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Studies on Stemphylium Leaf Spot of Broad Bean (*Vicia faba* L.)

3. Reaction of Broad Bean Varieties to Infection with *Stemphylium botryosum*

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The reaction of six different local and introduced varieties of broad bean (*Vicia faba* L.) towards *Stemphylium botryosum* showed that there was no source of high resistance within the studied varieties. All local varieties and introduced ones were subjected to infection. Generally, local varieties were more susceptible than the introduced ones. There was a positive correlation between percentage of penetrated stomata and susceptibility. Examination of stomatal apertures revealed that there was an apparent relationship between reaction to infection and stomatal pore widths. Relatively resistant varieties had a high frequency of narrow pores, whereas susceptible ones almost had wider stomata. However, both relatively resistant and susceptible varieties had similar pore lengths, thus indicating no correlation between stomatal pore length and disease reaction.

General survey on the Stemphylium leaf spot disease in Egypt indicated that broad bean variety "Acquadulce" was very susceptible while the local bean variety "Giza 2" was moderately susceptible with the same fungus[1]. Regarding fungal penetration, S. botryosum f. lacticum was found to penetrate the leaf tissues through the stomatal apertures[2,3]. Abdou[4] found a close relationship between the width of stomatal openings and susceptibility to infection. Pool and Mckay[5] found that stomata in young sugar beet leaves, which are not very susceptible to infection by Cercorpora beticola, are only about half as large as stomata in older, more susceptible leaves. They suggested that penetration of the smaller stomata is less likely because of the relatively large size of the germ-tubes of this parasite.

The present study was carried out to test the susceptibility of different local and introduced varieties of broad bean to S. botryosum and to find out the main factors, if any, responsible for disease resistance of the tested varieties.

Material and Methods

Conidial suspension required for artificial inoculation was prepared from 10 days old PDA culture plates. A casein sticker was added at the rate of 0.2 % (w/v) to give uniform infection over the entire plant. The inoculum was applied to the plants with the aid of hand atomizer, then plants were covered with polyethylene bags to maintain high relative humidity necessary for infection.

Healthy detached leaves were also inoculated under laboratory conditions. Inoculated leaves were incubated on filter paper in a moist chamber under room temperature.

Seeds of the local varieties Rebaya 40, Romy and Giza 2, as well as introduced varieties N.A. 117, N.A. 32 and N.A. 29, were used.

Disease readings were recorded, 10-15 days after inoculation, when clear symptoms appeared on the inoculated plants. On detached leaves, disease readings were recorded 3-6 days after inoculation.

Disease readings were recorded for each leaf according to disease severity rating which was calculated to include the size and frequency of the lesions/leaf. Readings were converted to disease index according to the equation suggested by Townsend and Heuberger(6), as the following:

$$\text{Disease index \%} = \frac{\text{sum } (n \times r)}{3\ N} \times 100$$

where, "n" is the number of leaves in each numerical rate, "r" and "N" is the total number of inoculated leaves multiplied by the maximum numerical rate "3".

Readings on stomatal number and measurement of stomatal pore sizes were made on fresh leaves. Plants used in this study were kept nearby in indirect sunlight and detached leaflets were stripped carefully and mounted in water on glass-slide, examined for not more than 5 min., then discarded to avoid any change in stomatal apertures. Stomatal pore size was measured using a calibrated ocular micrometer.

Results

Reaction of broad bean varieties to infection with *Stemphylium botryosum*

In this study, cultivated local varieties together with some introduced ones were tested for their susceptibility to infection with *S. botryosum*. Both leaf spot incidence and percentage of defoliation were determined.

It is clear from the results presented in Table 1 that the local varieties were generally more susceptible than the introduced ones. However, the local variety Giza 2 was somewhat resistant, whereas the introduced variety

N.A. 29 was moderately susceptible.

Results obtained on detached leaves were in conformity with those obtained on potted plants although disease index was somewhat higher.

Percentage of defoliation showed the same trend of leaf spot incidence except N.A. 29 variety which showed a low tendency of defoliation.

Table 1. Comparative reaction of different broad bean varieties to infection with S. botryosum

Varieties	Greenhouse experiment		Laboratory experiment
	Disease index (%)	Defoliatio (%)	Disease index (%)
Rebaya 40	15.8	11.2	14.5
Romy	18.6	17.4	40.0
Giza 2	5.0	3.2	8.4
N.A. 117	1.0	0.0	3.0
N.A. 32	1.8	1.8	4.0
N.A. 29	9.2	2.2	15.5
L.S.D. at 0.05	3.9	2.3	4.0

Mechanism of resistance to infection

In order to study the mechanism of resistance to infection, leaflets of some varieties of broad bean placed in moist chamber were inoculated with spore suspension of <u>Stemphylium botryosum</u>.

