BIOLOGICA NYSSANA
10 (1) September 2019: 17-21
DOI: 10.5281/zenodo.3463990

Phytopathogenic fungi causers fungal diseases of the faba bean (*Vicia faba* L.) in Serbia

Original Article

Tanja Vasić
Faculty of Agriculture, University of Niš, Kosančićeva 4, 37000 Kruševac, Serbia
tanjavasic82@gmail.com (corresponding author)

Sanja Živković
Faculty of Agriculture, University of Niš, Kosančićeva 4, 37000 Kruševac, Serbia
gajicsanja43@gmail.com

Jordan Marković
Institute for Forage Crops, 37251 Kruševac, Serbia
jordan.markovic@ikbks.com

Ivana Stanojević
Faculty of Agriculture, University of Niš, Kosančićeva 4, 37000 Kruševac, Serbia
stanojevic.ivana85@gmail.com

Sonja Filipović
Faculty of Agriculture, University of Niš, Kosančićeva 4, 37000 Kruševac, Serbia
sonjafilipovic86@yahoo.com

Dragan Terzić
Institute for Forage Crops, 37251 Kruševac, Serbia
dragan.terzic.agro@gmail.com

Received: June 24, 2019
Revised: September 8, 2019
Accepted: September 9, 2019

Abstract:

Faba bean (*Vicia faba* L.) is one of the oldest legume crops mainly grown as a valuable protein-rich food, both for human and animal consumption, where, in latter case, it provides an alternative to soybean meals in the temperate regions. There has not been systematic research of faba bean mycoflora in Serbia. This paper aims to present the results of preliminary investigation of mycopopulation of 10 different genotypes of faba bean. Total of 400 plant parts were examined, and 6 genera of fungi were isolated: *Fusarium, Rhizoctonia, Phoma, Sclerotinia, Alternaria* and *Ascochyta*. The results indicate that faba bean is vulnerable to a large number of phytopathogenic fungi that can have a significant impact on reducing its yield and quality.

Key words:

faba bean, mycopopulation

Apstract:

Fitopatogene gljive izazivači gljivičnih oboljenja boba (*Vicia faba* L.) u Srbiji

Bob (*Vicia faba* L.) je najstarija kultura koja se uzgaja kao hrana bogata proteinima, kako za ljudsku, tako i za stočnu ishranu, predstavljajući alternativu sojinim obrocima u umerenim područjima. S obzirom da nije bilo sistematskog istraživanja mikoflore boba u Srbiji, cilj ovog rada je da se predstave preliminarni rezultati mikopopulacije 10 različitih genotipova boba. Ispitano je ukupno 400 biljnih delova i izolovano je 6 rodova gljiva: *Fusarium, Rhizoctonia, Phoma, Sclerotinia, Alternaria* i *Ascochyta*. Dobijeni rezultati ukazuju da je bob osetljiv na veliki broj fitopatogenih gljiva koje mogu imati značajan uticaj na smanjenje prinosa i kvaliteta boba.

Ključne reči:

bob, mikopopulacija

Introduction

Faba bean is an annual plant from the genus of vetches (*Vicia*), the family of legumes (Fabaceae). It is originally of African-Asian origin, and it is grown all over the world. Faba bean was known as an annual field legume since ancient times. Faba bean was cultivated in ancient Egypt, Greece, and Rome, as confirmed by numerous records. It was used for human nutrition as well as for green fertilisation. Today, faba beans, both garden and fodder, are grown in many countries of the world. In Europe it is cultivated in England, Belgium, Scandinavian countries, Germany, Russia and Italy (Mišković, 1986). In our country faba bean is grown in a low capacity, especially as a fodder. Faba bean is a versatile fodder crop. The content of nutrients in the grain of the faba bean and the plant as a whole is significant. The grain contains about 85% dry matter, 25.1% of crude proteins, 46.8% of BEM, 1.6% of crude fat, 9.4% of raw cellulose and about 3.5% of crude ash. Because of this composition, faba bean serves as an excellent concentrate feed for most species of livestock. Also, fabae bean grain is used in human nutrition, like stew, as it has a high nutritional value (Vučković, 1999; Aleksić et al., 2015). *Vicia faba* L. can fix nitrogen through symbiosis with *Rhizobium leguminosarum* in its root nodules (Stoddard et al., 2010).

