a 0 to 3 severity scale. There was significant variability for resistance to *S. trifoliorum* among faba bean cultivars and among populations. Seven cultivars and 15 Greek faba bean populations showed a satisfactory level of resistance (≤ 1.50), whereas 22 cultivars and 25 populations were susceptible (> 2.00). However, all *V. narbonensis* populations were resistant to *S. trifoliorum* (disease severity from 1.11 to 1.44) without significant variability within populations. Variability in the resistance of faba beans to *S. trifoliorum* would allow selection within appropriate genotypes for breeding in an effort to provide an effective alternative for sclerotinia stem rot management in this crop.

[Évaluation de la résistance de la féverole à la pourriture à sclérotes causée par le *Sclerotinia trifoliorum*]

La pourriture à sclérotes, une maladie fongique causée par le *Sclerotinia trifoliorum* est souvent un grave problème pour la production de féverole (*Vicia faba*). À la suite d'une inoculation artificielle avec des morceaux de carottes colonisés par le champignon, l'intensité de la résistance à la pourriture à sclérotes a été évaluée en conditions contrôlées chez 23 cultivars de féverole provenant de divers pays européens, 18 cultivars de l'ICARDA, cinq cultivars canadiens, 58 populations issues de diverses provinces de Grèce et cinq populations de *V. narbonensis*. Une cote de résistance a été attribuée à chaque plante sur une échelle d'intensité de maladie allant de 0 à 3. Il y avait des différences significatives pour la résistance au *S. trifoliorum* parmi les cultivars et parmi les populations de féverole. Sept cultivars et 15 populations grecques de féverole ont démontré un degré satisfaisant de résistance (≤ 1,50), alors que 22 cultivars et 25 populations étaient sensibles (> 2,00). Par contre, toutes les populations de *V. narbonensis* ont été résistantes au *S. trifoliorum* (intensité de maladie entre 1,11 et 1,44) sans qu'il n'y ait de différence significative entre les populations. Les différences de résistance au *S. trifoliorum* dans la féverole signifient qu'il serait possible de procéder à de la sélection au sein de génotypes choisis afin d'offrir l'amélioration génétique comme moyen efficace de lutte contre la pourriture à sclérotes de cette culture.

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INTRODUCTION

Sclerotinia stem rot, also known as white mold, is a serious disease caused by fungi of the genus *Sclerotinia* with a wide host range. It is considered a major disease of many important crops such as dry bean (*Phaseolus vulgaris* L.), soybean (*Glycine max* L.), alfalfa (*Medicago sativa* L.), and many other legumes (Delclos *et al.* 1997; Halimi and Rowe 1998; Kim *et al.* 2000; Steadman *et al.* 1997). This disease is often a serious problem of faba beans (*Vicia faba* L.) in Greece (Roupakias 1983) caused by the fungus *Sclerotinia trifoliorum* Eriks. (Lithourgidis *et al.* 2003).

Several studies, using different evaluation methods under controlled conditions, indicated measurable genetic differences in the levels of resistance to *S. trifoliorum* in alfalfa (Halimi and Rowe 1998; Pierson *et al.* 1997; Pratt and Rowe 1994; Rowe and Welty 1984) and red clover (*Trifolium pratense* L.) (Delclos *et al.* 1997). Similar data were reported for a closely related disease caused by *Sclerotinia sclerotiorum* (Lib.) de Bary in dry bean (Hunter *et al.* 1981; Miklas *et al.* 1992a, 1992b; Steadman *et al.* 1997) and soybean (Boland and Hall 1986; Chun *et al.* 1987; Cline and Jacobsen 1983; Kim *et al.* 2000; Nelson *et al.* 1991; Wegulo *et al.* 1998). Although partial resistance to sclerotinia stem rot has also been identified with field evaluations in several crops, the results of these evaluations are often inconsistent due to specific environmental conditions (low temperatures and high level of moisture) required for disease development and the high spatial variability associated with this disease (Delclos *et al.* 1997; Kim *et al.* 2000). Chun *et al.* (1987), and Nelson *et al.* (1991) observed significant differences among soybean cultivars for lesion length, but these results frequently were not correlated with field ratings. Boland and Hall (1986), and Cline and Jacobsen (1983) reported significant differences among soybean genotypes using the limited-term inoculation method under controlled environmental conditions. Wegulo *et al.* (1998) reported that, despite poor repeatability across experiments, the detached-leaf inoculation method in soybean had the greatest correlation with field ratings. Kim *et al.* (2000) reported a significant correlation between resistance detected with the infested-oat-seed inoculation method and field results in soybean. Pratt and Rowe (1994) demonstrated an increase in the resistance of alfalfa genotypes to sclerotinia stem rot in the field and the greenhouse following one cycle of selection using a stem-lesion bioassay.

