Nitrogen requirements of Diplodia natalensis Pole Evans, the incitant of Diplodia stem-end rot of orange fruits*)

By B. B. Lal [1]) and V. N. Pathak [2])

Diplodia stem-end rot of orange fruits is a disease of world wide occurrence. While investigations on epidemiology and chemical control of the disease have been carried out in some detail in countries other than India, very little is known about the nutritional requirements of the incitant. The present paper reports the results of studies on nitrogen requirements of this organism.

Methods and results

Following nitrogen sources were employed in this study:

Nitrate nitrogen sources	— Sodium nitrate & Potassium nitrate
Nitrite nitrogen source	— Sodium nitrite
Ammonium nitrogen sources	— Ammonium phosphate, Ammonium sulphate and Ammonium chloride
Aliphatic monoamino carboxylic acids	— DL-alanine, DL-valine, DL-leucine, DL-serine, Glycine & DL-tryptophane
Aliphatic monoamino dicarboxylic acids	— DL-aspartic acid
Aliphatic diamino mono carboxylic acid	— L-arginine monohydrochloride
Amino acids containing sulphur	— L-cysteine and methionine
Aromatic derivative of amino acids	— DL-B-phenylalanine
Amides	— Asparagine, L-glutamine and urea

In earlier studies on growth of the fungus on various media, Richard's medium supported maximum growth of the fungus, the same was selected for the present investigations. Potassium nitrate in the Richard's medium was replaced with the equivalent amount

*) Portion of M. Sc. thesis submitted by senior author. 1 & 2, P. G. student and Reader, Department of Plant Pathology, University of Udaipur, Jobner (Rajasthan), India.

of inorganic or organic nitrogen compound. All nitrogen sources used were comparable on an equivalent 'utilizable nitrogen basis'.

The media were autoclaved at 10 lbs pressure for 10 minutes. The pH of different media was adjusted to 7.0 before autoclaving. Each flask was inoculated with a single disc of uniform size supporting peripheral growth of the fungus. The discs were cut out after 5 days growth of the fungus on PDA. The inoculated flasks were incubated at 30±1° C for 15 days. During incubation, flasks were regularly observed for detecting the presence of pycnidia, which did not form in any of the treatments up to 15 days. On 16th day, mycelial growth was harvested, dried and weighed. Tables 1 and 2 show the average dry weight of mycelium of four replicates of each treatment.

Table 1. Growth of *D. natalensis* on Richard's medium containing different inorganic sources of nitrogen

Nitrogen source	Average dry weight of the mycelium (mg)
Ammonium phosphate	198.00
Ammonium sulphate	204.000
Ammonium chloride	135.00
Sodium nitrite	170.00
Sodium nitrate	331.00
Potassium nitrate	393.00
Control (without nitrogen)	67.00

From Table 1, it is seen that Potassium nitrate supported maximum growth among all the inorganic nitrogen sources tried. Fungus did utilize ammonium nitrogen and among all the ammonium compounds, ammonium sulphate supported better growth. Fungus dis-

Table 2. Growth on Richard's medium with different organic sources of nitrogen

Nitrogen source	Average dry weight of the mycelium (mg)
DL-alanine	221.50
DL-valine	381.30
DL-leucine	275.30
DL-serine	356.30
Glycine	167.30
DL-tryptophane	242.60
DL-aspartic acid	373.60
L-arginine monohydrochloride	236.00
L-cysteine	502.00
DL-methionine	227.50
DL-B-phenylalanine	342.00
Asparagine	330.00
L-glutamine	388.00
Urea	201.00

played growth on sodium nitrite and it was better than that on ammonium chloride.

The results of Table 2, show that among the aliphatic monoamino carboxylic acids, DL-valine supported maximum growth, glycine supported poorest growth; it was also the poorest source of nitrogen among all the organic sources of nitrogen tried. DL-serine was next to DL-valine, other amino acids were intermediate in supporting growth of the fungus.

L-cysteine was best for growth among all the organic nitrogen sources tried. This amino acid contains sulphur, other such amino acid, DL-methionine was, however, comparatively poorer than L-cysteine. Among the amides, L-glutamine supported maximum growth, it was next to cysteine among all the organic sources of nitrogen.

Discussion

This fungus can be placed in group II of the classification put forth by Robbins (1937). Like many fungi, the present isolate preferred nitrate nitrogen for growth. It was incapable of utilizing ammonium chloride. It is interesting to note that sodium nitrite not only supported growth of the present isolate, but supported it better than certain other nitrogen sources. The explaination for this situation can be sought from the unpublished findings of Bhatnagar and Pathak (1969), who observed no growth of *D. natalensis*, isolated from mango fruits on nitrite in the acid range, but noted comparable growth of the fungus on nitrite and nitrate at pH 8.0. Since in the present investigations, media were buffered to pH 7.0 (the optimum pH for the present isolate), like *Diplodia* isolate of the above workers, this fungus also attained growth on nitrite at this pH. Wolf (1953) and Tandon and his coworkers (1961) reported very good growth of various fungi on DL-valine, the present isolate showed maximum growth on this amino acid among all the aliphatic monoamino carboxylic acids. Poorest growth was observed on glycine, similar observations on growth of *Helminthosporium gramineum* has been reported by Converse (1953). Cysteine supported maximum growth of the present isolate, good growth of *Phyllosticta* spp. on this sulphur containing amino acid has been reported by Tandon and his coworkers (1961).

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References

1. Bhatnagar, L. G. and V. N. Pathak, 1969: pH in relation to nitrate and nitrite utilization by *Diplodia natalensis* Pole Evans. Labdev. Sci. and Tech. (in press).
2. Converse, R. H., 1953: The influence of nitrogen compounds on the growth of *Helminthosporium gramineum* as culture. Mycologia 45: 335.
3. Robbin, W. J., 1937: The assimilation by plants of various forms of nitrogen. Amer. Jour. Bot. 24: 243—250.
4. Tandon, R. N., 1961: Physiological studies on some pathogenic fungi. Uttar Pradesh Sci Res. Committee Monograph.