NUTRITIONAL REQUIREMENTS OF ASCOCHYTA RABIEI AND A. PISI

MUKHTAR AHMAD AND ABDUL GHAFOOR KAUSAR*

Ascochyta rabiei and *A. pisi* grew well on the commonly used synthetic media and on the leaf and grain extract of gram and pea. Nitrogen source was relatively more important for the growth of two fungi than the carbon source. Of the sources of nitrogen studied, *A. rabiei* preferred peptone followed by asparagin and potassium nitrate. However, *A. pisi* preferred potassium nitrate followed by asparagin and peptone. The two fungi preferred sucrose as the carbon source and the colony diameter of the two increased with an increase in the quantity of sucrose in the medium. Maximum colony diameter of the two fungi was obtained with 40 gm. sucrose in the basal medium.

The germination of pycnospores of the two fungi took place fairly well in distilled water and 2 per cent glucose solution and was fairly high in leaf extract of gram and pea. The pycnospores of the two fungi germinated fairly well in lower concentrations of malic acid (N/100 and N/50), but was reduced appreciably at higher concentrations. Pycnospores of *A. pisi* were more sensitive to higher strength of malic acid than those of *A. rabiei*. The pycnospores of both the fungi germinated fairly well on leaves of gram and pea plants.

INTRODUCTION

Ascochyta rabiei (Pass.) Lab. and *A. pisi* Lib. are pathogenic on gram (*Cicer arietinum* L.) and pea (*Pisum sativum* L.) respectively, and cause serious blight diseases on their respective host plants. The identity and pathogenicity of these two fungi have often been confused, but the two fungi occurring on two related leguminous plants are now considered distinct species (Sprague, 1930; Sattar, 1933).

Adequate information on the nutritional requirements and mode of infection of these two well-known pathogenic fungi is not available. This information is obviously essential for a better understanding of these two pathogenic fungi and is highly desirable for further investigations on the basis of resistance to the blight diseases caused by these two fungi.

The present paper presents a comparative study of the nutritional requirements of *Ascochyta rabiei* and *A. pisi* for their growth and germination of pycnospores. The study was necessarily preliminary to further studies that may be made on the subject.

*Department of Plant Pathology, Faculty of Agriculture, West Pakistan Agricultural University, Lyallpur.

MATERIALS AND METHODS

The isolates of *Ascochyta rabiei* and *A. pisi* used in this study were obtained from blighted gram and pea plants collected from Lyallpur, Campbellpur and Sialkot. Isolations were made from leaves, pods and stems of the blighted gram and pea plants by the usual technique and were purified further by the isolation of single conidia.

Natural and synthetic media used in this study had the following composition :

Oat meal agar: Oat meal, 50 gm; agar, 20 gm; distilled water to make 1000 ml.

Richard's agar : Cane sugar, 50 gm; potassium nitrate, 10 gm; potassium dihydrogen phosphate, 5 gm; magnesium sulphate, 2.5 gm; agar, 20 gm; distilled water to make 1000 ml.

Brown's agar : Asparagin, 2 gm; glucose, 2 gm; magnesium sulphate, 2.5 gm; potassium phosphate, 1.25 gm; agar. 20 gm; distilled water to make 1000 ml.

Glucose nutrient agar : Glucose, 20 gm; peptone, 10 gm; sodium chloride, 5 gm; beaf extract, 4 gm; agar, 20 gm; distilled water to make 1000 ml.

Potoato-dextrose agar : Potato starch, 20 gm; dextrose, 20 gm; agar, 20 gm; distilled water to make 1000 ml.

Basal medium : Dextrose, 20 gm; potassium dihydrogen phosphate, 1.5 gm; magnesium sulphate, 0.5 gm; potassium nitrate, 3.12 gm; agar, 15 gm; distilled water to make 1000 ml.

Gram leaf extract agar : Gram leaf, 200 gm (extract); agar, 20 gm; distilled water to make 1000 ml.

Pea leaf extract agar : Pea leaf, 200 gm (extract); agar, 20 gm; distilled water to make 1000 ml.

EXPERIMENTAL RESULTS

1. Nutritional requirements for growth

(*a*) **Culture Media**. *Ascochyta rabiei* and *A. pisi* were grown on six culture media including Brown's agar, Richard's agar, Glucose nutrient agar, Basal medium, Potato-dextrose agar and Oat meal agar at 25°C. The colony diameter attained on six culture media (Table 1) indicated that the two fungi grew well on all culture media. *A. pisi* grew faster than *A. rabiei* on all the culture media studied. Maximum colony diameter was attained by *A. rabiei* on Richard's agar followed by Glucose nutrient agar, Potato-dextrose agar and Basal medium. The growth of this fungus was the least on Oat meal agar. On the other hand, *A. pisi*

had its best growth on Brown's agar followed by Richard's agar, Glucose nutrient agar and Basal medium. Colony diameter was the least on Oat meal agar.

