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DISEASE INCIDENCE OF CHOCOLATE SPOT OF FABA BEAN IN EL-DAKAHLIA AND EL-SHARKIA GOVERNORATES

Al-Laithy, B. E. A.; A. E. A. Ismail and S.M. El-Baz
Plant Pathology Res. Inst., Agric. Res. Cent., Giza, Egypt.

ABSTRACT

Faba bean chocolate spot disease caused by *Botrytis fabae* is the most important disease affecting the faba bean yield, the disease is widely spread in both El-Dakahlia and El-Sharkia governorate during winter of season 2000-2001 and 2001-2002. The highest percentage of infection and disease severity were recorded in Senbellawein district during 2000-2001 growing season, while the lowest percentage was found in Menia Al-Kamh district during 2002-2003 growing season. The causal pathogen of chocolate spot was isolated from infested leaves. Samples were collected from different locations of El-Dakahlia and El-Sharkia governorates. The causal organism fungal was isolated and identified as *Botrytis fabae*. All tested isolates of *B. fabae* were pathogenic with different degree to faba bean plants of the two tested cultivars Giza 3 and Giza 402.

Increased the inoculum concentration of *B. fabae* inoculum led to increase the disease incidence. There are significant differences in linear growth of different isolates of *B. fabae*. All the tested cultivars were different in their response to chocolate spot disease. Giza blanka cultivar was the most resistant followed by Giza 667, while Giza 402 and Giza 716 were high susceptible. On the other hand, Giza 3, Giza 429, Giza 643, Giza 667, Giza 714, Giza 716 and Giza 843 were moderately infested.

INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the most important legume crops in Egypt, it is infected by chocolate spot disease, which causing considerable losses in quantity and quality of the yield. Many investigators reported that *Botrytis fabae* Sard., is the causal organism pathogen of faba bean chocolate spot disease in Egypt (Mahmoud, 1985; Kamel *et al.*, 1989. Moreover, Abou-Zeid *et al.* (1998) isolated seven isolates of *Botrytis cinerea* and six isolates of *B. fabae* from faba bean leaf spot in Egypt. Differences in their morphological and physiological characters among them were detected. *B. fabae* was consistently isolated from infected faba bean leaves (Koike, 1998).

The present investigation aimed to study these points on; survey chocolate spot disease on faba bean plants in Dakahlia and Sharkia governorates, isolation, purification and identification of the causal pathogen, pathogenicity test, differentiation among isolates of *B. fabae* and inoculum potential. Abou-Zeid *et al.* (1998) tested thirty-five faba bean genotypes for their response to chocolate spot disease (*B. fabae*). They reported that ILB4 12A, ILB 417, ILB 4725 and ILB 4726 were resistant. Germplasm collection of *Vicia faba* have a good source of resistance to chocolate spot disease (Robertson *et al.*, 2000).

MATERIALS AND METHODS

1. Survey studies:

An extensive survey of faba bean foliar disease incidence and severity were carried out in two governorates, i.e. Al-Dakahlia (Sinbellawein, Meet Gamer and Temai Al-Amdeed district), and Al-Sharkia (Diarb Nigem, Menia El-Kamh and Zagazig district). Five villages were randomly selected for each district and five fields were taken in consideration for each. Five samples were collected from each selected field contained 100 plants. The percentage of infection was recorded for each sample. Disease severity was determined according to the methods described by Hanounik (1986).

Disease severity was calculated by using the following equation:-

$$\text{Disease severity \%} = \frac{N \times r}{9\,N} \times 100$$

Where:

r = Number of plants in every grade.
N = Total number of examined plants.
9 = Maximum disease grade.

2. Isolation, purification and identification of the causal pathogen:

Leaves of diseased faba bean plants collected from different locations of Al-Dakahlia and Al-Sharkia governorates. Samples were washed using tap-water, surface sterilized with 0.5% sodium hypochlorite for two minutes, then washed three times with sterilized distilled water. Samples were then dried between two layers of sterilized filter paper to remove the excessed water. The infested leaves were finally placed with their upper surface in moist chamber at 20±1°C, plenty of conidiophores were easily recognized within 3-4 days.

