





Figures 1-3. 1. Chromosomal organization of dividing nucleus. Note the nuclear membrane is still intact in *Terminalia* (nm, Nuclear membrane) (×1400); 2 and 3. Multinucleate fusiform initials of *Mangifera* and *Albizzia* respectively. Note the globular nuclei in the former and spindle-shaped nuclei in the latter (2. ×900; 3. ×350).

initials in T. grandis is confirmed and its presence in Albizzia, Dalbergia, Mangifera, Morinda and Terminalia is recorded for the first time.

The multinucleate condition is perpetuated even in the differentiation of vessel elements of *D. sissoo*<sup>9</sup> while Patel<sup>3</sup> observed the uninucleate xylem and phloem of *Solanum melongena*. This needs further studies. Occurrence of multinucleate condition in the ray cell initials is recorded for the first time here in *Albizzia*, *Dalbergia*, *Tectona*, *Terminalia*, *Mangifera* and *Morinda*.

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# CHARACTERIZATION OF SHEATH FROM PHOTOHETEROTROPHICALLY GROWING STRAIN OF CALOTHRIX MARCHICA LEMM.

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The filamentous cyanobacterium Calothrix marchica grew photo- and chemoheterotrophically in the presence of sucrose and fructose in the medium. A distinct sheath layer was developed around the trichome of photoheterotrophically grown strain of C. marchica<sup>1</sup>. It was thought that considerable proportions of the sugars were assimilated by C. marchica in certain specific biosynthetic process, (for example, in the synthesis of sheath layer around the trichome). The present work was undertaken to isolate and characterize the sheath from the heterotrophically growing strain of Calothrix.

Calothrix marchica Lemm, var, intermedia Rao strain Sahu 1978/3<sup>2</sup> was used as the experimental

material. The cyanobacterium was grown photoheterotrophically in the presence of 15 mM sucrose in Allen and Arnon's nitrogen-free medium<sup>3</sup>, irradiated continuously with white light from fluorescent tubes (2400 lux). The organism was harvested after 20 days of cultivation and washed once with 20 mM Tris-HCl buffer, pH 8 (used throughout sheath isolation). The sheath material was isolated from the cyanobacterium after homogenization with an 'ultrasonicator (4°C, 30 min). Discontinuous sucrose gradient (10 ml of 60% and 5 ml each 55, 50, 45 and 40%, w/w, sucrose in Tris/HCl buffer) was loaded with the cell homogenate and run in a swingout rotor (160,000 g, 4°C, 4 h). Sheaths were recovered from the 60% sucrose band and further purified by treatment with lysozyme (5 mg in 25 ml ammonium acetate buffer, pH 6.8, 37°C) followed by extraction with sodium dodecyl sulphate (2%, w/w in Tris/HCl buffer, 100°C, 15 min). The yield of the sheath material was 8.2% on a cell dry weight basis.

Hydrolysis of the carbohydrates and polypeptides and the determination of monomers have been described elsewhere<sup>4</sup>. The neutral sugars were separated, identified and quantified by gas liquid chromatography as alditol acetate derivatives (Varian aerograph model 1445-1, ECNSS-M column, 3% on Gas chrom Q, 100-200 mesh). Fatty acids were identified as methyl esters by gas liquid chromatography (EGSS-X column, 15% on Gas chrom P, 100-200 mesh). Uronic acids were quantitatively determined by colorimetric carbazole assay at 530 nm. Amino acids and amino sugars were separated (in 4N HCl, 110°C, 16 h) and quantitatively determined in an automatic amino acid analyser. Organic phosphorus content was determined following the method of Lowry et al<sup>3</sup>.

Chemical analysis of the isolated sheath of C. marchica showed that the neutral sugars were the major components of the sheath fraction; their content increased from 40.5% (gradient purified) to 84.5% (lysozyme + SDS). All fractions contained predominantly glucose and mannose. Absence of muramic acid, diaminopimelic acid and \(\beta\)-hydroxy fatty acids in the lysozyme + SDS extracted sheath fraction showed that it was free from the cell wall components. Glucosamine, uronic acids, fatty acids and phosphorus were detected only in trace in the purified sheath and their amount was 0.25% (w/w), 0.3% (w/w), 0.4% (w/w) and 0.2% (w/w) respectively (table 1). The sheath fraction from the sucrose gradient contained 10.8% (w/w) protein. The total amino acid content was not solubilized by treatment

Table 1 Chemical analysis of the sheath fractions of photoheterotrophically grown filaments of Calothrix marchica Lemm. var. intermedia Rao (Values represent per cent dry weight of the sheath material)