Screening under controlled conditions may provide rapid and accurate evaluation of resistance of a large number of genotypes in a relatively small space with great reproducibility (Hunter *et al.* 1981; Lithourgidis *et al.* 1989; Pierson *et al.* 1997). Moreover, with measurements of field resistance, it is impossible to distinguish between the level of resistance and avoidance expressed by crop plants (Miklas *et al.* 1992b). Thus, screening under controlled conditions is often preferred by researchers for determining the level of resistance to sclerotinia stem rot in various crops.

Despite the great number of crops and methods tested for identifying resistance to sclerotinia stem rot, the level of resistance to *S. trifoliorum* in faba beans has not been investigated. Artificial inoculation with carrot root pieces colonized by the fungus has been found to be a suitable method for rapidly screening large plant populations for resistance to sclerotinia stem rot in faba beans (Lithourgidis *et al.* 1989, 1991). This method can provide a more accurate evaluation of the level of resistance in plants because it can distinguish plants that have disease escape mechanisms.

The objective of this research was to investigate variability among and within various faba bean cultivars and populations for resistance to *S. trifoliorum* following artificial inoculation with carrot root pieces colonized by the fungus.

MATERIALS AND METHODS

Plant material

Twenty-three faba bean cultivars originating from four European countries, 18 cultivars from ICARDA, Syria, five cultivars from Canada, 58 populations originating from 17 provinces in Greece, and five *V. narbonensis* (L.) populations (Tables 1, 2, and 3) were evaluated for resistance to *S. trifoliorum* after artificial inoculation under controlled conditions.

Pathogen, inoculation, and incubation

An infected faba bean plant was collected from field at the Fodder Crops Research Institute located in Larissa (39°38′ N, 22°25′ E), Greece and *S. trifoliorum* was isolated on potato dextrose agar (PDA). Carrot root pieces (3 mm x 4 mm x 5 mm) were autoclaved (at 120°C and 1.5 atm for 20 min) and placed on PDA cultures of the fungus prior to the sclerotia formation. After incubation at 20°C for 72 h, the colonized root pieces were used as inoculum (Lithourgidis *et al.* 1989, 1991). Faba bean plants of each cultivar or population were grown in pots under controlled conditions (19-21°C, 12 h photoperiod). Pots were arranged in a completely randomized design. At least 25 4-wk old plants of each cultivar or population were inoculated with the colonized carrot root pieces, on the stem at the second internode from the top. Immediately after inoculation, the stems were wrapped at the point of inoculation with a piece of wet absorbent cotton, and fixed in position with parafilm to protect the inoculum and keep it in contact with the stem. Subsequently, the inoculated plants were placed in a growth chamber at 19-21°C under fluorescent light with a 16 h photoperiod. The plants were sprayed with water three times daily to ensure adequate moisture. After 72 h of incubation, the inoculum was removed and the plants were examined for lesions. Plants with no lesions were inoculated again to ensure that there were no inoculation failures during the experimental procedure.

Disease assessment

Each cultivar or population was evaluated based on the severity of the infection on each individual plant. A disease severity scale from 0 to 3 (Lithourgidis *et al.* 1989) was used as follows: 0 = no visible infection, 1 = early infection indicated by a small lesion (< 10 mm), 2 = moderate infection (10-20 mm), 3 = severe infection characterized by a large, water-soaked lesion (> 20 mm) or collapse of the stem. The severity scale was determined after long-term observations of the behavior of the disease in faba beans both in the laboratory and the field. Thus, plants with a disease severity rating of 1 grow normally (less than 5% mortality); plants with a disease severity rating of 2 may grow or die (about 50% mortality); and plants with a disease severity rating of 3 normally die (more than 95% mortality) (data not shown). Therefore, genotypes with a mean disease severity rating equal to or less than 1.50 were considered to be resistant; those with a rating between 1.51 to 2.00 were intermediate; and genotypes with a mean disease severity rating of more than 2.00 were considered susceptible.

