



Figures 1–4. 1. Proliferation of callus from cut margin of a leaf disc on MS + 2,4-D (1 mg/l) + kinetin (0.5 mg/l). Five-week old culture  $\times 1.4$ . 2. Induction of multiple shoot buds (sb) from leaf callus subcultured on MS + kinetin (1 mg/l). Four-week-old culture  $\times 1.5$ . 3. Rooted shoot on MS liquid medium with filter paper support. Eight-week-old culture.  $r$  = root  $\times 0.6$ . 4. Metaphase chromosomes ( $2n = 24$ ) from a squash preparation of root tip of an *in vitro* formed plant  $\times 1250$ .

treatment 24 cultures were raised and all experiments were repeated at least thrice.

The leaf discs cultured on MS containing 1.0 mg/l 2,4-D and 0.5 mg/l kinetin, produced callus from cut margin of the leaf disc after 4–5 weeks of culture (figure 1). Five-week old calli obtained from these cultures if subcultured on medium supplemented with 0.5, 1.0, 2.0 or 3.0 mg/l kinetin, grew well and formed a number of small green protuberances on their surfaces. After 4–5 weeks of subculture 8–12 shoot buds differentiated from the green tissues and developed into shoots (figure 2). The percentage of cultures showing shoot multiplication at 0.5, 1, 2 and 3 mg/l kinetin was 20, 27, 23 and 15 respectively. Application of 0.5, 1, 2 and 3 mg/l IAA alone stimulated only root

formation from the subcultured calli. In combination with kinetin (0.5 to 2.0 mg/l) IAA at lower concentration suppressed shoot bud differentiation, and on medium containing 2 mg/l IAA and 3 mg/l kinetin the calli remained completely unorganized.

For rooting the *in vitro* formed shoots were isolated and transferred to a filter paper bridge on MS basal liquid medium. Roots emerged directly from the base of shoot after 2 weeks of transfer resulting in the establishment of complete plantlet (figure 3). Diploid nature ( $2n = 24$ ) of *in vitro* formed plants was established by examination of acetocarmine squashes of root tips (figure 4).

The present study clearly demonstrates the possibility of vegetative multiplication of *S. torvum* in cultures via shoot differentiation from leaf callus.

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## BIOCHEMICAL ACTIVITIES OF SOME THERMOPHILIC FUNGI

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IN recent years, there has been considerable interest in utilizing thermophilic fungi in different industrial fermentation processes because of several advantages that high temperature fermentation affords. There are many reports on enzyme production<sup>1–5</sup> and antimicrobial activities<sup>6, 7</sup>, but none on organic acids and phenolic compounds produced by thermophilic fungi. An attempt was therefore made to study the extracellular products of four thermophilic fungi *viz*, *Nodulisporium thermoroseum* A Subrahm and

Mehrotra, *Mucor thermohyalospora* A Subrahm; an unidentified species of *Mucor* (ABCT) and *Talaromyces* sp, for total phenols, soluble protein, lactic and citric acids.

The test fungi maintained on PDA medium were grown on 100 ml static Czapek's Dox - liquid medium (with 3% sucrose) and incubated for seven days at 45°C. Spore suspension (0.1 ml) prepared aseptically by adding 5 ml sterile distilled water on to a healthy sporulating ten-day-old PDA culture slant grown at 45°C was used as the inoculum. Three replicates were maintained for each species and at the end of the incubation period the mycelium was separated by filtering through *Whatman* No. 1 filter paper. The culture filtrates were centrifuged at 3000 rpm for 5 min; 25 ml of this filtrate were refluxed with 80% ethanol for 10 min. The refluxed material was used to estimate total phenols<sup>8</sup>. The filtrate (75 ml) was used to estimate soluble proteins<sup>9</sup>, lactic<sup>10</sup> and citric<sup>11</sup> acids. Confirmatory tests for the presence of lactic and citric acids were carried out employing thin layer chromatographic method<sup>12</sup>.

The test fungi differed significantly in the extra-cellular products (table 1). The high protein content observed in *Talaromyces* sp, *Mucor* sp (ABCT) and *N. thermoroseum* respectively, shows their ability to produce large amounts of enzymes and thus a better survivor as observed for species of *Alternaria*<sup>13</sup> and *Curvularia pallescens*<sup>14</sup>.

Phenolic compounds are widely distributed in fungi<sup>15</sup> and the present investigation shows that thermophilic fungi are no exception. Probably, synthesis of these compounds may offer an additional advantage in survival and colonization of these thermophiles in a highly competitive environment. The ability of algae to produce lactic acid, and the amount formed have been found to be significant in the relationship between strains and species of *Chlorella*<sup>16</sup>. Though all the four thermophilic fungi produced

significant amounts of lactic and citric acids, *Mucor* sp, (ABCT), produced higher amounts.

The present study clearly indicates the industrial potential of these strains as elaborated from their chemical constituents of culture filtrates.

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**Table 1** Phenols, proteins and citric and lactic acids in culture filtrates of some thermophilic fungi (mg/ml)

Constituents	<i>Mucor</i> sp. ABCT	<i>N. thermo-</i> <i>roseum</i> (Tree)	<i>M. thermo-</i> <i>hyalospora</i> (MPV)	<i>Talaromy-</i> <i>ces</i> sp. (Yellow)
Total phenols	0.1332	0.2376	0.09	0.0477
Citric acid	0.280	0.100	0.210	0.150
Lactic acid	0.029	0.016	0.015	0.008
Protein	0.92	1.0	0.8	5.2

## SEXUALITY AND OXIDASE TESTS OF *HEXAGONIA APIARIA* (PERS.) FR.

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NOBLES<sup>1</sup> put forward the hypothesis that in Polyporaceae, species which possess tetrapolar type of