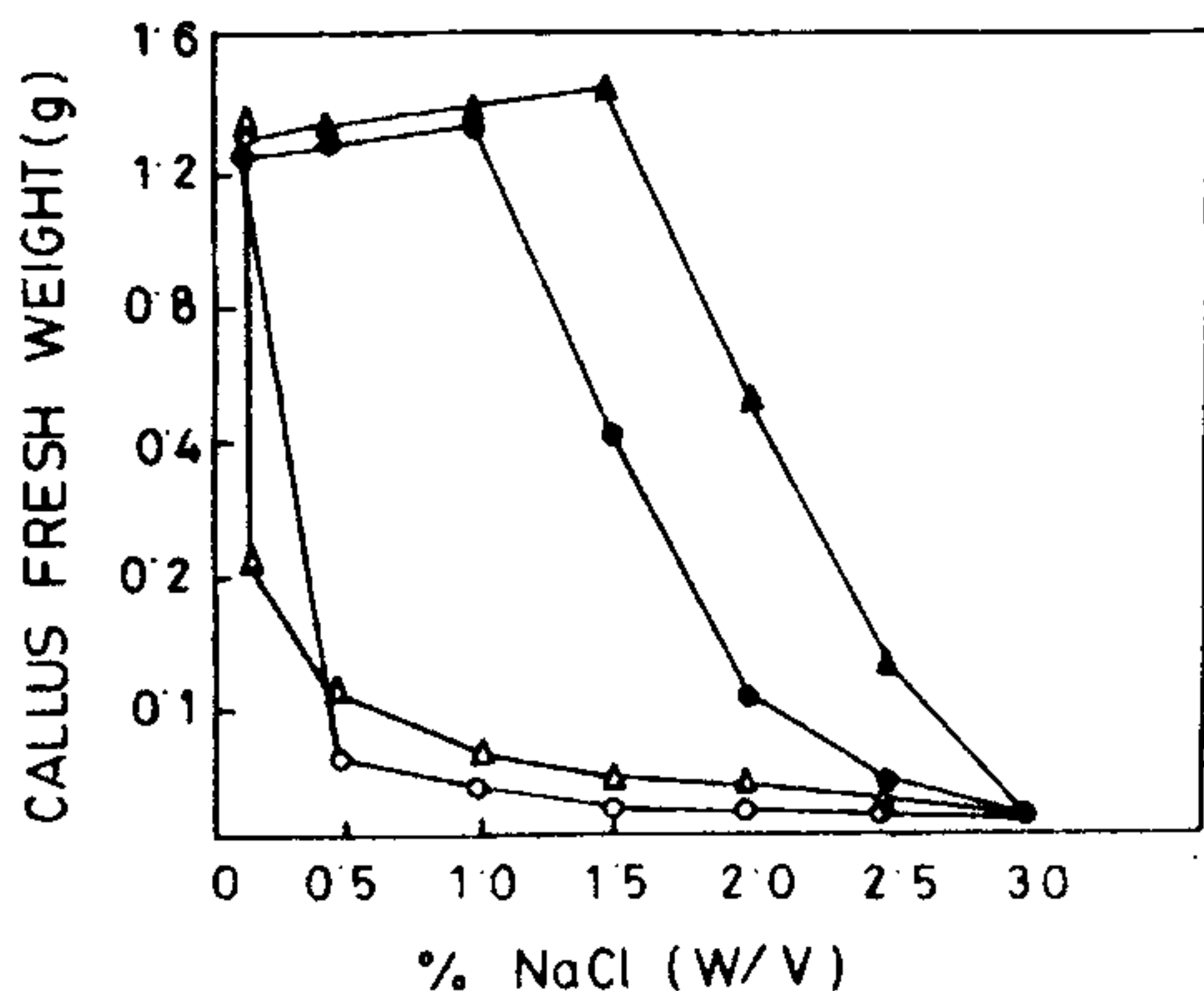


viability was tested by using fluorescence diacetate test<sup>8</sup>.