Mean disease severity and standard error for each cultivar or population were calculated based on the disease severity in each individual plant which was considered as a replicate. Cultivars and populations were ranked according to the mean level of resistance as determined by the disease severity scale used.

RESULTS AND DISCUSSION

There was significant variability in the level of resistance to *S. trifoliorum* among cultivars (Table 1) and Greek populations (Table 2) with disease severity of 1.18 to 2.73 in cultivars and 0.89 to 2.74 in Greek populations. Coefficients of variation in cultivars and

Table 1. Sclerotinia stem rot severity (0-3) in faba bean cultivars inoculated with *Sclerotinia trifoliorum*

Cultivar	Origin	Number of plants evaluated	Disease severity Mean ± SE
A-246	Italy	28	1.18 ± 0.18
A-90	ICARDA	122	1.24 ± 0.07
A-58	ICARDA	52	1.26 ± 0.12
A-161	France	40	1.41 ± 0.13
ILB-1814	Europe	66	1.44 ± 0.10
A-156	France	25	1.45 ± 0.19
ALTO	Europe	85	1.49 ± 0.09
A-168	Canada	27	1.52 ± 0.20
A-107	ICARDA	57	1.57 ± 0.09
VT	Europe	69	1.58 ± 0.18
TROY	Europe	83	1.60 ± 0.09
A-160	France	31	1.67 ± 0.13
A-142	ICARDA	28	1.68 ± 0.15
A-151	Spain	79	1.69 ± 0.10
Cluaro	Europe	53	1.70 ± 0.11
A-55	ICARDA	25	1.71 ± 0.20
A-131	ICARDA	30	1.73 ± 0.14
A-108	ICARDA	40	1.83 ± 0.11
A-73	ICARDA	27	1.89 ± 0.15
PAM	Europe	42	1.91 ± 0.14
A-72	ICARDA	30	1.93 ± 0.16
A-153	Spain	29	1.94 ± 0.19
A-37	ICARDA	48	1.95 ± 0.13
A-152	Spain	26	1.99 ± 0.14
A-74	ICARDA	33	2.01 ± 0.17
Gemini	Europe	76	2.05 ± 0.09
A-26	ICARDA	25	2.11 ± 0.16
A-148	ICARDA	26	2.11 ± 0.17
A-110	ICARDA	33	2.15 ± 0.12
A-62	ICARDA	25	2.15 ± 0.17
A-162	Canada	27	2.16 ± 0.19
Ms-125	Europe	70	2.17 ± 0.12
A-109	ICARDA	28	2.25 ± 0.16
A-157	France	30	2.27 ± 0.14
IDALO	France	46	2.29 ± 0.14
A-48	ICARDA	25	2.32 ± 0.20
A-147	Spain	31	2.33 ± 0.12
A-158	France	38	2.35 ± 0.15
A-25	ICARDA	25	2.35 ± 0.19
A-164	Canada	28	2.39 ± 0.17
A-247	Italy	93	2.41 ± 0.09
A-244	Canada	73	2.45 ± 0.11
Hybrid	Canada	30	2.47 ± 0.16
Polycarpe	Greece	60	2.58 ± 0.10
R-29T	Europe	27	2.63 ± 0.16
Tanagra	Greece	55	2.73 ± 0.10
Overall mean:			1.96
Standard error:			0.06
Coefficient of variation:			0.20

Table 2. Sclerotinia stem rot severity (0-3) in faba bean populations, from different provinces of Greece, inoculated with *Scelotinia trifoliorum*