The developed fungi were carefully transferred into slant of Potato Dextrose Agar (PDA) medium. Pure cultures were obtained for each isolated fungi using the single spore technique according to Hansen (1926) and hyphal tip technique according to (Brown, 1924). Detected fungi were transferred to PDA slant medium and kept at 5°C for further studies. identification of the isolated fungi were carried out according to Morgan (1971) and Munjal (1980).

3. Pathogenicity tests:

These experiments were carried out to study the pathogenicity of *Botrytis fabae* isolates using detached leaf technique.

Inoculum preparation:

Inoculum of *B. fabae* was prepared from cultures grown on PDA media for 7 days. The fungal suspension was prepared by adding deionized distilled water to the culture of 2-3 hr, then the culture surface was crushed to release spores. Spores concentration was adjusted microscopically with the aid of the hemicytometer technique to be 5×10^4 spores / ml.

Inoculation of detached leaves:

Healthy looking leaves of Giza 402 and Giza 716 faba bean cultivars were gathered from healthy plants grown under greenhouse conditions. Leaflets were placed in 15 cm Petri dishes with moistened filter paper. The upper surface of each of the leaflets was inoculated with 4 drops (30 ml) leaflet of each isolate. Other leaflets were inoculated with the same volume of deionized distilled water to serve as control.

The infected leaflets were incubated in a growth chamber at 18±2°C. Inoculated leaflets were examined daily for 10 days after inoculation, for the appearance of lesions. Number and diameter of necrotic lesions (mm) were determined and calculated as described by Cohen *et al.* (1993). The fungus was re-isolated from the aforementioned leaflets.

3.1. Differentiation between isolates of *Botrytis fabae*:

3.1.1. Linear growth:

The linear growth of the sixteen isolates, which previously identified of *B. fabae* was determined on PDA to differentiate between them. Sterilized Petri dishes (9 cm in diameter) contains 15-20 ml sterilized PDA were used. Sterilized equal filter paper disc (5 mm in diameter) was immersed in spore suspension (5×10^4 / ml) of each isolate of *B. fabae* for 2 min., then placed in the center of each plate. The inoculated plates were incubated at 25±2°C for 7 days, then the linear growth of isolates was measured in five replicates for each treatment.

3.1.2. Inoculum potential:

Healthy leaves of Giza 402 faba bean cultivar were gathered from the healthy plants under greenhouse conditions. The leaflets were placed on slides in Petri dishes (14 cm in diameter) containing filter paper saturated with 10 ml of sterile water. Four drops (30 ml) of the spore suspension 3×10^4, 5×10^4, 6×10^4 and 7×10^4 spores / ml of *B. fabae* were applied to the upper surface of each leaflet. The inoculated leaflets were incubated at 20±2°C for 10 days. Disease severity was estimated as mentioned before in pathogenicity test.

4. Varital reaction:

Detached healthy leaflets of different faba bean cultivars tested transferred to Petri dishes were inoculated with *B. fabae* as mentioned before in pathogenicity test. Leaflets on control treatments were treated with deionized distilled water. The infected leaflets were incubated as mentioned before. Number and diameter of necrotic lesions (mm) were calculated according Cohen *et al.* (1993).

RESULTS AND DISCUSSION

Data in Table 1 indicate that survey studies were carried out in different localities of El-Sharkia and El-Dakahlia governorates. Results in Table 1 indicate that faba bean chocolate spot disease was widespread in Al-Sharkia and Al-Dakahlia governorates. The percentage of the disease incidence and disease severity were higher in 2000-2001 growing season than that of 2001-

2002. Also, the highest percentage of infection and disease severity were recorded in Senbellawein region during 2000/2001 growing season. Differences in percentage of disease incidence and disease severity between the two growing seasons might be due to differences in environmental conditions during growth, crop rotation and agricultural practices. These results are in line with the results obtained by Kurowski *et al.* (1997) and Bouzhad *et al.* (1998).

Differences between districts in percentage of disease incidence and disease severity might belong to the differences in relative humidity, temperature, time of leaf witness, sowing cultivars, time of sowing and the race of pathogen (Cole *et al.*, 1996).

Isolated causal organism was identified as *Botrytis fabae* Sard. According to Morgan (1971) and Munjal (1980).

Table 1. Percentage of infection and disease severity of faba bean chocolate spot disease in different localities of El-Sharkia and El-Dakahlia governorates during two successive seasons (2000/2001) and (2001/2002).

