PATHOGENIC VARIABILITY OF *ASCOCHYTA LENTIS* IN BULGARIA

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Abstract
Ascochyta blight, caused by *Ascochyta lentis* Vassiljevsky, is an important foliar disease of lentil (*Lens culinaris* L.) with worldwide occurrence and is a serious threat to lentil production. The investigation involved 13 isolates of *Ascochyta lentis*. The pathogen was isolated from naturally infected lentil plants from various locations in Bulgaria, using an agar plate method. Inoculum was also produced by growing isolates on LDA. Ten plants (3 weeks old) of each of the cultivars were sprayed with a freshly prepared spore suspension (10^5 conidia mL-1). Disease symptoms were scored 14 days after inoculation. The virulence of the isolates was determined with the help of 11 lines and cultivars of lentil (ILL 358, ILL 5480, ILL 2429, ILL 5725, ILL 7537, Laird, Ilina, Bella, Nadejda, Naslada, Zornitsa). Based on the reaction of the used genotypes, the investigated isolates of *Asc. lentis* were grouped into seven pathotypes. The observations in the present study revealed a significant variation among the isolates of *Ascochyta lentis* for morphological traits as well as for pathogenicity.

Keywords: Pathotype, virulence, isolates, *Lens culinaris*.

Introduction

Ascochyta blight caused by *Ascochyta lentis* (Vassiljevsky) is an important disease on lentil (*Lens culinaris* L.) of wide distribution, causing serious damages (Nene *et al.,* 1988). The yield losses as a result from the disease vary from 40 to 70 % (Gossen and Morrall, 1983; Kaiser, 1992; Malik, 1983). The use of resistant varieties is considered to be the most cost-effective way for the control of the disease. Some resistant lines have been established (Singh *et al.,* 1982; Iqbal *et al*., 1990; Sugha *et al*.,1991; Erskine *et al*., 1996; Nasir and Bretag, 1996), but the presence of significant pathogenic diversity in the *Asc. lentis* population may lead to its overcoming (Ahmed *et al*.,1996). In their investigation, Kaiser *et al*. (1994) described differences in the cultural variability of *Asc. lentis* isolates from 24 countries. Ahmed and Morrall (1995) reported variation of *Asc. lentis* isolates from Canada, and Ahmed *et al.* (1996) found presence of sexually compatible types in isolates of *Asc. lentis*. Discrepancies were also observed in some lentil lines with regard to their reaction to ascochyta blight (Tay, 1989; Ahmed and Morrall, 1996; Nasir and Bretag, 1996). Any additional information on the variation of the virulence of *Asc. lentis.* can contribute to the development of cultivars with higher and more durable resistance. The aim of this investigation was to determine the pathogen diversity among the Bulgarian *Asc. lentis* isolates.

Material and methods

The pathogen *Asc. lentis.* was isolated from naturally infected lentil plants collected from different locations in Bulgaria. The isolation was carried out on PDA, and then the cultures were purified to monospores. All isolates were examined for pathogenicity. Single-spore lines of representative isolates were maintained on lentil seed extract agar (LSA) (Nasir and Bretag, 1997) at 4°C. Inoculum was also produced by growing isolates on LSA. Inoculated plates were incubated at 21°C and observed for colony color, diameter (mm), number of pycnidia. For quantification of pycnidia, 1 cm^2 was cut at a distance of 1 cm from the center of a well sporulating culture on LSA medium 12 days after incubation. The disc was observed under magnifying lens to count the number of pycnidia. The pathogenicity of the isolates was established by inoculating of 11 lens varieties (ILL 358, ILL 5480, ILL