The cells were then plated at a final density of  $10^6$  viable cells/ml in petridishes (8 cm diameter) containing 5 ml of semisolid MS medium supplemented with 2,4-D (2 mg/l) + CW (10% v/v) + CH (200 mg/l) and different concentrations of NaCl (0, 0.5%, 1%, 1.5%, 2%, 2.5%, 3%). An average of 20 plates per treatment were used. Plates were scored after 4 to 6 weeks by visual observation of cells or colonies which had a healthy appearance and able to grow despite exposure to higher level of salinity. These colonies were repeatedly transferred to the respective salt containing media for 48 to 50 subcultures (one subculture period is equivalent to 15 days). After the 45th subculture the callus gained some degree of salt tolerance. The salt-selected line of cells grew better than unselected cells at high level of salt. Growth patterns (by fresh weight measurement) of these salt selected calluses was virtually identical to that of control during the culture period. Among the two cultivars, Madhu was more tolerant (1.5% NaCl) than Kiran which achieved a tolerance level of NaCl upto 1% (figure 1).

Similar observations were also reported with alfalfa and rice callus cells<sup>9,10</sup>. Cells of *Capsicum annum* and *Nicotiana sylvestris*, progressively improved their growth in 1% of NaCl during the initial phase of



**Figure 1.** Growth characteristics of rice callus selected from suspension plating at different levels of NaCl in two cultivars of rice, Kiran (—●—) and Madhu (—▲—), corresponding the control (—○—, —△— respectively) cell lines from which they were selected.

culture<sup>11</sup>. NaCl-tolerant cell lines of *Cicer arietinum*, *Pisum sativum* and *Vigna radiata* were also isolated<sup>12</sup>.

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## REPETITIVE DNA AMOUNT IN *SOLANUM NIGRUM* GENOME

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EUKARYOTIC DNAs are characterized by repetitive sequences<sup>1</sup>, which might even go up to 87% in some grasses<sup>2</sup> or 90% in *Neturus*—an animal<sup>3</sup>. The association of high repetitive sequences with heterochromatic segments of chromosomes is well illustrated<sup>4-6</sup>, and it is assumed that most of them are inert sequences. However, interspersion of repeat and unique sequences, observed in many genomes<sup>7-9</sup>, hints at important regulatory functions of repetitive sequences.

Though some data are available from plant materials (angiosperms) about the distribution of repetitive sequences, yet the economically important plant genus *Solanum* is not well represented. We undertook a systematic study of this genus, and successfully labelled its DNA<sup>10</sup>. The present report illustrates that the amount of repetitive sequences is very low in one of its species.

Nuclear DNA was isolated from young shoot-tip of *S. nigrum* Linn., following Benedich and Bolton's method<sup>11</sup> in citrate buffer. DNA was purified over hydroxyapatite (HAP) column<sup>12</sup> followed by a further purification with RNase (Sigma), Pronase (Worthington) and chloroform extraction. Precipitated DNA, with chilled ethanol, was collected by centrifugation.

One microgram DNA was nicked with endonuclease (Hind III) and then labelled with dCTP<sup>32</sup> according to Chaudhuri *et al*<sup>10</sup>. This 'hot' DNA was then mixed with hundred times unlabelled *S. nigrum* DNA, sheared at 50 K psi in 0.12 M phosphate buffer (pH 6.8), denatured by boiling at 100°C and then incubated at 60°C. At 25  $C_0t$  (mole. sec. liter<sup>-1</sup>) aliquots were taken out, quickly frozen, diluted 30 times with 0.035 M PB and then put on HAP column, previously equilibrated with the same buffer at 60°C. The column was washed thoroughly with 0.035 M PB and then with a buffer containing 0.12 N PB and 0.4% SLS to remove the unhybridized fractions. The hybridized fraction was eluted with 0.48 M PB. Collected fractions were placed in scintillation vials and counted in a liquid scintillation counter.

The yield of *S. nigrum* DNA is 1 mg for 10 g fresh weight tissue. That DNA is free from chemical contamination is evident by more than 32% hyperchromicity, good absorption ratio (table 1), zero RNase lability, efficient nicking by Hind III<sup>10</sup>, gel electrophoresis and reassociation kinetics. The  $T_m$  of this DNA is 88.5°C which corresponds to 46.83 G-C mole%. This is an additional report for plant DNAs.

Occurrence of 11.56 repetitive sequences in *S. nigrum* genome at a  $C_0t$  value where fast to middle repetitive fractions of reported plant DNAs reassociate, finds a parallel in some algae, fungi and protozoan DNAs<sup>4</sup>. This low amount of repetitive sequences in *S. nigrum* is another striking departure, as is found in Dermestes, Mollusca and Rodentia where the ratio of repetitive to non-repetitive DNA amounts in the supplementary DNA fractions is 12.68, 0.31 and 0.31 respectively. It is apparent from reassociation rate of *S. nigrum* DNA that the rate is uniform up to 25  $C_0t$  (calculated according to Britten and Khone<sup>1</sup>)—thus indicating that it represents a single DNA fraction.

