with uninucleate cells. The connective tapetum is also differentiated (figure 1B). The tapetum degenerates at the pollen tetrad stage but the middle layer remains persistent (figure 1C). Simultaneous divisions in the microspore mother cells result in the formation of isobilateral and tetrahedral tetrads. Cytokinesis is by furrowing. Pollen grains are tri-aperturate and 3-celled at shedding (figure 1D). Degeneration of pollen occurs quite frequently from the microspore mother cell stage onwards.

The gynoecium is superior and the ovary is bicarpellary, syncarpous and bilocular with two orthotropous, unitegmic and tenuinucellate ovules in each locule on axile placentation (figure 1E). Integumentary tapetum is differentiated only on the micropylar region of the ovule and is multiseriate with uninucleate cells (figure 1G). A rudimentary aril is found on the funicular region of the ovule.

The hypodermal archesporial cell directly functions as the megaspore mother cell (figure 1F) which undergoes the usual meiotic divisions and gives rise to a linear tetrad of megaspores (figure 1H). The chalazal megaspore develops into an 8-nucleate embryo sac of the Polygonum type (figure 1I). Mature embryo sac is cylindrical in shape. Egg apparatus consists of an egg and two flask-shaped synergids. Polars fuse before fertilization. Antipodals are three in number and uninucleate. In rare instances, multiple embryo sacs are observed but only one reaches maturity. Pollen sterility was very high and this may be due to the early degeneration of the nutritive layer i.e. tapetum. Though a large number of flowers were studied not even a single case of fertilization of embryo sac was observed.

8 October 1986

## VARIABILITY OF ACETYLENE REDUCTION ASSAY IN THE ESTIMATION OF NITROGEN FIXATION IN TROPICAL FOREST SOILS

T. SAMBANDAM, V. MOHANKUMAR, N. MOHAN and A. MAHADEVAN
*CAS in Botany, University of Madras, Madras 600 025, India.*

Burns and Hardy[1] estimated that $4100 \times 10^6$ ha of earth's forests and woodlands account for 10 kg/ha/year of biological nitrogen fixation, which is about 28% of the total, estimated to occur in terrestrial ecosystems. Because of inadequate data from natural tropical and managed forests[2], the current estimates of nitrogen fixation are not reliable[3,4]. Therefore, we have been monitoring nitrogen fixation in Kalakad Reserve Forest, a tropical forest, located in the Western Ghats in Tamil Nadu, India. In the first instance, it was found that acetylene reduction assay (ARA) was highly variable and was influenced by soils, moisture, temperature and anaerobic conditions.

Soil samples from 10 places, from surface up to 5 cm depth, were collected from a 50 × 50 m plot in evergreen forest located at an altitude of 940 m and pooled. They were transferred to sterile polythene bags, sealed and assayed for acetylene reduction within 16 h of collection.

The soil sample (1 g) was transferred to a 250 ml Erlenmeyer flask fitted with 'Suba' seal stopper and flushed with argon for 30 sec. Immediately, 20 ml of the gas were removed from each flask by a hypodermic syringe and the same amount of acetylene was injected into it. All the experiments were duplicated.

Acetylene reduction was measured by withdrawing 0.5 ml of the gas from each flask by a gas tight plastic disposable syringe at prefixed intervals and injected into a Hewlett-Packard gas chromatograph (model 5840 A) fitted with Porapak-N column and hydrogen flame ionization detector. The temperatures of column, injection port and flame ionization detector were set at 60°, 100° and 100°C respectively. The carrier gas was nitrogen and its flow rate was maintained at 40 ml/min.

Since the temperature in the forest ranged from 22°C to 40°C, the effect of temperature on the nitrogenase activity was measured. The moisture level in the forest fluctuated from saturated condition to near dry status and the effect of moisture on ARA was also determined.

Flasks with 10 g of the soil with different moisture levels were flushed with argon and analysed for acetylene reduction.

The influence of nitrogen-free Jensen's modified medium[5] on nitrogenase activity of soil was ascertained. One set of 250 ml Erlenmeyer flask containing 1 g soil suspended in 100 ml medium and another set without the medium were used.