Localities	Percentage of disease infection			Disease severity %		
	2000/ 2001	2001/ 2002	(M)	2000/ 2001	2001/ 2002	(M)
El-Sharkia gov.:						
Diarb Nigem	84.66	74.66	79.67	62.23	60.03	61.13
Menia El-Kamh	73.33	62.33	67.83	57.83	52.63	55.23
Zagazig	76.66	64.50	70.58	52.83	49.20	51.02
Average	78.22	67.16	72.69	57.63	55.79	53.95
El-Dakahila gov.:						
Senbellawein	86.33	76.00	81.17	69.43	65.10	67.27
Miet Ghamr	80.50	70.50	75.50	61.30	56.80	59.05
Temay El-Amdied	84.61	73.00	78.50	67.60	63.20	65.40
Average	83.61	70.18	78.39	66.11	61.70	63.91
Main average	80.91	68.67	75.54	61.87	57.82	59.85
LSD at 0.05 for:						
Localities (L)			3.389			3.062
Season (S)			1.956			1.768
Interaction (L x S)			4.793			4.331

Data in Table 2 showed that all teseted isolates of *B. fabae* were pathogenic to each of the tested cultivars, i.e., Giza 3 and Giza 402. Also, isolate No. 9 was the most pathogenic, while isolate No. 11 was the lowest pathogenic one. Other isolates were moderately pathogenic. Similar results were obtained by Gorfu (1996) and Heweidy (1998).

Differences in the pathogenicity of isolates tested might return to one or more of genetic factors related with host variety and pathogen as far as their interaction. The most important factors those affecting the physiology and morphology of the host resulted from enzymatic activities of the pathogen. These activities affect host cell wall degradation, convert cell constituent to soluble nutrient as well as other metabolite and host parasite interactions (Jain and Thapliyal (1980), Radhakrishnan and Sen Bineeta (1986).

Table 2. Pathogenicity levels of different *Botrytis fabae* isolates on faba bean cultivars, i.e., Giza 402 and Giza 3.

Tested isolates	Mean diameter of lesions (cm)		Infected area (cm^2)	
	Giza 402	Giza 3	Giza 402	Giza 3
1	2.12	1.77	7.07	4.89
2	2.75	2.49	12.02	2.41
3	2.33	1.92	8.45	5.97
4	2.56	1.63	12.89	4.29
5	1.65	1.80	4.28	5.08
6	2.70	2.14	11.43	7.16
7	2.64	2.41	10.94	9.19
8	2.69	2.50	11.33	9.81
9	2.87	2.63	12.92	10.85
10	2.52	2.26	10.00	8.03
11	1.62	1.26	4.20	4.18
12	1.68	2.18	4.43	7.15
13	2.16	2.13	7.05	7.22
14	1.80	1.64	5.05	9.73
15	2.12	2.14	7.02	7.12
16	2.31	2.31	8.34	8.39
Mean	2.28	2.08	8.58	6.97

LSD at 0.05 for:

Cultivars (Cv)	**0.072**	**0.54**
Isolates (I)	**0.204**	**1.53**
Interaction (Cv x I)	**0.288**	**2.16**

Data in Table 3 revealed that there are differences among linear growth level of tested isolates of *B. fabae*. Isolate No. 4 gave the highest linear growth, while isolate No. 6 gave the lowest linear growth.

Table 3. Linear growth of *Botrytis faba* isolates.

Isolates	Linear growth (mm)
1	21.3
2	31.0
3	31.3
4	50.0
5	26.3
6	24.0
7	33.3
8	36.0
9	26.0
10	29.6
11	28.6
12	37.3
13	27.6
14	30.0
15	28.5
16	25.6

LSD at 0.05 for:

Isolates	**4.79**

These results may be due to the differences in their genetic structure. Similar results were reported by Gorfu (1996) and Abou-Zeid *et al.* (1998).

Data in Table 4 indicate that mean number of lesions, mean diameter of lesion of infected leaves were increased by increasing concentration of inoculum from 3 x 10^4 to 7 x 10^4 spore / ml. These results might belong to concentration of spore reach inoculum potential. Similar results were obtained by Ben-Yephet *et al.* (1996) and Devay *et al.* (1997).

