sinus close and flat up to 2/3 across and roundly open outside; 33–41 μm long, 25–26 μm wide, isthmus 8.0–8.5 μm, 15–16 μm wide in lateral view.

Iconotype: Figure 6.

Among different taxa of *C.anceps* Lund, the present alga can be compared with f. *crispula* Nordst[9] in having flat apical margin, but differs much in shape of semicells and marginal curvatures being not deep in the present form.

Cosmarium nygaardii Bongale sp. nov. (figure 7) Semicellulae rhombeae a fronte et fere circulares a latere visae; granula in serieb :s verticalibus deposita; tubercula 3 apicalia et unum sub isthmo sunt; sinus clausus; 29–30μm longae,22.0–23.5μmlatae; isthmus 5·0–5·5 μm, a latere visae 16·5 μm crassae.

Iconotypus : Figure 7.

Semicells rhomboid in front and nearly circular in side view; granules arranged in vertical rows; three apical and one subisthmal tuberculations; sinus open; 29–30 μm long, 22.0–23.5 μm wide, 5.0–5.5 μm isthmus, 16.5 μm broad in side view.

Iconotype : Figure 7.

Shape of the present alga resembles that of *C. bituberculatum* Fritsch et Rich[10], but differs much in having three subapical (instead of two) and additional subisthmal tuberculations; it can also be compared slightly in shape with *C. subnudiceps* West et West[11], but both these taxa are without granular walls. In view of the above distinctive characters, a new taxon is raised and is named after the algologist Nygaard Gunnar.

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MYCELIAL AMINO ACID COMPOSITION OF DIFFERENTIALLY VIRULENT ISOLATES OF *RHIZOCTONIA SOLANI*

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RHIZOCTONIA SOLANI is a versatile pathogen and has gained considerable importance as it attacks an array of important crops causing severe losses. Sheath blight incited by *R. solani* is one of the major diseases of rice leading to serious damage of the crop in all rice growing countries.

Reddy and Rao[1] earlier reported greater production of amino acids by a virulent *R. solani* isolate from groundnut, than by a non-virulent isolate. Ramalingam[2] obtained a positive correlation between the toxin (*p*-HPAA) production by *R. solani* isolates and their virulence rating. Aromatic amino acids, L-phenylalanine and L-tyrosine, have been known as the main precursors of certain toxic metabolites produced by *R. solani*[3]. Hence, a preliminary investigation was carried out on the possible correlation between the mycelial amino acid composition of five *R. solani* isolates from rice and their relative virulence.

Five differentially virulent *R. solani* isolates from rice[4], designated as R1, R2, R3, R4 and R5 (ATCC No. 48502, 48503, 48570, 48504 and 48505, respectively) were used in the present study.

Mycelial mats, from 8-day-old potato dextrose broth grown cultures (static condition), were harvested after filtering through a four-layered cheese cloth. Any sclerotia found were removed with a forceps and the mats were repeatedly washed with glassdistilled water. The amino acids were extracted by boiling the mycelia in water for 15 min and the suspension was centrifuged. The pellet was reex-

tracted with water and centrifuged[5]. The supernatants were pooled, evaporated to dryness on a water bath and the residue was dissolved in a known volume of glass distilled water (0.3 ml/100 mg mycelial dry weight).

The cell wall bound amino acids were extracted following the method of Mani[5]. The fungal mycelia were ground in a pre-chilled mortar and pestle with NaCl. The resulting suspension was centrifuged at 10,000 rpm for 15 min and the pellet was washed five times with water to remove NaCl. The sediment was then taken in 25 ml of water containing sodium lauryl sulphate (1%, V/W). This mixture was incubated for 18 hr under shaking condition at 25°C, after which it was centrifuged. The residue was washed twice with water, then with 50%, 75% ethanol and finally absolute ethanol and dried in an incubator at 60°C. A known weight (100 mg) of this dry residue was taken in 6 N HCl and hydrolyzed at 120°C for 3 hr in sealed vials. After hydrolysis, HCl was removed by evaporation and the residue was taken in a known amount of water.