The results of this experiment showed that the common culture media are quite adequate for the growth of these two fungi. This conclusion was further verified by growing them on the natural media containing gram and pea leaves and grain extract, and by varying the constituents of a synthetic medium.

TABLE 1. *Colony diameter of* Ascochyta rabiei *and* A. pisi *on six culture media at* 25°C. *after* 14 *days.*

Culture media	Average colony diameter (millimeters)	
	A. rabiei	*A. pisi*
Brown's agar	24.6	100.6
Richard's agar	42.0	83.6
Glucose nutrient agar	39.1	77.1
Basal medium.	27.4	71.5
Potato-dextrose agar	27.5	53.0
Oat meal agar	18.0	48.7

(*b*) **Natural media.** *Ascochyta rabiei* and *A. pisi* were grown on natural media containing extracts of leaves and grains of gram and pea in petri dishes at 25°C. *A. rabiei* attained a little better colony diameter on gram leaf extract than on gram grain extract (Table 2). Similarly, *A. rabiei* grew a little better on grain

TABLE 2. *Colony diameter of* Ascochyta rabiei *and* A. pisi *on natural media containing extracts from gram and pea leaves and grains at* 25°C. *after* 14 *days.*

Medium	Average colony diameter (millimeters)	
	A. rabiei	*A. pisi*
Gram grain extract agar	32.6	75.5
Gram leaf extract agar	33.7	73.1
Pea grain extract agar	28.5	74.3
Pea leaf extract agar	27.3	84.1

and leaf extract of gram than on leaf and grain extract of pea, but in general, the differences were not marked. Likewise, *A. pisi* grew a little better on leaf extract of pea than pea grain extract. The colony diameter of *A. pisi* grown on leaf and grain extract of pea were not markedly different from the colony diameter on leaf and grain extract of gram, except that growth on pea leaf extract was better than the other media.

(*c*) **Relative importance of different constituents of the basal medium.** *Ascochyta rabiei* and *A. pisi* were grown at 25°C. on the basal medium and the basal medium lacking in dextrose, potassium nitrate, magnesium sulphate and potassium dihydrogen phosphate respectively. The colony diameter attained by *A. rabiei and A. pisi* on these media was materially affected by the omission of potassium nitrate (Table 3). The omission of dextrose influenced the colony diameter to some extent, but in general the effect was not so marked. This indicated that the nitrogen source was relatively more important for the growth of these two fungi than the carbon source.

TABLE 3. *Colony diameter of* Ascochyta rabiei *and* A. pisi *on the basal medium and the medium lacking in constituent at* 25°C *after* 14 *days.*

Medium	Average colony diameter (millimeters)	
	A. rabiei	*A. pisi*
Basal medium	27.4	71.5
Basal medium lacking dextrose	24.9	65.4
Basal medium lacking potassium nitrate	18.4	36.0
Basal medium lacking magnesium sulphate	21.0	71.3
Basal medium lacking postassium dihydrogen phosphate	21.6	66.8

(*d*) **Sources of nitrogen.** *Ascochyta rabiei* and *A. pisi* were grown at 25°C. on the basal medium containing ammonium nitrate, ammonium sulphate, potassium nitrate, asparagin, peptone and urea at the rate of 42 mg. nitrogen per litre. The basal medium with potassium nitrate served as the check.

The colony diameter of *A. rabiei* was the maximum on the basal medium with peptone as source of nitrogen followed by asparagin and potassium

nitrate (Table 4). On the other hand, *A. pisi* had maximum colony diameter on the basal medium with potassium nitrate followed by asparagin and peptone. The least colony diameter of the two fungi on the basal medium was with ammonium sulphate as the source of nitrogen.

TABLE 4. *Colony diameter of* Ascochyta rabiei *and* A. pisi *on the basal medium with different sources on nitrogen at* 25°C. *after* 14 *days.*

Nitrogen sources in the Basal medium	Average colony diameter (millimeters)	
	A. rabiei	*A. pisi*
Ammonium nitrate	15.2	23.7
Ammonium sulphate	12.6	10.7
Potassium nitrate	18.2	63.5
Asparagin	20.7	47.0
Peptone	27.4	41.6
Urea	13.9	27.6

(*c*) **Sources of carbon.** *Ascochyta rabiei* and *A. pisi* were grown at 25°C. on the basal medium with sucrose, glucose, lactose, maltose and starch as sources of carbon. Maximum colony diameter of *A. rabiei* was on the basal medium with sucrose, followed closely by the basal medium with glucose and lactose (Table 5). Similarly, best growth of *A. pisi* was on the medium with sucrose followed by maltose, glucose and lactose. The best colony diameter of the two fungi was attained on the basal medium with starch as source of carbon.