Nitrogen-fixing activity as measured by ARA was high at 24°C and higher temperatures reduced it (figure 1). Hardy *et al*[6] observed that the nitrogenase activity increased at incubation temperature from 10 to 20°C, reached a maximum between 20 and 30° C and declined thereafter. Akkermans[7] and Wheeler[8] found a maximum nitrogenase activity in soils between 20 and 25° C followed by a sharp

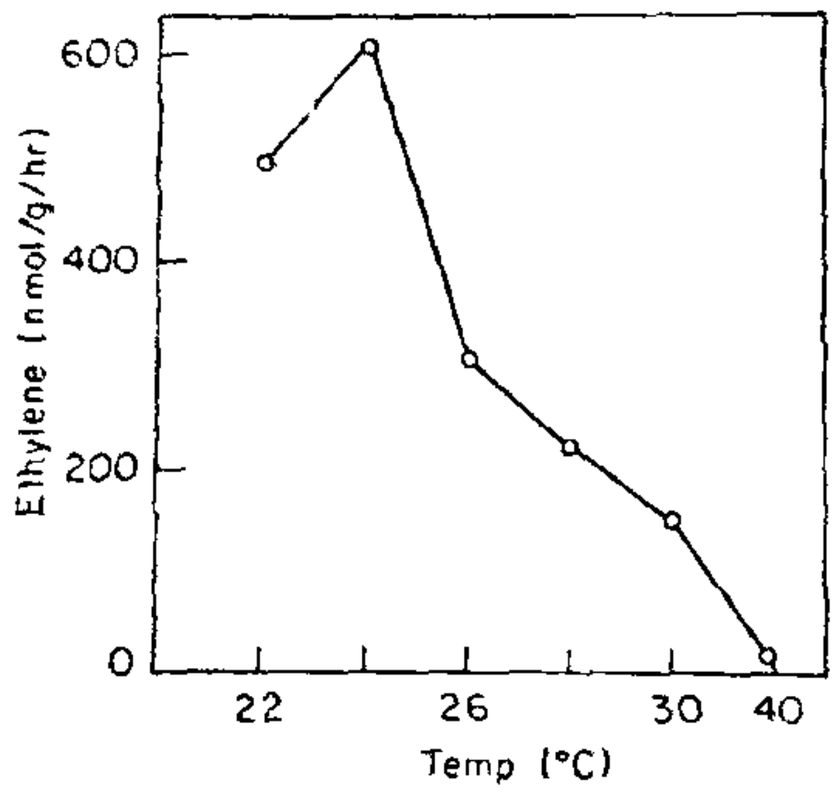


**Figure 1.** Acetylene reduction by evergreen soil influenced by temperature.

decline at higher temperatures. Schwertzer and Harper[9] also noticed that nitrogenase activity was highly influenced by the soil temperature.

Moisture level of the soil profoundly influenced nitrogenase activity (table 1). Moisture level at 25% substantially reduced ARA, while 50% increased it. Water-logging affected ARA. Indeed Rice and Paul[10] claimed that acetylene reduction was slow in water-logged soil. Increasing the moisture level beyond 50% affected the nitrogen-fixing activity in the forest soils[11]. In soils with reduced water content, nitrogenase activity was not detected[12] or reduced[13]. In silty clay and loam soils, 50% moisture content was optimum for the maximum nitrogenase activity[12]. Moisture does affect the nitrogen-fixing bacteria. Koach and Oya[12] found that the total count for nitrogen-fixing bacteria was very low in silty clay and loam soils, having 30% water content. In contrast, in rice soils, flooding activated nitrogen fixation[14] and this was attributed to the increased multiplication of nitrogen-fixing anaerobic micro-organisms. Presumably, the contributions by anaerobic micro-organisms in nitrogen fixation may not be important, as water-logging seldom occurs.

**Table 1** *Nitrogenase activity at different moisture level in evergreen forest soil measured at the end of 1 h*

| Moisture level (%) | Ethylene formed (nmol/g/h) |
|---|---|
| Air-dried soil | 11 |
| Soil + 25% moisture | 5 |
| Soil + 50% moisture | 21 |
| Soil + 100% moisture | 11 |
| Submerged soil | 6 |

**Table 2.** *Influence of medium on nitrogenase activity, using soil collected from evergreen forest*

| Time (h) | Ethylene formed (nmol/g/h) | |
|---|---|---|
| | Soil | Soil + medium* |
| 1 | 103 | 40 |
| 24 | 12 | 71 |
| 48 | 9 | 91 |
| 72 | 6 | 110 |
| 96 | 5 | 145 |
| 120 | 10 | 142 |

*Modified Jensen's nitrogen-free medium, 100 ml in 250 ml Erlenmeyer flask; incubated at 28°C.

