incorporated into the soil seven days after sowing, finally providing the same treatments as described above. After 40 and 50 days of plant growth, five plants from each treatment were harvested for determining the percentage of root segments showing the mycorrhizal fungus and the number of vesicles or spores per one cm root in a random root sample of 20 to 30 (one cm) bits[7]. The data on per cent colonization and the average vesicle/spore number were statistically analysed employing test for equality of proportions and test for equality of means respectively as mentioned earlier[9].

The plants infected with *G. mosseae* alone exhibited greater mycorrhizal formation (table 1). When the mycorrhizal fungus and *F. solani* were inoculated simultaneously, no significant reduction in root colonization was observed. But, at the end of 50 days of plant growth, sporulation by the fungus was significantly affected under the influence of the pathogen. On the other hand, both colonization and vesicle and/or spore formation by the mycorrhizal fungus were inhibited significantly due to the impact of *R. solani*, even by 40 days. Thus, the antagonistic effect of this fungus led to only about half the development of mycorrhiza compared to that observed with only *G. mosseae* inoculation.

The development of mycorrhiza was not affected when the two fungi were introduced separately into the soil after inoculation with *G. mosseae*. It was reported earlier that *G. mosseae* colonized the roots of groundnut after eight days of sowing[10]. The present investigation clearly shows that the root-infecting fungi do not exert any antagonistic effect on the mycorrhizal endophyte once the latter colonizes and establishes in roots.

Table 1 *Impact of F. solani and R. solani inoculation on the development of mycorrhiza in groundnut*

Inoculant	Mycorrhizal development (days after plant growth)			
	Experiment 1		Experiment 2	
	40	50	40	50
Glomus mosseae	42[a](4.9)[b]	48 (5.3)	38 (4.1)	42 (4.6)
G. mosseae + *F. solani*	28 (2.6)	32 (3.0)*	36 (3.9)	38 (4.3)
G. mosseae + *R. solani*	24*(2.1)*	28*(2.5)*	34 (3.6)	38 (4.0)

VAM fungus and the root-infecting fungus were introduced simultaneously in experiment 1; VAM fungus was introduced one week earlier than the root-infecting fungus in experiment 2

[a]Per cent root segments showing the VAM fungus, [b]Number of vesicles or spores per one cm root

*Significantly different ($P < 0.05$) from the corresponding value with *G. mosseae* inoculation alone

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1. Mosse, B., *Annu. Rev. Phytopathol.*, 1973, **11**, 171.
2. Baltruschat, H. and Schonbeck, F., *Phytopathol. Z.*, 1972, **74**, 358.
3. Dehne, H. W. and Schonbeck, F., *Phytopathol. Z.*, 1979, **95**, 105.
4. Zambolin, L. and Schenck, N. C., *Phytopathology*, 1981, **71**, 267.
5. Ross, J. P., *Phytopathology*, 1972, **62**, 896.
6. Davis, R. M., Menze, J. A. and Zentmeyer, G. A., *Phytopathology*, 1978, **68**, 1614.
7. Parvathi, K., Venkateswarlu, K. and Rao, A. S., *Curr. Sci.*, 1984, **53**, 1254.
8. Parvathi, K., Ph.D. Thesis, Nagarjuna University, 1984.
9. Parvathi, K., Venkateswarlu, K. and Rao, A. S., *Trans. Br. Mycol. Soc.*, 1985, **84**, 29.
10. Parvathi, K., Venkateswarlu, K. and Rao, A. S., *Proc. Indian Acad. Sci.*, 1984, **93**, 105.

SHIKIMATE-SENSITIVE ISOZYME OF 3-DEOXY-D-ARABINOHEPTULOSONATE-7-PHOSPHATE SYNTHASE IN THE CYANOBACTERIUM *NOSTOC LINCKIA*

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THE regulatory isozymes of 3-deoxy-*d*-arabinoheptulosonate 7-phosphate synthase from the cyanobacterium *Nostoc linckia* were studied. One isozyme was sensitive to shikimic acid, and the other two isozymes were sensitive to phenylalanine and tyrosine respectively. No inhibition of DAHP synthase activity was observed in the presence of tryptophan. The observed inhibition of DAHP synthase activity by shikimate was due to the shikimate-sensitive isozyme and not due to the intermediate metabolite in the biosynthesis of aromatic amino acids