TABLE 5. *Colony diameter of* Ascochyta rabiei *and* A. pisi *on the basal medium with different sources of carbon at* 25°C. *after* 14 *days.*

Carbohydrate sources in the Basal medium	Average colony diameter (millimeters)	
	A. *rabiei*	*A. pisi*
Surcose	24.2	54.1
Glucose	23.3	50.0
Lactose	21.4	49.0
Maltose	19.0	52.0
Starch	17.0	43.8

(*f*) **Quantities of sucrose in the basal medium.** *Ascochyta rabiei* and *A. pisi* were grown at 25°C. on the basal medium with 10, 20, 30 and 40 grams sucrose per litre. The basal medium with no sucrose served as check. The colony diameter of *A. rabiei* and *A. pisi* increased with an increase in the quantity of sucrose in the basal medium (Table 6). The maximum colony diameter of the two fungi was with 40 grams sucrose in the basal medium.

TABLE 6. *Colony diameter of* Ascochyta rabiei *and* A. pisi *on the basal medium with different quantities of sucrose after* 14 *days at* 25°C.

Quantities of sucrose in the Basal medium	Average colony diameter (millimeters)	
	A. rabiei	*A. pisi*
0 gm.	13.1	16.8
10 gm.	21.3	29.1
20 gm.	22.3	31.5
30 gm.	24.3	31.4
40 gm.	32.4	36.4

2. Nutritional Requirements for Germination of Pycnospores

(*a*) **Germination in nutrient solutions.** The germination of pycnospores of *A. rabiei* and *A. pisi* was studied in distilled water, different concentrations of glucose, gram leaf extract and pea leaf extract. The pycnospores of the two fungi germinated fairly well in distilled water. Maximum germination of *A. rabiei* was obtained in 2 per cent glucose solution which was reduced in higher concentrations of glucose. On the other hand, the germination of the pycnospores of *A. pisi* increased with higher concentrations of glucose. The germination of the pycnospores of *A. rabiei* was a little higher in gram leaf extract than in pea leaf extract, but germination in pea leaf extract was fairly high. Similarly, the germination of *A. pisi* was a little higher in pea leaf extract than in gram leaf extract, but the germination in gram leaf extract was also fairly high (Table 7).

Thus, the pycnospores of *A. rabiei* and *A. pisi* germinated fairly well in the leaf extract of gram and pea, but the germination of the pycnospores of the two fungi was a little better in leaf extract of their respective host plants.

TABLE 7. *Germination of the Pycnospores of* Ascochyta rabiei *and* A. pisi *on different solutions at* 25°C. *after* 48 *hours.*

Nutrient solution	Percentage germination of the pycnospores	
	A. rabiei	*A. pisi*
Distilled water	84.6	88.3
Glucose		
0.5 per cent.	55.6	63.2
1.0 per cent.	71.6	87.5
2.0 per cent.	91.0	93.0
3.0 per cent.	67.0	95.0
4.0 per cent.	18.0	100.0
5.0 per cent.	7.0	100.0
Gram leaf extract	95.5	80.3
Pea leaf extract	72.0	92.1

(*b*) **Germination in malic acid.** The germination of the pycnospores of *A. rabiei* and *A. pisi* in different concentrations (N/100, N/50, N/25, N/12 and N/6) of malic acid after 48 hours at 25°C. are tabulated in Table 8.

TABLE 8. *Germination of the pycnospores of* Ascochyta rabiei *and* A. pisi *in different concentrations of malic acid at* 25°C. *after* 48 *hours.*

Strength of malic acid	Percentage germination of the pycnospores	
	A. rabiei	*A. pisi*
N/100	83.0	72.0
N/50	93.0	43.3
N/25	75.6	16.0
N/12	15.6	5.0
N/6	Nil	Nil

Maximum germination of the pycnospores of *A. rabiei* was observed in N/50 malic acid followed by N/100 malic acid. The germination of pycnospores decreased markedly in malic acid of concentrations higher than N/50, and no germination took place in N/6 malic acid.

Almost similar were the results with pycnospores of *A. pisi*. Maximum germination of pycnospores was obtained in N/100 malic acid and decreased as the concentration of malic acid was increased. There was poor germination of pycnospores of *A. pisi* in N/12 malic acid and pycnospores of *A. pisi* did not germinate at all in N/6 malic acid.

