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Influence of Some Nitrogen and Carbohydrate Sources on Growth, Sporulation and Pathogenicity of *Bipolaris* Species

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Isolates of *Bipolaris sacchari* and *B. australiensis* that had been isolated from stem base and roots of wheat grown in Hamadan province, have shown tendencies to give a differential pathogenic response when used to inoculate the root and foliage of several cereals including barley and wheat cvs. They grew well on a number of inorganic and organic nitrogen and carbohydrate sources. There was some evidence of a differential pathogenicity of *B. sacchari* and *B. australiensis* on wheat and barley, with inoculum grown on media containing different amino acids. However, inoculum grown on media containing argenine produced less leaf and stem-base lesions on barley and wheat cultivars in comparing with others, even the control. In the case of *B. australiensis*, the most pathogenic response on foliage and stem-base occurred with inoculums grown on peptone, whereas in the case of *B. sacchari* inoculum grown on media containing galactose and glucose were more effective. All nitrogen and carbon sources tested, supported some growth with the possible exception of arginine and asparagine. Maximum growth of fungal colony occurred on peptone as well as mycelium dry weight on media containing glucose and cellubiose.

Key Words: Amino acid, Carbohydrates, Pathogenicity, *Bipolaris sacchari*, *B. australiensis*.

INTRODUCTION

Agricultural chemistry is the study of both chemistry and biochemistry which are important in agricultural production, the processing of raw products into foods and beverages and in environmental monitoring and remediation. These studies emphasize the relationships between plants, animals and microorganisms and their environment.

Bipolaris is one of the most important soilborne fungal pathogens which develops in field crops, especially on cereals and causing different diseases such as foliar spot blotch, root rot, as well as head blight[1]. Losses due to this disease in South Asia, where nearly 1200000 ha are affected, amounts to 16–23%[2]. *B. sacchari* is an important pathogen on most cereals and many other grasses and can cause root rot, leaf spot and head blight.

The nutritional basis for differential pathogenicity has been investigated in a number of fungi but little attention has been paid to *Bipolaris* species[3-5]. Several reports have indicated that certain nitrogen sources and trace elements stimulated the growth of this form genus and that a combination of some vitamins showed no influence on growth of *Cochliobolus sativus*[3, 6].

The present paper reports investigations carried out on isolates of two species of this form genus, *Bipolaris sacchari* and *B. australiensis*, known to have different pathogenic capabilities. They have been compared for their relative ability to utilize different nitrogen and carbon sources and for influence of these chemicals on their growth, sporulation as well as their pathogenicity.

EXPERIMENTAL

Isolates of *Bipolaris sacchari* and *B. australiensis* were obtained from surface sterilized (NaOCl) wheat root. Two single-sporing isolates of these fungi were used to infect four varieties of wheat and three varieties of barley. The basal medium was similar to that used by Clark[3] and contained nitrogen, 0.245 g; sucrose, 38 g; KH_2PO_4, 0.3 g; K_2HPO_4, 1.2 g; $MgSO_4 \cdot 7H_2O$, 0.3 g; $FeCl_3 \cdot 6H_2O$, 1 ppm; $ZnSO_4 \cdot 7H_2O$, 1 ppm; $CuSO_4 \cdot 5H_2O$, 1 ppm; $MnSO_4 \cdot 5H_2O$, 1 ppm; water, 1 L. This medium was used because it had been shown that trace elements were important for this fungus[3]. Previous reports[3] indicated that sodium nitrate was a good source of nitrogen for this fungus and from then on it was used as the inorganic nitrogen source and included in all experiments as a control.

For media preparation double distilled water was used, and all media were sterilized throughout the experiment as well as the acid-washed glassware. Experiments were carried out with both liquid and solid media, using purified agar added at the rate of 17 g per L. The nitrogen sources were some amino acids including asparagine, L-cysteine, arginine, peptone and also urea and sodium nitrate. The carbohydrate sources were sucrose, glucose, galactose, xylose, lactose and cellubiose. They were prepared as stock solutions and sterilized separately, and then added in the appropriate amounts to previously autoclaved basal medium. All the growth studies on liquid media were down in 125 mL Erlenmeyer flasks with 25 mL of medium. Inoculum for both agar and liquid media was obtained from 7-day-old agar cultures containing basal medium and sodium nitrate as a nitrogen source. A 5-mm diskc of inoculum was cut from the edges of the cultures and used to inoculate the various liquid media.

Most cultures were incubated for 14 days, but some were harvested after 7 days to compare early growth. Four replicates were harvested in all experiments. Growth of fungus on liquid medium was determined by harvesting the mycelium produced, separating it from the media by filtering the contents of each flask and drying it overnight in an oven at 65°C and weighing it to the nearest milligram.