Amino acids were resolved by two-dimensional thin layer chromatography[7] (TLC) on 0.5 mm thick silica gel plates, previously activated at 120°C for 30 min. The chromatograms loaded with 25 μl of the extracts representing 8.33 mg mycelial dry weight, were run first in *n*-butanol: acetic acid: water (4:1:1) and subsequently with phenol: water (75:25) to a distance of 10 cm. The chromatograms were developed by spraying with ninhydrin (300 mg dissolved in a mixture of 3 ml glacial acetic acid and 100 ml *n*-butanol), and incubating at 60°C for 30 min. The individual amino acids were counted by eluting the ninhydrin colour with 50% acetone and measuring the optical density at 560 nm. Quantification was done by intrapolating the results from the respective standards and is expressed as mg/g dry weight.

The free and bound mycelial amino acids of the five isolates ranged between 5–8 and 6–10 respectively. Of the mycelial free amino acids identified, only alanine, glutamic acid and aspartic acid were common to all the isolates and no specific trend was observed with either the presence or absence of other amino acids. However, lysine was present only in highly virulent isolates, R4 and R5; glutamine was present only in R1 and one unidenified amino acid in R1 and R2, the isolates of least virulence (table 1).

Noticeable variation in the quantity of bound amino acids was observed between the five differentially virulent isolates (table 2). Bound amino acids were conspicuously low in the isolate R5. Previously Reddy and Rao[1] reported greater quantitative rather than qualitative differences between culture filtrate, mycelial (free and bound) amino acids of two *R. solani* isolates from groundnut. Among the cell wall amino acids quantified, valine, alanine, glutamic acid and aspartic acid were present in all the five *R. solani* isolates (table 2). Glycine was present in R4 and R5; however only traces of serine were detected in R5. Although L-phenylalanine and L-tyrosine are the main precursors for the

Table 1 *Qualitative occurrence of mycelial free amino acids in R. solani isolates*

Amino acids	Isolates R_1	R_2	R_3	R_4	R_5
Leucine	+	+	+	–	+
Valine	+	+	+	–	+
Alanine	+	+	+	+	+
Glutamic acid	+	+	+	+	+
Aspartic acid	+	+	+	+	+
Histidine	–	+	+	+	+
Glutamine	+	–	–	–	–
Arginine	+	–	–	–	+
Lysine	–	–	–	+	+
Unidentified	+	+	–	–	–

+ : Present; – : Absent

Table 2 *Quantitative estimation of cell wall amino acids in R. solani isolates*

Amino acid	Isolates R_1	R_2	R_3	R_4	R_5
	(mg/g dry wt)				
Leucine	10.65	10.05	7.5	10.65	—
Valine	3 60	3.75	3.75	3 60	1.50
Alanine	4.50	2.50	1.20	1.05	0 60
Histidine	25.80	12.00	—	—	T
Glutamic acid	6.75	0.45	2.55	T	T
Aspartic acid	1.50	0.30	31.50	T	T
Arginine	1.35	—	2.10	0 90	—
Lysine	1.95	—	4.20	1.20	T
α-Aminobutyric acid	—	—	1.50	3.00	—
Glutamine	—	—	3.00	—	—
Glycine	—	—	—	7.50	2.70
Serine	—	—	—	—	T
Phenylalanine	—	—	—	T	—

T : Traces; – : Absent

production of *R. solani* toxin, neither of them was detected in mycelial amino acids of *R. solani* isolates, but for R4 in which traces of phenylalanine were detected in the cell wall amino acid fraction. Interestingly, in the infected rice tissue also neither of these amino acids could be detected despite their presence in the healthy tissue. The ability of *R. solani* isolates from rice to convert phenylalanine and tyrosine to metabolites such as meta-hydroxyphenyl acetic acid (HPAA), ortho-HPAA and para-HPAA respectively, was shown by Ramalingam[2] using labelled precursors in replacement culture studies.

In the present investigation, the five *R. solani* isolates from rice exhibited greater differences than similarities in their mycelial amino acid composition (qualitative and quantitative), but did not reveal any specific correlation with their relative virulence rating, contrary to the reported[1] greater production of amino acids by the virulent *R. solani* isolate from ground nut.