Population	Province	Weight (g) of 1000 seeds	Number of plants evaluated	Disease severity Mean ± SE
		Northern Greece		
E-47	Kavala	1446	54	1.92 ± 0.14
E-12	Kavala	1575	31	1.30 ± 0.15
E-114	Kavala	1538	27	1.70 ± 0.15
E-117	Kavala	1204	25	2.24 ± 0.19
E-118	Kavala	1288	36	2.23 ± 0.12
E-10	Serres	1569	26	1.40 ± 0.17
E-50	Serres	1223	25	1.16 ± 0.15
E-70	Serres	1442	26	2.04 ± 0.18
		Central Greece		
E-87	Larissa	1357	25	1.44 ± 0.16
E-102	Larissa	2166	25	2.23 ± 0.17
E-104	Larissa	1333	26	1.54 ± 0.14
Kv-188	Larissa	—	28	2.41 ± 0.16
Kv-189	Larissa	—	29	2.24 ± 0.13
Kv-191	Larissa	—	30	2.33 ± 0.15
Kv-192	Larissa	—	30	2.13 ± 0.14
Kv-193	Larissa	—	31	2.36 ± 0.15
E-77	Trikala	1316	25	1.33 ± 0.21
E-97	Trikala	1100	27	1.95 ± 0.17
E-66	Karditsa	1300	34	1.82 ± 0.16
E-67	Karditsa	1578	26	1.50 ± 0.16
E-94	Karditsa	1305	25	1.25 ± 0.17
E-107	Karditsa	921	25	1.07 ± 0.17
E-121	Fthiotida	2211	26	1.62 ± 0.16
		Southern Greece		
E-18	Evia	1429	26	2.01 ± 0.16
E-69	Evia	1633	25	1.91 ± 0.17
E-110	Evia	1600	25	2.52 ± 0.18
E-22	Viotia	1705	28	1.14 ± 0.18
E-82	Viotia	1870	26	1.92 ± 0.18
E-112	Viotia	1916	33	2.19 ± 0.14
E-1	Korinthia	1730	30	1.80 ± 0.17
E-76	Argolida	1629	30	1.83 ± 0.14
E-92	Argolida	1779	29	2.56 ± 0.14
E-101	Argolida	1757	27	0.89 ± 0.20
E-30	Arcadia	637	29	1.95 ± 0.17
E-61	Arcadia	2323	32	1.59 ± 0.14
E-14	Messinia	1311	29	1.17 ± 0.13
E-109	Messinia	1556	35	2.74 ± 0.19
E-113	Messinia	1591	28	1.79 ± 0.16
E-3	Lakonia	428	25	2.01 ± 0.18
E-5	Lakonia	1582	27	1.72 ± 0.17
E-27	Lakonia	1065	27	1.60 ± 0.18
E-60	Lakonia	1081	26	2.42 ± 0.15
E-88	Lakonia	574	27	2.59 ± 0.14
		Island of Crete		
E-7	Chania	563	26	2.14 ± 0.19
E-16	Chania	634	29	2.39 ± 0.15
E-71	Chania	1321	32	1.50 ± 0.13
E-90	Chania	1309	26	1.50 ± 0.17
E-123	Chania	1852	29	1.76 ± 0.13
E-65	Rethimno	960	27	2.30 ± 0.16
E-72	Rethimno	1681	50	1.49 ± 0.13
E-103	Rethimno	778	60	2.34 ± 0.10
E-105	Rethimno	927	48	1.32 ± 0.15
E-115	Rethimno	778	25	2.26 ± 0.19
E-11	Iraklio	1557	35	2.10 ± 0.16
E-48	Iraklio	758	28	1.99 ± 0.17
		Island of Rhodes		
E-62	Rhodes	1006	28	1.95 ± 0.16
E-78	Rhodes	1037	25	2.08 ± 0.19
E-99	Rhodes	1341	31	2.31 ± 0.17
Overall mean:				1.88
Standard error:				0.06
Coefficient of variation:				0.23

populations were 0.20 and 0.23 respectively. Only seven of the cultivars and 15 of the Greek populations exhibited a high level of resistance to the disease (disease severity ≤ 1.50). Among cultivars, A-246, and among populations, E-101, showed the highest level of resistance with disease severity of 1.18 and 0.89 respectively. Regardless of the level of resistance, there was significant variability within cultivars and within Greek populations tested, as indicated by the values of standard error ranged from 0.07 to 0.21 (Tables 1 and 2). All of the *V. narbonensis* populations showed a higher level of resistance to *S. trifoliorum*, compared with cultivars and Greek populations of *V. faba* (Table 3). Disease severity in these populations was relatively low (1.11-1.44) and the coefficient of variation also was low (0.10).