Constituent	From sucrose gradient	After lysozyme + SDS treatment	
Sugars		····· <u> </u>	
Ribose	3.3	<del></del>	
3-O-methyl pentose	0.4		
Mannose	5.4	1.3	
Glucose	31.4	83.2	
Total sugars	<u>40.</u> 5	<u>84.</u> 5	
Total amino acids	10.8	4.2	
Fatty acids			
C 14	0.1	0.04	
C 16	0.4	0.2	
C 16:1	0.1	0.03	
C 18	0.1	80.0	
C 18:1	0.1	0.05	
β-C 16-OH	0.3	_	
Total fatty acids	<u>1.1</u>	0.4	
Glucosamine	1.7	0.25	
Muramic acid	0.76	_	
Diaminopimelic acid	1.14	<del></del>	
Uronic acids	0.68	0.3	
Phosphorus Phosphorus	3.4	0.2	

<sup>-</sup> = absent.

with lysozyme and hot SDS, however, was reduced to 4.2% (w/w). A high proportion of glutamic acid and asparatic acid was observed in the purified sheath fraction (not shown in the table).

Chemical analysis of the isolated sheath fraction of photoheterotrophically grown Calothrix filaments showed that it was predominantly composed of sugars (84.5%, w/w). Formation of sugar-rich sheath layers in the heterotrophic culture of the cyanobacterium, which in various other cyanobacteria is also composed mostly of neutral sugars<sup>4,6,7</sup>, may be the consequence of a specific biosynthetic process which is involved in the utilization of exogenous carbon compounds. By utilizing radioactive exogenous carbon sources, various steps of the biosynthetic process can be further characterized.

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## FUSARIUM SPECIES ASSOCIATED WITH GRAIN MOULD AND STALK ROT OF SORGHUM AND THEIR EFFECT ON SEED GERMINATION AND GROWTH OF SEEDLINGS

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SORGHUM [Sorghum bicolor (Linn.) Moench] is an important food crop. Among several diseases infecting sorghum, grain moulds or head moulds and stalk rot caused by species of Fusarium are of great economic importance. In recent years they are serious in all tropical sorghum-producing areas.

Curvularia sp., F. monliforme, F. oxysporum and C. lunata, Helminthosporium tetramera, F. moniliforme and F. semitectum Berk and Rav. were isolated<sup>1,2</sup> from mouldy grains of sorghum. The association of F. roseum, F. solani and F. oxy-

sporum with root zones of gramenaceous plants and F. moniliforme with stalk rot of sorghum was reported earlier<sup>3,4</sup>.

In the present investigation an attempt was made to isolate and identify the species of Fusarium associated with sorghum grain, peduncle, stalk and root and their effect on seed germination and growth of seedlings. The different species of Fusarium were isolated following the blotter technique<sup>5</sup>. The cultures were subjected to reisolation by single spore isolation method and maintained on PDA slants at  $27 \pm 1$ °C. The individual species of Fusarium were identified based on colony characters, growth rate, pigmentation, and full range of spore forms<sup>6</sup>.

The species of Fusarium were identified as F. moniliforme sheld, F. semitectum Berk and Rav. and F. oxysporum Schtect. Among these F. semitectum was isolated from grains, F. moniliforme from grains, stalk and peduncle, and F. oxysporum from roots of sorghum.

The pathogenicity of these species of Fusarium to sorghum earheads, peduncle and stalk was proved by cross-inoculation studies by using sorghum genotypes CSH-1 and CSH-5. The study indicated that all the three species of Fusarium were potentially pathogenic on sorghum genotypes CSH-1 and CSH-5 and produced distinct symptoms on grains, peduncle and stalk. It is clear that species of Fusarium involved in sorghum grain mould are also pathogenic to stalk and peduncle and the species of Fusarium involved in peduncle rot, stalk rot and root rot are pathogenic to grain.

The effect of each species of Fusarium on seed germination in soil and on moist blotter was tested separately using CSH-1 seeds by smearing the sorghum seeds with equal quantity of eight-day-old pure culture of each species of Fusarium. The per cent germination ranged from 59.5 to 69.8 and 80.1 to 93.2 in soil and on moist blotter respectively

Table 1 Effect of species of Fusarium on seed germination and growth of seedlings of CSH-1 sorghum (all values in %)

	In soil			On moist blotter in petri plate		
Fungí	Germination on 7th day	Pre-emergence death on 7th day	Post-emergence death of exis- ting seedlings on 16th day	Germination on 7th day	Pre-emergence death on 7th day	Post-emergence death of exis- ting seedlings on 16th day
F. moniliforme	61.6	38.4	100.0	80.1	19.9	86.7
F. semitectum	59.5	40.5	83.3	91.5	8.5	73.8
F. oxysporum	69.8	30.2	76.6	93.2	6.8	69.2
Control	95.5	0.5	0:0	99.8	0.2	0.0