2429, ILL 5725, ILL 7537, Laird, Ilina, Bella, Nadejda, Naslada, Zornitsa). The seeds of each variety were planted in plastic pots (ø 12 cm) containing soil-and-sand mixture at ratio 1:1. Ten plants of the genotype have been grown in the pots at 18-20° C in the greenhouse. Three weeks after emergence, the plants were sprayed with spore suspension (10^5 conidia mL-1). Plants sprayed with sterile distilled water were used as a check. After inoculation, the plants were placed in a moist chamber at 95-100 % relative humidity and temperature 20°C ± 2°C. After 72 h, they were transferred again to the greenhouse and were sprayed with water twice a day to maintain humidity. Disease symptoms were scored 14 days after inoculation using the following scale (Iqkbal et al. 2006): 1 – no spots; 3 – small spots on the leaves; 5 – many spots on the leaves with or without a halo around them and small spots on the stem; 7 – large spots on the leaves and defoliation, numerous spots on the stem and occurrence of fruit bodies in them; 9 – spots merging and covering the stem, perishing of the plants. The lines with score 1-3 were marked as resistant (R), while those with score above 3 were considered susceptible (S).

Results and discussion

The investigation showed significant differences in the linear growth of the isolates. The radial growth of the colonies of the investigated 13 isolates varied from 53.9 to 88.6 mm 14 days after incubation. The colonies of seven of the isolates were with faster growth rate, and the other six - with lower. Isolates AL 10, AL 5, AL 11, AL 9, AL 3, AL 1 and AL 13, with respective radial growth of the colonies 88.6, 87.3, 82.1, 81.5, 80.1, 77.7 and 75.9 mm were with fast growth rate. The radial growth of the rest of the isolates was less than 75.0 mm. Differences were also observed in the color of the colonies and the density of the pycnidium among the investigated isolates (Table 1). No correlation was found between the growth rate of the colonies and the virulence of the isolates, nor between the strength of sporulation and the virulence of the isolates. The investigation showed that there was variation in the morphological and cultural characteristics of the investigated isolates. Such variation among the *Asc. lentis* isolates has also been found by Kaiser *et al*. (1994) and Ahmed and Morrall (1995). In 1984, Grewal reported that the comparatively rapidly growing isolates with low sporulation were less virulent, while the isolates with slow growth and abundant sporulation were with higher virulence. Our investigation did not confirm these correlations.

Table 1. Cultural variability among the 13 Bulgarian isolates of *Ascochyta lentis*

Izolates	Origin	Radial growth mm	Colony color	pycnidial density /cm^2
AL 1	Selanovtsi	77.7	Brown	61
AL 2	Dobrich	68.9	Dark grey	59
AL 3	Ruse	80.1	Black	85
AL 4	Pordim	67.0	Dark grey	56
AL 5	Pordim	87.3	Grey	69
AL 6	Lipnitsa	73.3	Brown	43
AL 7	Spasovo	56.8	Dark brown	77
AL 8	Dobrich	59.7	Dark brown	82
AL 9	Vardim	81.5	Dark brown	74
AL 10	Brashlen	88.6	Black	68
AL 11	Selanovtsi	82.1	Dark brown	83
AL 12	Vardim	62.2	Black	87
AL 13	Sitovo	75.9	Dark brown	79

The disease reaction of the investigated lentil accessions 14 days after inoculation varied from 1 to 9. All tested isolates caused the symptoms typical of the disease on the susceptible accessions. The data from the investigation showed that none of the used genotypes possessed complex resistance to the involved isolates (Table 2). Cultivars Ilina and Bella demonstrated resistance to eight of the investigated isolates. Cultivar Nadezhda and line ILL 358 showed resistance to seven isolates. Line ILL

5480 and cultivars Zornitsa and Laird exhibited resistant reaction to six isolates. Line ILL 2429 showed resistance to five isolates, while line ILL 5725 was resistant to only one isolate. Line ILL 7537 was susceptible to all tested isolates. According Iqbal et al. (2006), line ILL 358 was resistant to all isolates. In our study, line ILL 358 has resistance to seven isolates. The discrepancy between our results and those of Iqbal et al. (2006) is probably due to the differences between virulence of the pathogen populations in Bulgaria and Pakistan.