The nitrogenase activity of soil was maximum at the end of 1 h but with the medium, the maximum activity was recorded at 96 h of incubation (table 2). Nohrstedt[11] claimed that the addition of carbon source enhanced nitrogenase activity by selectively favouring the multiplication of nitrogen-fixing micro-organisms.

The authors agree with Van Berkum and Bohlool[4] that the results of ARA test are variable and profoundly influenced by temperature, soil moisture, additives in the medium and efficiency of argon flush. Therefore investigators studying forest ecosystems for nitrogen fixation employing ARA should give cognizance to these factors.

The authors thank DST and UGC, New Delhi for financial assistance.

31 January 1987; Revised 7 September 1987

1. Burns, R. C. and Hardy, R. W. F., *Nitrogen fixation in bacteria and higher plants*, Springer-Verlag, New York, 1976, p. 510.
2. Paul, E. A., *Ecol. Bull.*, 1978, **26,** 282.
3. Dawson, J. O., *Can. J. Microbiol.*, 1983, **29,** 979.
4. Van Berkum, P. and Bohlool, B. B., *Microbiol. Rev.*, 1980, **44,** 491.
5. Brown, M. E., Burlingham, S. K. and Jackson, R. M., *Plant Soil*, 1962, **17,** 309.
6. Hardy, R. W. F., Holsten, R. D., Jackson, E. K. and Burns, R. C., *Plant Physiol.*, 1968, **43,** 1185.
7. Akkermans, A. D. L., Doctoral Thesis, University of Leiden, Leiden, 1971.
8. Wheeler, C. T., *New Phytol.*, 1971, **68,** 675.
9. Schweitzer, L. E. and Harper, J. E., *Plant Physiol.*, 1980, **65,** 51.

10. Rice, W. A. and Paul, P. A., *Can. J. Microbiol.*, 1971, **17,** 1049.
11. Nohrstedt, H. O., *Soil Biol. Biochem.*, 1984, **16,** 19.
12. Koch, B. L. and Oyą, *Y., Soil Biol. Biochem.*, 1974, **6,** 363.
13. Huang, C. Y., Boyer, J. S. and Vanderhoef, L. N., *Plant Physiol.*, 1975, **76,** 222.
14. Rao, V. R. and Rao, J. L. N., *Plant Soil*, 1984, **81,** 111.

## THE FIRST CYTOTAXONOMIC REPORT IN *CHARA BRAUNII* F. *NOVI-MEXICANA* (DIV. CHAROPHYTA)

S. K. BHATNAGAR
*Department of Botany, Bareilly College. Bareilly 243 003, India.*

*CHARA BRAUNII* is characterized by ecortication of stem and branchlets. Wood and Imahori[1] located in the section *Charopsis* along with other ecorticated forms *C. corallina* and *C. socotrensis* and identified only seven forms of *Chara braunii*. Subsequently Wood and Mason[2] added one more form. Of the eight forms of *C. braunii* complex only seven forms viz. *C. braunii* f. *braunii*[3–19], *C. braunii* f. *schweinitzii*[15], *C. braunii* f. *coromandalina*[15,20], *C. braunii* f. *kurzii*[15,21], *C. braunii* f. *oahuensis*[12,22], *C. braunii* f. *Perrottetii*[23] and *C. braunii* f. *novi-mexicana*[18] have so far been investigated for their cytological characters. Karyological investigations in *C. braunii* f. *novi-mexicana* have been undertaken for the first time while studying the charophytes of Rohilkhand division in Uttar Pradesh. The morpho-karyological studies are made by the present author on this taxon.

The plants *C. braunii* f. *novi-mexicana* were collected from a temporary pond on Sambhal road in the Moradabad District of Rohilkhand division during September-December, 1977 and 1980, and identified in accordance with the earlier provided description[1].