The influence of different media on the pathogenicity of the isolates was determined in two ways. The comparisons were made by detached leaves by removing the second and third emerging leaves from 3-week-old seedlings of the wheat and barley cultivars, inoculated with spore suspension in petri dishes and incubated for 5 days. Then they were rated for the extent of lesion development.

Additional comparisons were done by inoculating the surface sterilized seeds of barley and wheat *cvs* with spore suspensions, placed on moist sterile paper in the petri dishes for 7 days. According to Singleton *et al.*[7] scale, disease ratings were determined at the end of this time by estimating the amount of damage done to the seedlings using the disease index rating (DIR) explained in Table-1.

TABLE-1
BIPOLARIS DISEASE INDEX RATING (DIR)

Symptoms	% of tissue covered	Numerical value of disease category
No symptoms	0	0
Slight discolouration	1–25	1
Moderate	26–50	2
Severe	51–75	3
Dead plants	>76	4

RESULTS AND DISCUSSION

The two fungal isolates grew well on a number of inorganic and organic nitrogen and carbohydrate sources. Both carbon and nitrogen sources were more indusive on growth rate of *B. australiensis* rather than on *B. sacchari* isolate (Fig. 1). However, comparing the control the least effect was observed by galactose.

The majority of the chemicals supported some growth on two isolates with the possible exception of arginine, peptone and asparagine (Figs. 1, 2). In all cases much more growth occurred during the first 7 days than in the next 7. Maximum growth of radial fungal growth was indicated on agar media containig peptone as well as mycelium dry weight on media containing glucose and cellubiose. The responses of *B. australiensis* isolate to glucose, galactose, sucrose and cellubiose were more inducive in comparison with the other carbon sources in liquid media (Fig. 1).

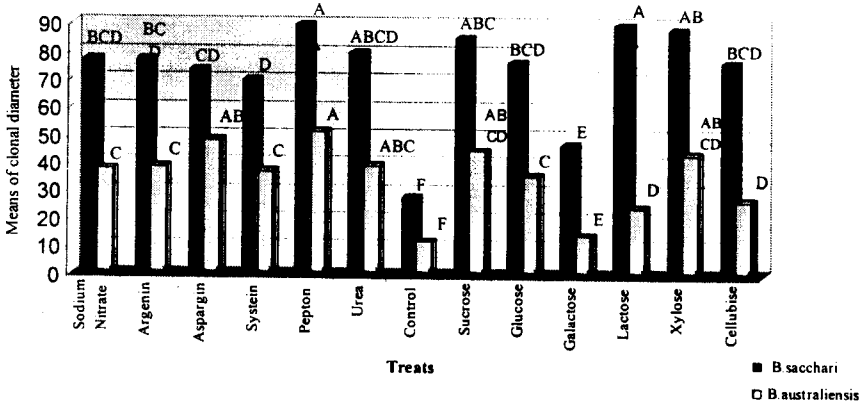

Fig. 1. Influence of some carbon and nitrogensources on clonal diameter of fungi

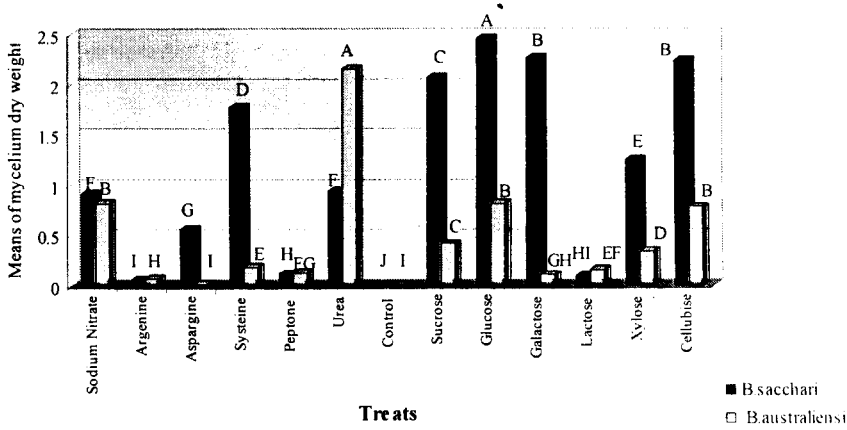

Fig. 2. Influence of some carbon and nitrogen sources on mycelium dry weight

The mycelium dry weight of *B. australiensis* was more affected by the carbon sources rather than on the nitrogen one (Fig. 2). In the case of *B. sacchari,* urea was most effective on growth and mycellium dry weight, whereas, L-cysteine was the most effective amino acid regarding mycelium dry weight. The least effect on mycellium dry weight of both fungal isolates was due to arginine, peptone and asparagine (Fig. 2). In some cases the amount of sporulation by the isolates was changed by certain chemicals, but it was not significant comparing the control.