These results indicated that the pycnospores of the two fungi germinated fairly well in low concentrations of malic acid and that germination of the pycnospores of the two fungi was reduced at high concentrations. Pycnospores of *A. pisi* appeared to be more sensitive to high concentrations of malic acid than the pycnospores of *A. rabiei*.

(c) **Germination on gram and pea leaves.** The germination of pycnospores of *A. rabiei* and *A. pisi* was also studied on the leaves of gram and pea plants. The suspension of pycnospores of the two fungi in distilled sterilized water were placed on the leaves of gram and pea by means of a dropper. These gram and pea plants were then covered with disinfested bell jars to maintain high humidity. The drops of water containing pycnospores in suspension of two fungi were removed with a pipette and observations on the germination indicated that the pycnosopores of the two fungi germinated on gram and pea leaves.

DISCUSSION

A comparative study of nutritional requirements of *Ascochyta rabiei* and *A. pisi* have not indicated marked differences. Both the fungi were able to maintain themselves on the natural media from the two host plants containing leaf and grain extracts of gram and pea. The only difference in the nutritional requirements of the two fungi was in their nitrogen requirements. *A. rabiei* grew a little better on peptone and *A. pisi* flourished a little better on potassium nitrate. The three best sources of nitrogen for the two fungi were similar.

The pycnospores of *A. rabiei* and *A. pisi* could germinate readily on the two host plants. Evidently, germination of conidia of the two fungi is possible on leaves of gram and pea plants in nature. Moreover, germination of pycnospores of *A. rabiei* and *A. pisi* was favoured by N/100 and N/50 malic acid and was reduced at higher concentrations of the acid. Pycnospores of *A. pisi* appeared to be more sensitive to higher concentrations of malic acid than those of *A. rabiei*. It may be concluded that germination of *A. rabiei* and *A. pisi* may be possible on young gram plants on account of lower concentrations of malic acid. But at higher concentrations, the conidia of *A. pisi* may not be able to germinate on the gram plants on account of higher concentrations of malic aid.

Gram plant differs from pea plant in giving out acid secretions from glandular hairs, consisting mostly of malic acid with traces of oxalic and acetic acids (Sahasrabuddhe, 1914). The germination of pycnospores of *A. rabiei* is favoured by malic acid (N/50-N/25), and higher concentrations of the acid are detrimental to the germination of pycnospores of the fungus (Sattar, 1933). Malic acid secretions from gram plants have been shown to increase with age of the plant and higher concentrations of the acid detrimental to the germination of pycnospores of *A. rabiei* have been considered to be the basis of resistance in gram types to blight caused by the fungus (Hafiz, 1952).

The identity and pathogenicity of *A. rabiei* and of *A. pisi* have often been confused but these two fungi occurring on two related leguminous plants have been considered distinct species (Sprague, 1930; Sattar, 1934). However, Sattar (1934) also reported that Indian form of *A. pisi* infected 6 out of the 42 gram plants inoculated, and the culture of *A. rabiei* isolated from stem lesion of gram from Madrid, Spain infected 3 pea plants out of 18 inoculated. This indicates that *A. rabiei* and *A. pisi* may not be too highly specialized in pathogenicity on their respective host plant.

Almost similar nutritional requirements of *A. rabiei* and *A. pisi* for the germination of conidia and growth in culture indicates that any degree of specialization in pathogenicity that may be shown by *A. rabiei* and *A. pisi* on gram and pea plants may be explained partially on account of concentrations of malic acid on the leaves of gram plants detrimental to conidia of *A. pisi* and on account of the absence of malic acid secretions by leaves of peas which may limit the germination of conidia of *A. rabiei*. Studies of the two fungi on these aspects should result in a better understanding of the degree of specialization in pathogenicity in these two fungi.

LITERATURE CITED

Hafiz, A. 1952. Basis of resistance in gram to Mycosphaerella blight. *Phytopath.* **42** : 422-424.

Sahasrabuddhe, D. L. 1914. The acid secretions of the gram plant, *Cicer arietinum*. *Agr. Res. Inst. Pusa. Bull.* 45.

Sattar, A. 1933. On the occurrence, perpetuation and control of gram (*Cicer arietinum*) blight caused by *Ascochyta rabiei* (Pass.) Lab. with special reference to Indian conditions. *Ann. Appl. Biol.* **20** : 612-662.

Sattar, A. 1934. A comparative study of the fungi associated with blight diseases of certain cultivated leguminous plants. *Trans. Brit. Mycol. Soc.* **18** : 276-301.

Sprague, R. 1930. Notes on *Phyllosticta rabiei* on chick pea. *Phytopath.* **20** : 591-593.