The most important and widespread fungal diseases observed at all locations in the world are: rust (*Uromyces fabae*), chocolate spot (*Botrytis fabae* and *B. cinerea*), ascochyta blight (*Ascochyta fabae*), leaf

spots (*Alternaria alternata* and *Cercospora fabae*) and wilt/root rot complex (*Fusarium oxysporum* and *Macrophomina phaseolina* dominating). Other diseases of apparent minor importance recorded at low incidence levels at most locations in the world are Sclerotinia blight (*Sclerotinia sclerotiorum*), downy mildew (*Peronospora viciae*) (Akem and Bellar, 1999; Sillero et al., 2010; Stoddard et al., 2010). In Ethiopia chocolate spot (*Botrytis fabae* and *B. cinerea*) and rust (*Uromyces fabae*) are the major diseases which can reduce yield by about 61 and 21%, respectively (Tegen, 2017). In Croatia two *Fusarium* species were determined on the seed vetch, *F. verticillioides* and *F. proliferatum* (Miličević et al., 2013).

Since faba bean has become very important in recent years as a fodder crop in Serbia, the aim of this paper was to determine the phytopathogenic fungi disease causative agents in faba bean for more precise understanding of the problems (plant die-off, reduction of yield and quality, etc.) that arise as a result of the presence of phytopathogenic fungi in faba bean.

Materials and methods

The samples were collected between March and June 2016-2017 at the location of the Institute for forage crops in Globoder. The samples of faba bean seed for the study of mycoflora were taken from different sites: the Rasina region (Kruševac 1, Kruševac 2, Gaglovo 2), Zaječar region (Crni kao), Nišava region (Praskovče, Rujevica, Šarbanovac, Sokobanja) and Pomoravlje region (Jagodina 3, Oparić). Parts of plants are carefully washed under running water. After washing, the parts of stem and roots were cut to pieces of 0.5-1 cm in size. Prepared samples of roots and stems were disinfected with 96% ethanol for 10 seconds and with 1% sodium hypochlorite (NaOCl) for 1 minute and then washed three times in sterile distilled water. They were then dried on sterile filter paper and placed on potato dextrose agar (PDA) with streptomycin. Five pieces of the plant parts (root and stem) were each placed in Petri dish in four replications. They were kept in a thermostat at 25°C in 12 h light / 12 h dark regime. The observations were performed every 3 days, and the majority of mycelium samples were developed up to 14 days. Developed mycelia were screened to a new PDA substrate and, after an initial grow, the peak part of the mycelium was reseeded on PDA again. Microscopic examination was performed using microscopes Olympus CX31. Morphological identification of fungi to the genus was carried out using a standard key. The frequency of isolation was calculated in percents according to the formula by Vrandečić et al. (2011):

$$\text{(\%) Isolation frequency} = \frac{\text{Number of segments containing the fungal species}}{\text{Total number of segments used in the isolation}} \times 100$$

Results and discussion

In these studies, the mycopopulation of faba bean genotypes was examined in a total of 400 plant parts. The isolation frequency of the pathogens from diseased samples largely confirmed the field diagnosis based on visual symptoms. There were clearly expressed symptoms on the leaves in the form of necrotic spots and lesions in all the plants where fungi were isolated. Fungi of genera *Ascochyta* and *Alternaria* were isolated from these plants (**Tab. 1**).

Likewise, necrosis with the presence of white, aerial mycelium was observed on stems in a large number of plants. Fungi from the genus *Sclerotinia* were isolated from the lower third of the stems of these (**Tab. 1**).

Symptoms in the form of light to dark brown necrosis were present at the plant root system, and from these plants were isolated fungi from the genera *Fusarium, Phoma* and *Rhizoctonia* (**Tab. 1**).

In these studies, there was a difference in the isolation frequency of some genera of phytopathogenic fungi in faba bean genotypes originating from different regions of Serbia.