Amongst eukaryotes, *Aspergillus* represents the lowest amount of repetitive sequences<sup>13</sup>, i.e. 2 to 3%. Many angiosperms, particularly legumes, exhibit small amounts (30%) of repetitive DNAs<sup>14</sup>, at an identical  $C_0t$  value, though *Lathyrus* shows 56 to 70% repetitive sequences. Murray *et al*<sup>15</sup> while working on pea and mung bean genomes, observed that small lengths of unique sequences might give an uniform reassociation rate, and that might be responsible for smaller amount of repetitive sequences in a genome. Ingle *et al*<sup>16</sup>, however, noticed that the absence of satellite DNA

Table 1 Characteristics of *S. nigrum* DNA—both labelled and unlabelled

Particulars	Native DNA (unlabelled)	Denatured DNA (labelled & unlabelled)	Reassociated DNA (labelled)	Remarks
$A_{260}/A_{280}$	1.85	1.80	1.80	Uniform
Hyperchromicity	0%	32%	30% (when denatured)	Decrease after reassociation
Temperature melting	88.5	0	Not studied	
G-C mole per cent	46.83			
Per cent hybridization at 25 $C_0t$			11.56	Front peak (0.12M) 82,284 cpm Back peak (0.48 M) 10,790 cpm



might influence the per cent repeats of a genome. However, the true significance of low amount of repetitive sequences in *S. nigrum* genome is not clearly understood.

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## ON MESOSPORA SCHMIDTII WEBER VAN BOSSE (RALFSIACEAE, PHAEOPHYCEAE) FROM THE ANDAMAN ISLANDS

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THE genus *Mesospora* was established by Weber van Bosse<sup>1</sup> with *M. schmidtii* as the type on the basis of material collected in Indonesia. Later it was reported from Malayan Archipelago<sup>2</sup>, Vietnam<sup>3</sup>, Solomon Islands<sup>4</sup> and Japan<sup>5</sup>. This is the first report of this genus from Indian coasts.

During a visit to the Andaman islands the authors collected *Mesospora schmidtii* Weber van Bosse from Chiryatapu, about 20 km from Port Blair. The alga grows epiphytically on roots of *Rhizophora* sp (figure 1) as thin, circular to irregular brownish crusts with marginal growth (figure 2), about 335  $\mu\text{m}$  thick and 2-3 cm in diameter. The thallus has a basal disc composed of 1-4 layers of cells, from which somewhat clavate erect filaments arise (figure 3). The branching of filaments of the basal disc can be seen by scrapping the erect filaments. The diverging basal filaments laterally adhere to form a disc (figure 4). Erect filaments are held together loosely and are easily separated by applying slight pressure. Cells of the basal layers are radially cylindrical or barrel-shaped, 10-11  $\mu\text{m}$  long and 6-10  $\mu\text{m}$  high. The erect filaments are composed of upto 28, slightly barrel-shaped cells, which are about 6  $\mu\text{m}$  broad and 12  $\mu\text{m}$  high. The tip cell of each erect filament is slightly enlarged, about 9  $\mu\text{m}$  broad and 9-18  $\mu\text{m}$  high. Each cell has a single plate-like chromatophore and several fucosan vesicles (figure 5). Hairs in groups are produced in the place of erect filaments from the basal layer (figure 6). Plurilocular sporangia are intercalary in position on erect filaments, located 1-3 cells below the apex of erect branches. Each plurilocular sporangium may consist of a single linear series of two to four cells, each of which appears to divide by both longitudinal and transverse walls. The plurangium measures 22-31  $\mu\text{m}$  in length and 6-8  $\mu\text{m}$  breadth (figure 7). It must be stated that totally mature sporangia have not been observed. Examination of lower parts of the erect filaments revealed the presence of unilocular sporangia on plants distinct from those having plurilocular sporangia, had a small stalk cell, and were club-shaped, 71-99  $\mu\text{m}$  long and 25-35  $\mu\text{m}$  broad (figure 8).

Habitat: Growing on roots of *Rhizophora*, collected at Chiryatapu, Port Blair on 4.3.1982. Leg. V.