After 24 hr incubation at 24°C, the percentage of germinated spores, growth extension of germ tube, the number of penetrated stomata and the average diameter of the penetrated stomata were determined.

Data presented in Table 2 show that percentages of spore germination on the upper leaves surfaces of different broad bean varieties were almost identical ranging from 60 to 70 %. The same was true regarding growth extension of germ tubes except on varieties Romy, N.A. 32 and N.A. 29 where they grew vigorously and reached a maximum length of 176.5 u.

Table 2. Germination of conidia on the upper leaf surface of different broad bean varieties and percentage of penetrated stomata.

Varieties	Germination (%)	Average length of germ tube (u)	Penetrated stomata (%)	Average diameter of penetrated stomata (u)
Robaya 40	64	108.4	14	9.00 x 27.50
Romy	64	173.3	15	9.00 x 27.50
Giza 2	70	108.4	5	5.25 x 22.00
N.A. 117	60	108.4	3	5.25 x 24.75
N.A. 34	66	176.5	3	6.66 x 24.75
N.A. 29	76	173.1	12	7.70 x 27.50

However, when the percentage of readly penetrated stomata was determined for each variety, a clear positive correlation was found. The nnmber of penetrated stomata was higher in the more susceptible varieties (i.e. Rebaya 40, Romy and N.A. 29), while low frequency of penetrated stomata was observed in the other resistant ones. A similar trend was also found concerning the diameter of stomatal pores.

Frequency distribution of stomatal width and length in different varieties of broad bean

According to the above observations, the frequency of distribution of stomatal aperture sizes was determined in different varieties of broad bean.

It is evident (Table 3) that the more resistant varieties Giza 2, N.A. 117 and N.A. 32 had a stomatal widths ranging from 0.53 and 6.66 u, with a high frequency of stomatal width of 2.75 u or less; while, the more susceptible Rebaya 40 and Romy varieties had a high frequency of wider stomata ranging from 6.66 to 9.00 u.

When comparing the frequency of stomatal lengths of different varieties (Table 4) it was found that the resistant N.A. 32 and N.A. 117 varieties had the majority of

Table 3. Frequency distribution of stomatal width in different varieties of broad bean.

Aperture width (u)	Rebaya 40	Romy	Giza 2	N.A.117	N.A.32	N.A.29
0.53	0	0	18	12	32	0
1.05	0	0	14	28	26	0
2.75	0	0	32	26	20	0
3.50	0	0	20	10	8	12
4.50	0	12	6	18	0	28
5.25	2	18	6	2	6	34
6.66	10	32	4	4	8	10
7.70	30	22	0	0	0	16
9.00	50	16	0	0	0	0

Table 4. Frequency distribution of stomatal lengths in different varieties of broad bean.

Aperture length (u)	Rebaya 40	Romy	Giza 2	N.A.117	N.A.32	N.A.29
13.75	0	0	0	0	0	0
14.85	0	0	30	0	0	0
16.50	0	0	32	24	16	0
19.25	12	26	28	24	26	10
22.00	32	26	10	20	24	18
23.10	0	0	0	18	18	0
24.75	20	18	0	14	16	44
27.50	36	30	0	0	0	28

stomatal lengths ranging from 19.25 and 27.50 u. On the other hand, the susceptible Rebaya 40, Romy and N.A. 29 varieties also had a high frequency of stomatal lengths lying in the same range as the above two resistant varieties.

Discussion

The present study showed that there is no source of high resistance against S. botryosum within the different varieties of broad bean. All local varieties as well as introduced ones were subjected to infection. Generally, local varieties were more susceptible than the introduced ones, however, Giza 2 was more resistant than N.A. 29.

There was no apparent differences between different varieties in their effect on spore germination of S. botryosum. There was, however, a clear positive correlation between the percentage of penetrated stomata, as the more susceptible varieties had the high percentages of penetrated stomata. A similar trend was observed concerning the diameter of stomatal pores. It was found from the study of stomatal aperture size in different varieties that there was a positive correlation between susceptibility to infection in such varieties and stomatal widths. While there was no apparent relationship between susceptibility to infection and stomatal lengths. In this respect, Abdou[4] found a close relationship between the width of stomatal openings and susceptibility to infection in *Arachis* sp. to infection by *Cercospora personata* and *Cercospora arachidicola* the causal organisms of peanut leaf spot. Highly susceptible peanut species have high frequency of wide stomata, moderately susceptible have low frequency of wide stomata, and the immune ones have only narrow stomata.

In spite of the degree of resistance in some of the tested varieties, more broad bean varieties must be tested to search for a better degree of resistance to the disease.

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