The first reaction in the pathway of aromatic amino acid biosynthesis is catalyzed by the enzyme 3-deoxy-*d*-arabinoheptulosonate 7-phosphate (DAHP) synthase. The regulation of its activity has been the subject of considerable interest. A comparative study[1] showed that the production of differentially regulated isozymic species of DAHP synthase is a common mechanism for the regulation of the aromatic pathway. The role of shikimic acid as a common intermediate metabolite in the biosynthesis of aromatic amino acids has been recognized for quite some time[2-5]; its involvement in cyanobacterial metabolism is not known, and previous studies[6] of DAHP synthase regulation in the cyano-bacteria did not suggest the presence of shikimate-sensitive isozyme species. Here we report the presence of shikimate-sensitive isozyme of DAHP synthase in *N. linckia.*

N. linckia, a rapidly growing, sporulating, nitrogen-fixing filamentous cyanobacterium, was the test organism used. The alga was grown axenically in Allen and Arnon's[7] medium, free of combined nitrogen sources. For DAHP synthase assay, cells (1 mg protein) were harvested in mid-exponential phase and were concentrated in 0.5 ml of medium, treated with 0.1 ml of toluene for 5 minutes at 37°C. The whole cell assay was performed as described by Gollub *et al*[8]. The reaction mixture contained 2.5 mM each of phosphoenol pyruvate and erythrose 4-phosphate and 50 mM potassium phosphate buffer (pH 7.5) in a final volume of 0.6 ml. Maximum inhibition of DAHP synthase activity was attained at 100 μg ml^{-1} concentration of L-phenylalanine and shikimic acid, whereas maximum inhibition by L-tyrosine was attained at a concentration of 150 μg ml^{-1}. No inhibition of activity was observed in the presence of tryptophan.

It can be concluded from table 1 that at least 31 % of the total activity was due to the phenylalanine-sensitive isozyme, 52.4 % was due to tyrosine-sensitive isozyme and the remaining 16.6 % activity was due to the shikimate-sensitive isozyme.

Table 1 *Effect on supplementation on DAHP synthase activity of Nostoc linckia.*

Strain control	Specific activity 50	100 (μg ml^{-1})	150
Nostoc linckia.			
L-phenylalanine	2 3	2 0	2 0
L-Tyrosine	1 95	1.70	1 38
L-Tryptophan	2.9	2 9	2 9
Shikimic acid	2 8	2.42	2 42
Phenylalanine + tyrosine	–	0 48	–

Specific activities are expressed as nmol of product formed min^{-1} mg protein^{-1} (All values are mean of three replicates)

Shikimic acid can serve as a precursor of aromatic amino acids for bacteria and fungi, provided earliest clues are known as to the nature of the intermediates involved in aromatic amino acid biosynthesis[2]. Shikimic acid substituted for the aromatic amino acid requirements of many auxotrophs indicates that it was the common precursor of at least five different aromatic compounds[2]. However, the organization and control of the enzyme activities and the relevant genetic relationships differ markedly from one group of organisms to another. Subtle variations may also exist even between very closely related species[6]. The inhibitory effect of shikimic acid was attributed to the inhibition of DAHP synthase activity in *N. linckia* which then leads to starvation for pre-cursors of phenylalanine, tyrosine and tryptophan. Shikimate-inhibited cultures also contained decreased level of phycocyanin pigments[10], a characteristic, previously correlated with amino acid starvation in the cyanobacteria.

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1. Jensen, R. A., Nasser, D. S. and Nester, E. W., *J. Bacteriol.*, 1967, **94,** 1582.
2. Davis, B. D., *J. Biol. Chem.*, 1951, **191,** 315.
3. Smith, L. C., Ravel, J. M., Lax, S. R. and Shive, W., *J. Biol. Chem.*, 1962, **237,** 3566.
4. DeMoss, J. A., *J. Biol. Chem.*, 1965, **240,** 1231.
5. Weber, H. L. and Bock, A., *Arch. Mikrobiol.*, 1968, **61,** 159.
6. Hall, G. C. and Jenson, R. A., *J. Bacteriol.*, 1981, **148,** 361.
7. Allen, M. B. and Arnon, D. I., *Plant Physiol.*, 1955, **30,** 366.
8. Gollub, E., Zalkin, H. and Sprinson, D. B., *J. Biol. Chem.*, 1967, **242,** 5323.
9. Tatum, E. L. and Bonner, D. M., *J. Biol. Chem.*, 1943, **151,** 349.
10. Kumar, H. D., Mishra, D. K. and Rai, L. C., *DAE Symposium on biological nitrogen fixation*, IARI, New Delhi, 1982, (accepted)