In the samples from the Rasina region (Krusevac 1, Krusevac 2, Gaglovo 2), on the leaves, the infection by genus *Alternaria* was 10-40%, and by genus Ascochyta was represented in all tested samples at 30% (**Tab. 1**). Fungi from genera *Fusarium* with 66.67% and *Phoma* with 40% were dominant in the crowns of the root in the faba bean plants from Rasina region (**Tab. 1**). In the Zaječar region (Crni Kao), the most represented fungi were *Ascochyta* with 40% in leaves, and *Sclerotinia* with 43.33% it roots (**Tab. 1**). In the Nišava region (Praskovče, Rujevica, Šarbanovac, Sokobanja), the prevalent species were from the genus *Alternaria* with 80% in leaves, while in the root system, fungi from genus Rhizoctonia were the most abundant with 46.67%. Genera *Alternaria* with 80% and *Sclerotinia* with 60% were the most prevalent in Pomoravlje region (Jagodina 3, Oparić) (**Tab. 1**).

The genera *Fusarium, Phythophthora, Rhizoctonia, Phoma, Verticillium, Alternaria* and *Sclerotinia* were dominant in annual and perennial legumes in the world (Tivoli et al., 2006; Villegas-Fernández and Rubiales, 2011; Salam et. al., 2011; Sillero et al., 2014; O'Sullivan and Angra, 2016). Miličević et al. (2013) determined two *Fusarium* species, *F. verticillioides* and *F. proliferatum* in vetch seed in Croatia. *Rhizoctonia solani* Kühn is a soil parasite that can

Table 1. Frequency of fungal isolation on *Vicia faba* L.

Genotypes	Number of samples (Plant part)		Fungi species (leaf)	(%) Isolation frequency	Fungi species (root)	(%) Isolation frequency
	Leaf	Root				
3/II Jagodina 3	10	*30*	*Alternaria* sp.	80	*Sclerotinia* sp.	60
19 red Rujevica-Sokobanja	10	*30*	*Alternaria* sp.	80	*Sclerotinia* sp. *Rhizoctonia* sp.	26.67 20
41 red Kruševac 1	10	*30*	*Alternaria* sp. *Ascochyta* sp.	30 30	*Fusarium* sp.	66.67
7/II Oparić	10	*30*	*Alternaria* sp. *Ascochyta* sp.	30 10	*Fusarium* sp. *Sclerotinia* sp.	23.33 6.67
15 red Šarbanovac-Sokobanja	10	*30*	*Alternaria* sp. *Ascochyta* sp.	60 10	*Fusarium* sp. *Sclerotinia* sp.	43.33 16.67
20 red Crni Kao	10	*30*	*Alternaria* sp. *Ascochyta* sp.	20 40	*Sclerotinia* sp.	43.33
Kruševac 2	10	*30*	*Alternaria* sp. *Ascochyta* sp.	10 30	*Phoma* sp. *Rhizoctonia* sp.	40 23.33
10 red Gaglovo 2	10	*30*	*Alternaria* sp. *Ascochyta* sp.	40 30	*Rhizoctonia* sp. *Fusarium* sp.	33.33 26.67
4 red Sokobanja	10	*30*	*Alternaria* sp. *Ascochyta* sp.	50 20	*Rhizoctonia* sp.	46.67
13 red Praskovče	10	*30*	*Alternaria* sp. *Ascochyta* sp.	30 30	*Fusarium* sp. *Sclerotinia* sp. *Rhizoctonia* sp.	16.67 13.33 13.33

cause serious problems on many legumes, especially on faba bean (Assunção, 2011). In Canada, 304 faba bean genotypes were tested for resistance to *R. solani* and only five were identified with high resistance (Rashid and Bernier, 1993). *Sclerotinia* stem rot, a fungal disease caused by *Sclerotinia trifoliorum*, is often a serious problem in faba beans (*Vicia faba*) in Greece (Lithourgidis et al., 2005).

Chocolate spot is caused by *Botrytis cinerea* Pers. and *B. fabae* Sard., the latter being the most important since its action can result in serious plant damage, which is not usually the case with *B. cinerea*. Chocolate spot is especially severe in humid areas, having been reported to be the cause of heavy reductions in yields in places such as the Maghreb, Southern China, Egypt, UK or France (Tivoli et al., 2006).