Susceptibility of different faba bean cultivars against the infection with *B fabae* was studied by detached leaf technique. Data obtained from this study indicated that Giza blanka was the most resistant cultivar followed by Giza 667, while Giza 402 was the highest susceptible one. Other tested cultivars were moderately resistant and moderately susceptible. These results agreed with those obtained by Hashim *et al.* (1997) and Knott (1997).

Differences between cultivars in their susceptibility may be due to the different in their genetic make up and their effect on some morphological characters and chemical components of plants. In addition difference in genetic make up between cultivars as well as the environmental conditions may effect on the host pathogen relationship, which may play a role in the cultivar susceptibility (Walker, 1975).

Table 4. Effect of different concentrations of inoculum on chocolate spot disease of Giza 402 cv using detached leaf technique.

Spore concentrations	Mean No. of lesions	Mean diameter of lesions (cm)	Leaf infected area (cm^2)
7 x 10^4	2.00	3.33	17.45
6 x 10^4	2.33	2.90	15.40
5 x 10^4	2.67	1.95	7.90
4 x 10^4	3.33	1.15	3.39
3 x 10^4	4.33	0.833	2.42
Mean	2.93	2.03	9.31
LSD at 0.05 for:			
Concentration	**0.94**	**0.54**	**3.54**

Table 5. Susceptibility of some faba bean cultivars against chocolate spot disease using detached leave technique.

Cultivars	Reaction Type*	Mean No. of lesions	Mean diameter of lesions (cm)	Infected area (cm^2)
Giza 3	MR	2.33	2.62	12.34
Giza 402	S	3.67	2.75	18.43
Giza 429	MS	3.33	2.33	14.14
Giza 643	MR	3.67	2.02	11.54
Giza 667	R	3.67	1.72	8.48
Giza 714	MR	4.00	2.02	12.39
Giza 716	S	2.67	2.54	15.89
Giza 843	MR	3.67	2.10	12.47
Giza blanka	R	4.00	1.32	5.29

*** MR: Moderately resistance** **S: Susceptible**
MS: Moderately susceptible **R: Resistance**
LSD at 0.05 for: Cultivar **0.954** **0.257** **2.531**

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حدوث مرض التبقع الشيكولاتى للفول البلدى فى محافظتى الدقهلية والشرقية

بهاء الكردى أحمد الليثى – عادل الصادق أحمد إسماعيل – سحر محمد الباز
معهد بحوث أمراض النباتات – مركز البحوث الزراعية – الجيزة – مصر

يعتبر مرض التبقع البنى (الشيكولاتى) من أخطر الأمراض التى تصيب الفول البلدى فى ج.م.ع. وقد وجد أن المرض واسع الإنتشار فى محافظتى الدقهلية والشرقية وقد سجلت أعلى نسبة إصابة وشدة مرض فى مركز السنبلاوين دقهلية فى الموسم الزراعى ٢٠٠٠-٢٠٠١ فى حين أن أقل نسبة إصابة وشدة مرض قد سجلت فى مركز منيا القمح شرقية فى الموسم الزراعى ٢٠٠٢-٢٠٠٣ .

ولقد تم عزل عزلات مختلفة من المسبب المرضى فطر البوترايتس فابى وتم تنقيتها وتعريفها وقد إختلفت هذه العزلات فى قدرتها المرضية إلا أنها كانت جميعها ممرضة لصنفى الفول البلدى جيزه ٣ وجيزه ٤٠٢ ، وقد تبين أن نسبة وشدة الإصابة بالمرض تزداد بزيادة تركيز اللقاح للفطر الممرض ، وقد وجد أن هناك إختلاف واضح فى معدل النمو الخيطى الميسيليوم للعزلات المختلفة لفطر البوترايتس فابى. ووجد أن الصنف جيزه بلانكا أكثر مقاومة للمرض بينما الصنف جيزة ٤٠٢ كان أكثر حساسية فى حين أن الأصناف جيزة ٤٢٩ ، جيزه ٦٤٣ ، جيزه ٦٦٧ ، جيزه ٧١٤ وجيزه ٧١٦ والصنف ٨٤٣ كانت متوسطة الإصابة .

وهذه النتائج التى تم الحصول عليها تجعلنا نوضح الصورة أمام الزراع ليختاروا مايتناسب معهم من هذه الأصناف المقاومة للمرض حتى يحصلوا فى النهاية على أعلى وأجود محصول.