

MACROMOLECULAR SYNTHESIS AND CONIDIAL GERMINATION OF *DRECHSLERA ORYZAE* SUB.

BY

GITASREE GHOSH AND SUBHENDU CHAUDHURI

Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya
Kalyani-741 235

Synthesis of macromolecules - protein, RNA and DNA, registered measurable increase at about 3 hours coinciding with germ tube emergence. Studies with specific inhibitors of macromolecular synthesis revealed essentiality of protein synthesis for germination during first 90 minutes of incubation. Proteins that were synthesized thereafter till germ tube emergence were probably not essential for germination. Synthesis of nucleic acids, which proceeds during this period, was probably not essentially required for germination. The ungerminated conidia have a pool of free amino acids, which changed qualitatively during germination, to sustain the protein synthesis. Conidial germination of *D. oryzae* is dependent on synthesis of essential protein (s), the synthetic components for which are probably present in ungerminated conidia.

INTRODUCTION

Drechslera oryzae Sub. (*Cochliobolus miyabeanus* Ito et Kurib. Drechs. et Dastur) is a non-specialized perthophytic pathogen of rice causing self-limited necrotic lesions on seed, coleoptile and leaves. The multicelled conidia of the fungus germinate on activated endogenous metabolism on water, normally from two polar cells. Relation of macromolecular synthesis to germination of conidia of the fungus was investigated.

MATERIALS AND METHODS

The strain of *D. oryzae*, used in the study was isolated from leaf lesions of rice following usual methods of isolation. A monoconidial isolate was maintained on PDA. Synchronously produced conidia on filter paper cultures (Lukens, 1960 ; Oku, 1971) were harvested dry and used for all experiments.

Estimation of macromolecules

Total protein of *D. oryzae* conidia was estimated with Folin phenol reagent (Lowry *et al.*, 1951). RNA was extracted and purified by Kirby's phenol method (Kirby, 1956). Total RNA was determined by the orcinol procedure (Schneider, 1957) using pure yeast RNA as standard. DNA was extracted from the

residue left after RNA extraction (Kirby, 1956). DNA was assayed with diphenyl amine reagent according to the procedure of Burton (19:6) using pure calf thymus DNA as standard.

Estimation on intracellular free amino acids

The pool of free amino acids in *D. oryzae* conidia was separated by two dimensional paper chromatography on Whatman No. 1 papers, with water saturated phenol and butanol : acetic acid : water (4:1:1) as solvents. Quantitative estimations were made according to Block *et al.* (1958).