The type of *Alternaria tenuissima* was detected on the broad bean in Japan. The disease was found in all surveyed fields. The initial lesion was brown, water-soaked, circular to slightly irregular. Then the lesion enlarged and became concentric. Mature leaves had coalescing necrosis surrounded by yellowing. Older leaves of the plant were particularly affected. In a later stage of the disease, the leaves became blighted from the margin to the centre and most of the diseased plants defoliated (Rahman et al., 2002). Ascochyta blight, caused by *Ascochyta fabae* Speg., is a common and destructive disease of faba bean (*Vicia faba* L.) in the Middle East, Europe, Canada, New Zealand (Díaz-Ruiz et al., 2009). In Syria *Ascochyta* blight were frequently isolated from infected samples showing typical chocolate spot symptoms (Akem and Bellar, 1999). Symptoms occur on leaves, stems and pods of infected plants, and can be confused with the early stages of chocolate spot (*Botrytis fabae*). On leaves, small, circular, dark-brown spots appear first. As the disease develops, lesions enlarge and turn light and then change to dark grey. They become irregular in shape, often zonate, and may coalesce to cover most of the leaf surface. Leaf tissue next to the lesions may become black and necrotic. Within the lesions, numerous pinhead-sized black fruiting bodies (pycnidia) of

the fungus develop. These appear only under moist conditions and are often concentrically arranged (El-Komy, 2014). Ascochyta blight is a common disease that causes up to 90% yield losses in susceptible cultivars when environmental conditions are favourable for disease development (Díaz-Ruiz et al., 2009).

Conclusion

This paper presents preliminary results of mycopopulation of 10 experimental faba bean genotypes. The obtained results indicate that faba bean is susceptible to the attack by a large number of phytopathogenic fungi that can significantly reduce its yield and affect its quality. Faba bean has more and more significance in our country, becoming an important fodder crop as livestock feed. This paper is the beginning of a more comprehensive study of phytopathogenic fungi in faba bean. So far, there has been no significant research in this field in Serbia, so future investigations related to the selection of genotypes with increased tolerance to the disease-causing agents will be conducted. Selection to disease resistance can be used as part of the Integral Plant Protection program (IPP), which can include biological and agronomic measures, as well as cultivation of resistant cultivars and hybrids, and which aims to prevent economically significant damage and preserve the environment. Principles and practice of IPP include the monitoring or other detection methods, accurate identification of the target pest (phytopathogenic fungi), population monitoring, rotation of products with different mechanisms of action, and the use of plant protection products when the target pest population (phytopathogenic fungi) threshold at the local level reaches economically significant level.

References

Akem, C., Bellar, M. 1999: Survey of faba bean (*Vicia faba* L.) diseases in the main faba bean-growing regions of Syria. *Arab Journal of Plant Protection*, 17 (2): 113-116.

Aleksić, J., Banović, B., Miljuš-Đukić, J., Jovanović, Ž., Mikić, A., Ćupina, B., Zlatković, B., Anđelković, S., Spanu, I., Jelić, M., Maksimović, V. 2015: A rapid and cost-effective procedure for delineation and utilization of genomic microsatellites for paralleled genotyping in *Vicia faba*. *Czech Journal of Genetics and Plant Breeding*, 51 (1): 36–39.

Assunção, I.P., Nascimento, L.D., Ferreira, M.F., Oolivira, F.J., Michereff, S.J., Lima, G.S.A. 2011: Reaction of faba bean genotypes to *Rhizoctonia solani* and resistance stability. *Horticultura Brasileira* 29: 492-497.