Table 2. Variation in the virulence of *Asc. lentis* isolates collected from different locations in Bulgaria[a]

Genotypes	AL1	AL2	AL3	AL4	AL5	AL6	AL7	AL8	AL9	AL10	AL11	AL12	AL13
ILL 358	R	S	R	S	S	R	R	R	S	R	S	S	R
ILL 5480	S	R	R	R	S	S	R	R	R	S	S	S	S
ILL 2429	S	R	R	R	S	S	R	R	S	S	S	S	S
ILL 5725	S	S	R	S	S	S	S	S	S	S	S	S	S
ILL 7537	S	S	S	S	S	S	S	S	S	S	S	S	S
Laird	S	R	R	R	S	S	R	R	S	R	S	S	S
Ilina	R	R	R	R	S	R	R	R	S	S	S	S	R
Bella	R	R	R	R	S	R	R	R	S	S	S	S	R
Nadejda	S	R	R	R	S	S	R	R	S	R	S	S	S
Naslada	R	R	R	R	S	R	S	S	S	R	S	S	R
Zornitsa	R	S	R	S	S	R	R	R	S	S	S	S	R

[a] – Based on a 0- to- 9 scale, where 0 to 3 = resistant (R) and 3.1 to 9 = susceptible (S)

Among the 13 isolates included in the investigation, AL5, AL 9, AL 11 and AL 12 were with the highest virulence. Isolate AL 3 was with lowest virulence, followed by AL 7 and AL 8. Only some of the isolates demonstrated identical reaction to the genotypes involved in this investigation. Based on the reaction of the used genotypes, the investigated *Asc. lentis* isolates were grouped into seven pathotypes (*Pt*) (Table 3). Patotype *1* was with the lowest virulence, it was virulent to only one genotype. The virulence of *Pt* 7 and *Pt* 6 was the highest. Pathotype 7 was virulent to all investigated genotypes, while *Pt* 6 – to ten genotypes. The presence of isolates belonging to *Pt* 7 which overcame the resistance of all investigated genotypes, implies a necessity to search for new sources of resistance.

Table 3. Pathotype groupings of 13 *Ascochyta lentis* isolates based on disease reaction of 11 lentil genotypes

Pathotype	Nomb. of isolates	Differential line or cultivar										
		ILL 358	ILL 5480	ILL 2429	ILL 5725	ILL 7537	Laird	Ilina	Bella	Nadejda	Naslada	Zornitza
1	1	**R**	**R**	**R**	**R**	S	**R**	**R**	**R**	**R**	**R**	**R**
2	2	**R**	**R**	**R**	S	S	**R**	**R**	**R**	**R**	S	**R**
3	2	S	**R**	**R**	S	S	**R**	**R**	**R**	**R**	**R**	S
4	3	**R**	S	S	S	S	S	**R**	**R**	S	**R**	**R**
5	1	**R**	S	S	S	S	**R**	S	S	**R**	**R**	S
6	1	S	**R**	S	S	S	S	S	S	S	S	S
7	3	S	S	S	S	S	S	S	S	S	S	S

The reactions of the 11 lentil genotypes to individual isolates of *Asc. lentis* revealed differences in the virulence of the isolates. Although the isolates in this investigation differed by their virulence, they were not designated as races because there is no unified methodology for racial identification of *Asc. lentis*. The few publications, presenting researches on the resistance to *Asc. lentis,* have used

different differential lines and score scales, as well as different techniques for inoculation and incubation, which makes the results from these different investigations difficult to compare (Gossen et al., 1986; Kaiser and Hannan, 1986; Ahmed and Morrall, 1995).

Conclusions

There is variation in the morphological and cultural characteristics of *Ascochyta lentis* isolates. Seven pathotypes of *Asc. lentis* were identified in North-East Bulgaria, the most frequent of them being *Pt* 4 and *Pt* 7. None of the investigated genotypes possessed complex resistance to the 13 isolates of *Asc. lentisi* included in the investigation.