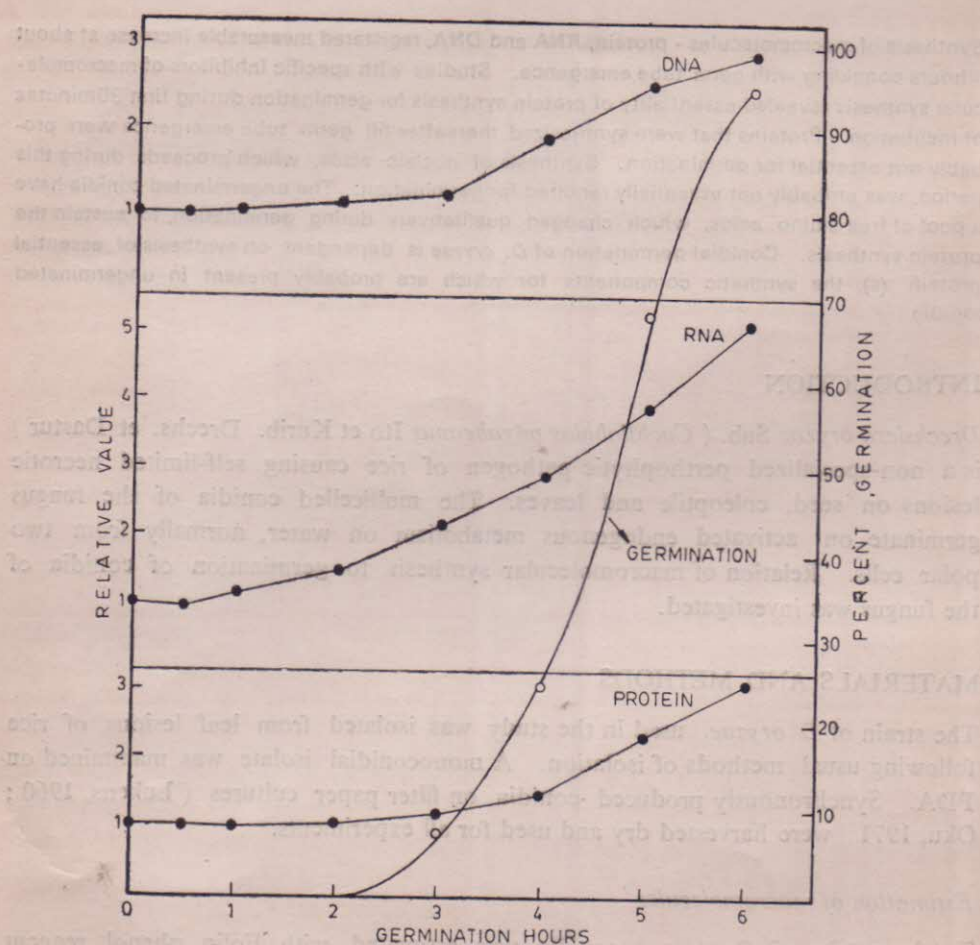


Fig. 1. Synthesis of macromolecules in the germinating conidia of *D. oryzae*: Ordinate values are relative to 0 h value considered 1 (actual 0 h values were, protein—145.5, RNA—32.0, DNA—4.35 $\mu\text{g}/\text{mg}$ dry spores).

RESULTS

Net syntheses of macromolecules during germination

Changes in net syntheses of protein, RNA and DNA in the germinating conidia were measured by actual estimation of macromolecules at various times after the onset of germination metabolism. Increase in net synthesis of protein, RNA, DNA during germination was evident at about 3 hours, almost coinciding with the start of emergence of germ tubes. Earliest measurable increase in net synthesis was for RNA first (2h), then protein (3h) and then DNA. There were approximately 3.5 and 3 fold increases in total protein, RNA and DNA respectively at 6 hours when most conidia had germinated (Fig. 1).

Changes in the free amino acid pool

Consequent to germination, the free amino acid pool in the conidia showed both qualitative and quantitative changes. The normal pool of eleven free amino acids changed to fifteen detectable amino acids (and amides) and two unidentified ninhydrin positive substances. Except for glutamine and aminobutyric acids registered increase in 3h germinated conidia. Total free amino acid pool, however, remained constant (Table 1).

Table 1. *The pool of free amino acids in the germinating conidia of Drechslera oryzae*

Total nitrogen of ungerminated conidia (mg/g dry wt.)	54.6	
Total free aminonitrogen of conidia (% of total N)	Ungerminated	4.2
	Germinated	4.6
Amino acids	*Relative amounts in conidia	
	Ungerminated	Germinated ^(a)
Cyst (e) ine	1.8	2.6
Aspartic acid	6.2	7.5
Glutamic acid	18.5	23.8
Serine	1.4	2.3
Glycine	—	1.6
Threonine	—	1.4
Alanine	15.8	18.4
Lysine-Histidine	—	1.4
Arginine	1.9	2.6
γ -aminobutyric acid	8.8	2.8
Valine - Methionine	3.3	4.2
Phenyl alanine	4.2	5.6
Tryptophan	—	1.2
Leucin - Isoleucin	3.9	4.6
Glutamine	15.6	3.3
NPS 1	—	1.8
NPS 2	—	1.2

* Amino nitrogen of individual amino acids as percentage of total amino N.

(a) Conidia germinated for 3 hours.