Díaz-Ruiz, R., Satovic, Z., Ávila, C.M., Alfaro, C.M., Gutierrez, M.V., Torres, A.M., Román, B. 2009: Confirmation of QTLs controlling *Ascochyta fabae* resistance in different generations of faba bean (*Vicia faba* L.). *Crop & Pasture Science*, 60: 353–361

El-Komy, Mahmoud H. 2014: Comparative analysis of defense responses in chocolate spot-resistant and -susceptible faba bean (*Vicia faba*) cultivars following infection by the necro-trophic fungus *Botrytis fabae*. *The Plant Pathology Journal,* 30 (4): 355-366

Lithourgidis, A.S., Roupakias, D.G., Damalas, C.A. 2005: Inheritance of resistance to sclerotinia stem rot (*Sclerotinia trifoliorum*) in faba beans (*Vicia faba* L.). *Field Crops Research*, 91 (2–3): 125–130.

Miličević, T., Kaliterna, J., Ivić, D., Stričak, A. 2013: Identification and occurrence of *Fusarium* species on seeds of common vetch, whithe lupine and some wild legumes. *Agriculture*, 19 (1): 25-32.

Mišković, B. 1986: *Krmno bilje*. Naučna knjiga, Beograd. 503.

O'Sullivan, D.M., Angra, D. 2016: Advances in Faba Bean Genetics and Genomics. *Frontiers in Genetics*, 7 (150): 1-12.

Rashid, K.Y., Bernier, C.C. 1993: Genetic diversity among isolates of *Rhizoctonia solani* and sources of resistance in *Vicia faba*. *Canadian Journal of Plant Pathology,* 15: 23-28.

Rahman, M.Z., Honda, Y., Islam, S.Z., Muroguchi, N., Arase, S. 2002: Leaf spot disease of broad bean (*Vicia faba L.)* caused by *Alternaria tenuissima* a new disease in Japan. *Journal of General Plant Pathology*, 68 (1): 31-37.

Salam, M.U., Davidson, J.A., Thomas, G.J., Ford, R., Jones, R.A.C., Lindbeck, K.D., MacLeod, W.J., Kimber, R.B.E., Galloway, J., Mantri, N., van Leur, J.A.G., Coutts, B.A., Freeman, A.J., Richardson, H., Aftab, M., Moore, K.J., Knights, E.J., Sillero, J.C., Rojas-Molina, M.M., Emeran, A.A., Rubiales, D. 2011: Rust resistance in faba bean. *Grain Legumes*, 56: 27-28.

Sillero, J.C., Villegas-Fernàndez, A.M., Thomas, J., Rojas-Molina, M.M., Emeran, A.A., Fernàndez-Aparicio, M., Rubiales, D. 2010: Faba bean breeding for disease resistance. *Field Crops Research,* 115 (3): 297–307.

Sillero, J.C., Rubiales D. 2014: Response of *Vicia* species to *Ascochyta fabae* and *Uromyces viciae-fabae*. *Czech Journal of Genetics and Plant Breeding*, 50: 109–115.

Stoddard, F.L., Nicholas, A.H., Rubialesc, D., Thomas, J., Villegas-Fernàndez, A.M. 2010: Integrated pest management in faba bean. *Field Crops Research,* 115: 308–318.

Tegegn, A. 2017: Effect of aerated and non-aerated compost seepages on the severity and incidence of major fungal diseases of faba bean; *Botrytis fabae, Uromyces vicia fabae* and *Ascochyta fabae*. *Plant*, 5 (6): 85-92.

Tivoli, B., Baranger, A., Avila, C.M., Banniza, S., Barbetti M., Chen W.D., Davidson J., Lindeck, K., Kharrat, M., Rubiales, D., Sadiki, M., Sillero, J.C., Sweetingham, M., Muehlbauer, F.J. 2006: Screening techniques and sources of resistance to foliar diseases caused by major necrotrophic fungi in grain legumes. *Euphytica*, 147: 223–253.

Villegas-Fernández, A.M., Rubiales, D. 2011: Chocolate spot resistance in faba bean. *Grain Legumes*, 56: 29-30.

Vrandečić, K., Ćosić, J., Jurković, D., Poštić, J. 2011: Mycopopulation of medicinal plants in Croatia . *Agriculture*, 17 (2): 18-21.

Vučković, S. 1999: *Krmno bilje*. Institut za istraživanja u poljoprivredi Srbija, Beograd; Bonart, Stara Pazova. 553.