References

1. Ahmed, S.K. and R.A.A. Morrall, 1995. Population changes in *Ascochyta fabae* f.sp. *lentis* over 15 years of lentil cultivation in Saskatchewan. *Second European Conference on Grain Legumes,* Pp: 76-77. 9-13 July, 1995 Copenhagen-Denmark
2. Ahmed, S. and R.A.A. Morrall, 1996. Field reactions of lentil lines and cultivars to isolates of *Ascochyta fabae* f.sp. *lentis*. *Canadian J. Pl. Pathol.,* 18: 362–9
3. Ahmed, S., R.A.A. Morrall and J.W. Sheard, 1996. Virulence of *Ascochyta fabae* f.sp. *lentis* on lentils. *Canadian J. Pl. Pathol.*, 18: 354-361
4. Erskine, W., B. Bayaa and M.C. Saxena, 1996. Registration of ILL 5588 lentil germplasm resistant to vascular wilt and Ascochyta blight. *Crop Sci.,* 36: 1080
5. Gossen, B.D. and R.A.A. Morrall, 1983. Effect of Ascochyta blight on seed yield and quality of lentils. *Canadian J. Pl. Pathol.,* 5: 168-173.
6. Gossen, B.D., J.W. Sheppard, C.J. Beauchamp and R.A.A. Morrall, 1986. *Ascochyta lentis* renamed *Ascochyta fabae* f.sp. *lentis*. *Canadian J. Pl. Pathol.,* 8: 154–60
7. Grewal, J.S. 1984. Evidence of physiologic races in Ascochyta rabiei of chickpea. In: Proceedings of the Workshop on Ascochyta Blight and Wintering of Chickpeas. (Eds.): M.C. Saxena and K.B. Singh. ICARDA, Aleppo, Syria, 55-65.
8. Iqbal, S.M., A. Bakhsh and B. A. Malik, 1990. Identification of resistant sources to Ascochyta blight in lentil. *LENS Newsleter,* 17: 26-27.
9. Iqkbal, S.M., C.A. Rauf and S. Ali, 2006. Pathogenic variability among the isolates of *Ascochyta lentis*. *International journal of agriculture & biology,* 618-620, http://www.fspublishers.org
10. Kaiser, W.J. and R.M. Hannan, 1986. Incidence of seed borne *Ascochyta lentis* in lentil germplasm. *Phytopathology*, 76: 355–60
11. Kaiser, W.J., 1992. Fungi associated with the seeds of commercial lentils from the US Pacific Northwest. *Pl. Disease,* 76: 605-610.
12. Kaiser, W.J., R.M. Hannan and J.D. Rogers, 1994. Factors affecting growth and sporulation of *Ascochyta fabae* f.sp. *lentis. Pl. Disease,* 78: 374-379
13. Malik, B.A., 1983. Discussion remarks on lentil breeding II. *In:* Saxena, M.C. and S. Verma, (eds.) *Proceedings of International Workshop on Faba bean, Kabuli Chickpea and Lentils in 1980s,* P: 105 May 1983, Aleppo, Syria. International Center for Agricultural Research in Dry Areas.
14. Morrall, R.A.A. and J.W. Sheppard, 1981. Ascochyta blight of lentils in Western Canada: 1978-1980. *Canadian Pl. Disease Survey,* 61: 7–13
15. Nasir, M. and T.W. Bretag, 1996. Screening lentil for resistance to Australia isolates of Ascochyta blight. *LENS Newsletter,* 23: 7-9
16. Nasir, M. and Bretag, T.W, (1997) - Prevalence of *Ascochyta fabae* f sp. *lentis* on lentil seeds from Victoria, Australia. *Australasian Plant Pathology 26:* 117-120.
17. Nene, Y.L., S.B. Hanounik, S.H. Qureshi and B. Sen, 1988. Fungal and bacterial foliar diseases of peas, lentils, faba bean and chickpea. *In:* Summer, R.J. (ed.), *World Crops: Cool Season Food Legumes,* Pp: 577-89. Kluwer Academic Publishers, Dordrecht, The Netherlands.
18. Singh, G., K.Singh, A.S. Gill and J.S. Brar, 1982. Screening of lentil varieties/lines for blight resistance. *Indian Phytopathol.,* 35: 678-679.

19. Sugha, S.K., B.M. Singh and S.K. Sharma, 1991. Performance of lentil varieties/germplasm liens against blight. *LENS Newsletter* 18: 34-35.
20. Tay, J., 1989. Inheritance of resistance to Ascochyta blight of lentil. *M.Sc. Thesis,* Department of Crop Science and Plant Ecology, University of Saskatchewan, Saskatoon, Canada