The significant variability within faba bean cultivars and populations tested in this study would allow selection for resistant individuals within each cultivar or population, so it is not necessary to resort to *V. narbonensis* populations to find genes for resistance to *S. trifoliorum*. This is very important since all previous attempts to obtain interspecific hybrids between *V. faba* and *V. narbonensis* were unsuccessful (Lazaridou and Roupakias 1993).

Evaluation of dry bean cultivars under controlled environmental conditions indicated that there was also significant variability in resistance to *S. sclerotiorum* (Hunter *et al.* 1982). However, these cultivars were very susceptible to the disease. Only 6.5% of the cultivars were partially resistant. On the other hand, more than 15% of the faba bean cultivars tested in this study were resistant. The percentage of resistant Greek populations was much greater (25.8% of the populations tested). This may be attributed to the fact that the Greek populations, unlike the cultivars, have never been subjected to selection and, consequently, genes for resistance have not been lost. In the two neighboring provinces of Trikala and Karditsa in central Greece, none of the six populations evaluated were susceptible to the disease (Table 2). The widespread and continuous cultivation of other hosts, such as alfalfa and red clover, probably created an extensive infected area in which only the relatively resistant genotypes of faba beans eventually survived in local populations.

Miklas *et al.* (1992a), studying partial physiological resistance to white mold in dry beans, found significant differences in mean lesion length among small- and medium-seeded dry bean genotypes. However, in our study, seed size of faba beans was not correlated with the level of resistance to the disease ($r = -0.21$, NS). Some populations with small seed size were susceptible (e.g., E-16, E-88, E-115), whereas others of the same group were resistant (e.g., E-27, E-105, E-107). Similarly, populations with large seed size were susceptible (e.g., E-92, E-109, E-110) or resistant (e.g., E-12, E-22, E-61).

The high variability observed within each cultivar, regardless of whether it was ranked as resistant, susceptible or intermediate, may be attributed to the fact that faba beans are about 35% cross-pollinated (Bond and Pope 1974). Thus, genotypes resistant to *S. trifoliorum* could be detected and selected at the individual plant level even within a susceptible cultivar. The differentiation among cultivars is maximum at 72 h (Lithourgidis *et al.* 1991), so any plant that shows resistance (disease severity 0 or 1) after more than 72 h of contact with the fungus should be considered resistant to the disease.

Field experiments to evaluate the productivity and the level of resistance of the selected cultivars did not show the same disease rating detected under controlled conditions, and this was probably the result of unfavorable conditions prevailing during these growing seasons (Lithourgidis 1991). Differences for resistance to sclerotinia stem rot disease between controlled and field conditions have been previously reported (Chun *et al.* 1987; Delclos *et al.* 1997; Kim *et al.* 2000; Nelson *et al.* 1991). Selection for resistance to sclerotinia stem rot in the field is difficult considering that the disease requires high moisture and moderate temperature, conditions that are not always present. Evaluation of the level of resistance in faba beans is therefore necessary under controlled conditions.

Data of this study provide an evaluation of the resistance variability of faba beans to *S. trifoliorum* under controlled conditions. To our knowledge, similar evaluation studies with faba beans do not exist in the literature. Considering the absence of research

Table 3. Sclerotinia stem rot severity (0-3) in *Vicia narbonensis* populations inoculated with *Sclerotinia trifoliorum*

V. narbonensis population	Origin	Number of plants evaluated	Disease severity Mean ± SE
A-201	Turkey	47	1.34 ± 0.13
A-202	Greece	31	1.44 ± 0.17
A-204[a]	—	46	1.11 ± 0.13
A-205[a]	—	28	1.36 ± 0.21
A-207[a]	—	28	1.41 ± 0.18
Overall mean:			1.33
Standard error:			0.06
Coefficient of variation:			0.10

[a] Obtained from Institut P. Kul. Gatersleben of GDR.

with faba beans in this area, these data might be useful for further research on resistance of this crop to sclerotinia stem rot. For this purpose, cultivars or populations with different levels of resistance could be crossed in order to study the inheritance of disease resistance genes, and to select high yielding and resistant genotypes.

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