The results described in Fig. 3 confirm that the pathogenicity of *B. australiensis* isolates which have been grown on media containing nitrogen sources was greater than the carbon sources. Also there was some evidence of a differential pathogenicity of *B. sacchari* and *B. australiensis* on wheat and barley, with inoculum grown on media containing different amino acids (Figs. 3 and 4).

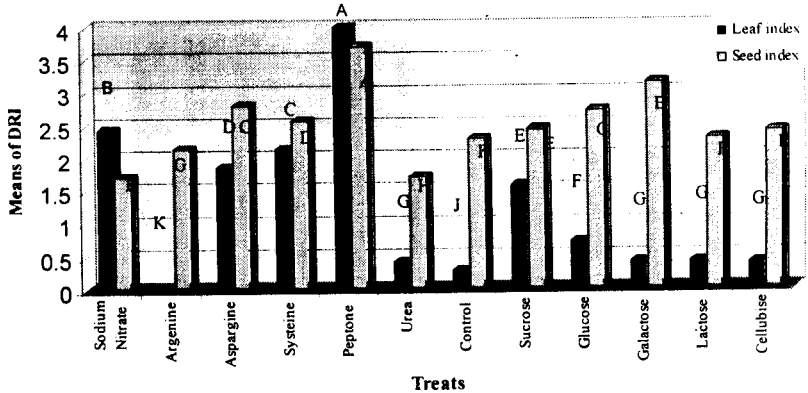

Fig. 3. Influence of some carbon and nitrogen sources on pathogenicity of *B. australiensis*

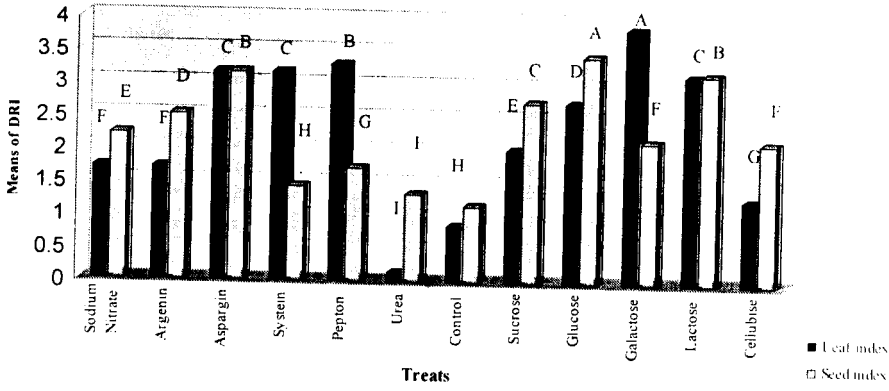

Fig. 4. Influence of some carbon and nitrogen sources on pathogenicity of *B. sacchari*

However, inoculum grown on media containing arginine produced less leaf and stem-base lesions on barley and wheat cultivars in comparison with others, even the control. This is in close agreements with the results reported by Deadman *et al.*[4] that higher nitrogen content of wheat straw was responsible for increasing the incidence and greater severity of wheat stem-base disease caused by Fusarium foot rot and eyespot. Both Fusarium foot rot and eyespot diseases have already been reported as being enhanced by ammonium salts[4, 5].

In the case of *B. australiensis*, the most pathogenic response on foliage and stem-base occurred with inoculum grown on peptone, whereas in the case of *B. sacchari*, with inoculum grown on media containing galactose and glucose. This is in close agreement with the results reported, regarding positive correlation between glucose content of rice leaves and disease severity caused by *B. specifera*[6]. Asparagine, the amide form of aspartic acid, L-cysteine and peptone had increased the pathogenesis ability of *B. sacchari* on both wheat and barley cultivars (Fig. 4).

From these results it is clear that nitrogen and carbohydrate sources could be able to enhance the pathogenicity and growth of these pathogens, so it would be regarded by plant breeders and growers while using different cereal cultivars in their practices. From the agro-chemical point of view, plant root exudates contain so many carbohydrate and amino acid compounds, which are secreted into the rhizospheric zone, where the soilborne pathogens including *Bipolaris* species are present. Further investigations are needed to clarify the detail of interactions between the plants and their soil environmental microorganisms.

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