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A NEW ORCHID FROM COORG DISTRICT, KARNATAKA

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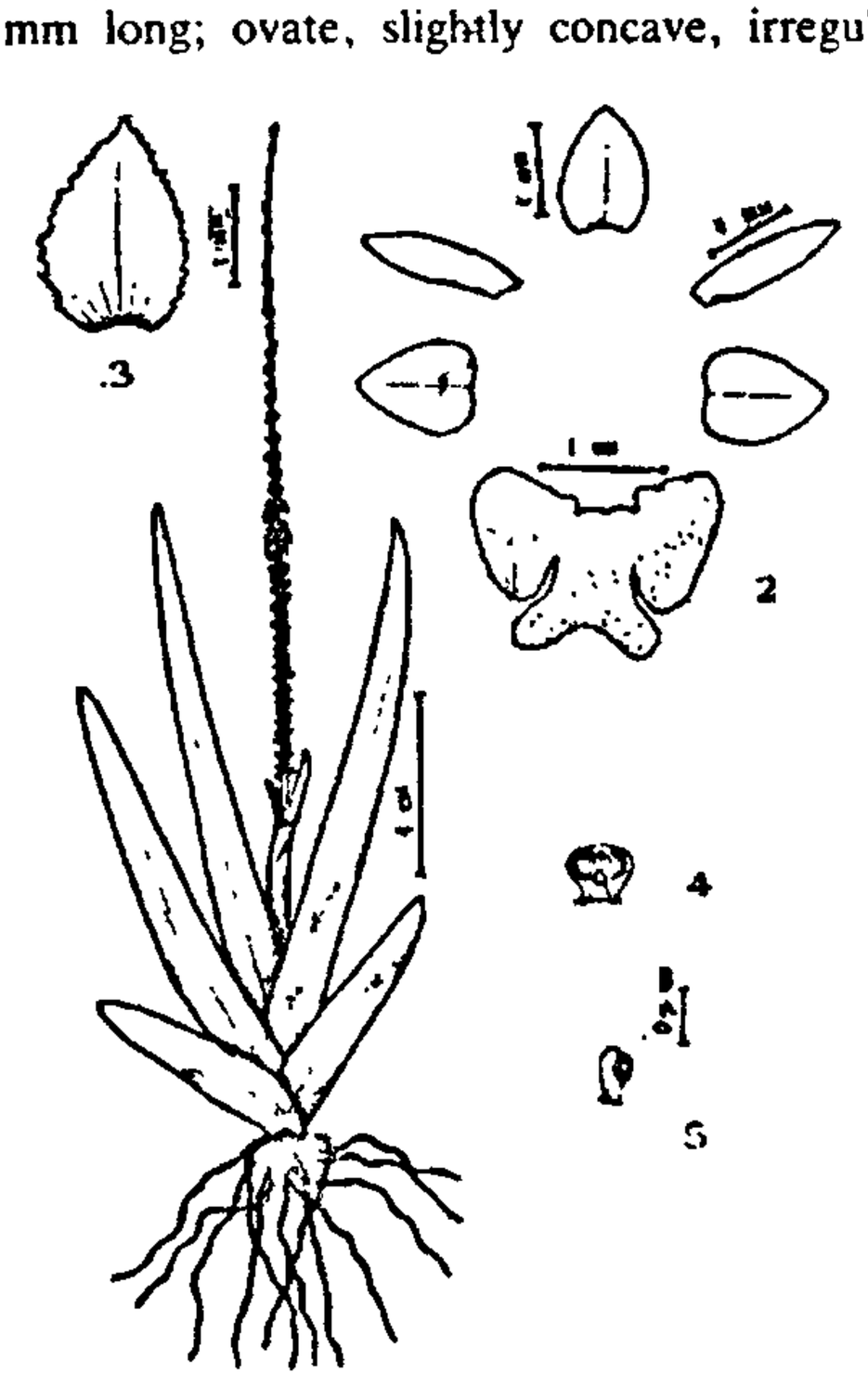
CRITICAL studies carried out on the flora of Coorg District, Karnataka revealed a new species of *Oberonia* Lindl., (Orchidaceae) which is described.

Oberonia rangannaiana sp. nov.

Oberonia brunoniana Wt. affinis sed flores manifeste pedicellatis bracteis longioribus irregulariter et lobis labii minute dentatis et papillatis differt.

Allied to *Oberonia brunoniana* Wt. but differs in having distinctly pedicellate flowers, longer and irregularly dentate bracts and lip with minutely toothed and papillate lobes.

Epiphytes. Leaves 5– 14 × 0.7 –1.1 cm, linear-oblong, slightly falcate, acute at apex, yellowish-green. Flowers 2 to 2.5 mm long, in 14 to 22 cm long spikes; pedicels 0.5 to 0.75 mm long, bracts 2 to 2.5 mm long; ovate, slightly concave, irregularly



Figures 1 to 5: *Oberonia rangannaiana* sp. nov. **1.** Plant with inflorescence; **2.** sepal, petals and lip spread out; **3.** bract; **4.** operculum; **5.** column.