NPS 1 and NPS 2 - Ninhydrin positive substances remaining unidentified.

Table 2. *Effect of various inhibitors of macromolecular synthesis on conidial germination of D. oryzae*

Conc. $\mu\text{g/ml}$	Inhibition (%)	
	Germination ^a	Germ tube growth ^b
Actidione (cycloheximide)		
0	0	0
10	24.5	32.8
20	62.3	70.0
40	100	100
80	—	—
Proflavin sulfate		
0	0	0
10	3.8	15.0
20	20.2	46.0
40	47.5	84.0
80	68.0	95.5
6-Mercaptopurine		
0	0	0
10	0	0
20	6.2	14.0
40	19.6	42.5
80	35.2	63.0
160	61.3	92.5

Average of 5 x 200 conidial counts

Table 3. *Inhibition of germination of D. oryzae conidia by time lapse addition of actidione*

Time of addition (Minutes)	Germination inhibition (%)	Reduction in germtube growth (%)
0	100	100
10	100	100
20	100	100
30	100	100
40	96.2	97.4
50	73.5	90.3
60	54.2	82.0
70	28.8	76.5
80	11.7	70.2
90	5.2	65.2

Average of 200 x 3 conidial counts

Macromolecular synthesis and germination

Both initiation and development of germtubes were sensitive to low concentrations of the antibiotic, cycloheximide (Table 2). Conidial germination was sensitive to only relatively high concentrations of proflavin sulfate and 6-mercaptopurine. Proflavin at such high concentration may inhibit, besides RNA polymerase, both DNA polymerase activity (Franklin and Snow, 1971) and also protein synthesis (Waring, 1966). Growth of germtubes was more sensitive to proflavin and 6-mercaptopurine than initiation of germ tube (Table 3).

Relationship of protein synthesis to germination was further elaborated by time lapse addition of cycloheximide to germinating conidia. Results showed that cycloheximide when added during 0-30 min. of incubation caused total inhibition of germination. But addition of cycloheximide at 40 min. and thereafter caused only partial inhibition and when added at 90 min of incubation, cycloheximide failed to inhibit germination at all.

DISCUSSION

Increases in the net synthesis of macromolecules in the germinating conidia of *D. oryzae* were almost concomitant to the initiation of germ tubes. This and the apparent sequence of increases in net synthesis of macromolecules (RNA first, then protein and then DNA) as evidenced by analytical methods of estimation, were comparable to the similar findings for few such multinucleate conidia already reported (Lovett, 1976). The observed concomitance of increased net synthesis of protein and RNA to germ tube emergence, however, do not reveal the actual metabolic events during germination. The later experiments on germination inhibition with cycloheximide, had shown increased protein synthetic activity in conidia quite prior to germ tube emergence.

Essentiality of protein synthesis for conidial germination was established from the results of germination inhibition with low concentration of cycloheximide. The data with regard to RNA synthesis, although not very conclusive, suggested that nucleic acid synthesis, particularly of RNA, may not be essential for germination. Concentrations of 6-mercaptopurine and proflavin that caused germination inhibition of conidia were not expected to inhibit RNA synthesis specifically. That low concentrations of these compounds had no significant inhibitory activity to conidial germination suggested independence of germination to nucleic acid, particularly RNA, synthesis. Evidence with regard to proflavin sensitivity of another porospore conidia, that of *Alternaria solani*, provided similar conclusions (Hollomon, 1970).

Although protein synthesis appeared essential for germination, not all proteins that are synthesized prior to germination in conidia are essential for germination.

It appeared that proteins essential for germination are synthesized at very early stages of germination, at least prior to 90 minutes of germination incubation. Analysis of amino acid pool in the germinating conidia revealed that a pool free amino acids are present in that conidia that may sustain the essential protein synthesis during initial hours of germination.

It seems that *D. oryzae* is included within the general category of fungi where protein synthesis is essentially required for germination and the transcriptional, translational and other components of synthetic activities reside in the ungerminated conidia.

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