Plants are monoecious, 8–20 cm high. Axes 560–870 $\mu$m in diameter. Heavily incrusted. Internodes equal to branchlets. Haplostephanous. Branchlets 9–11 with coronary termination. Segments 3–4, gametangia conjoined sometimes in conjugate pairs. Oogonia 1–2, bracts 4, large, bracteoles 2, oogonia 560–833 $\mu$m long, 334–588 $\mu$m wide. Oospores black, 364–470 $\mu$m long, 250–430 $\mu$m wide. Convolutions 8–11. Antheridia 360–383 $\mu$m in diameter.

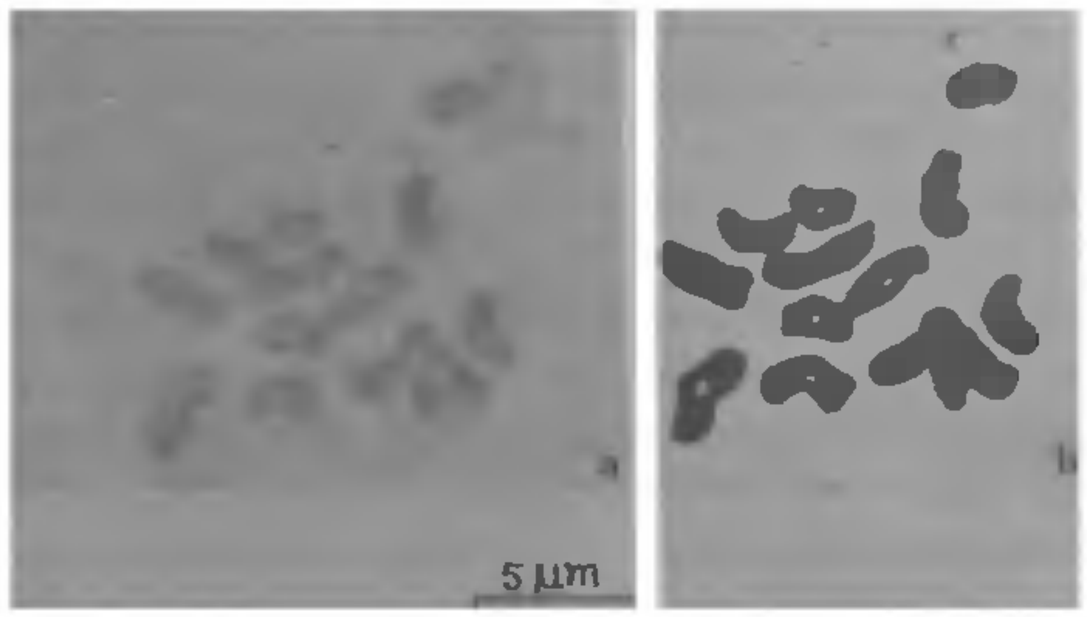


**Figure 1a, b. a.** Metaphase plate of *Chara braunii* f. *novi-mexicana* (A. Br.) R. D. W. showing 14 chromosomes; **b.** Drawing of the same.

Cytological studies revealed that the resting nucleus is spherical, 6.4–7.4 $\mu$m in diameter, nucleoli 2, 1.6–2.3 $\mu$m in diameter, chromocentres 1–2 (–3), chromosome number $n = 14$ (figure 1A, B). Chromosomes small to medium, 1.5–3.6 $\mu$m long, 0.8–1.2 $\mu$m wide. Colchicine pretreated plants exhibit 3 metacentric and 11 submetacentric chromosomes (figure 2).

The earlier studies on *Chara braunii*[3–24] complex indicate the occurrence of 7, 12, 14, 28, 35 and 42 chromosomes but the majority of *C. braunii* forms possess 14 chromosomes. The occurrence of 14 chromosomes in *C. braunii* f. *novi-mexicana* is in conformity with the previous reports in *C. braunii* complex but for the 'forma', it is a new record.

Natural polyploidization in *C. braunii* complex has resulted in the formation of polyploid races within this complex ranging from $n = 7$ to $n = 42$. Polyploidy is always associated with evolution. In the case of *C. braunii* complex, high ploidy level prevails which is an indication of advancement over other ecorticated forms of *Chara*. Wood and Imahori[1] have already assigned an advanced status to *C. braunii* on the basis of phenotypic difference and absence of dioecious taxa in this complex. Thus the phylogeny provided by Wood and Imahori[1] to *C. braunii* complex seems to be correct on cytological grounds.

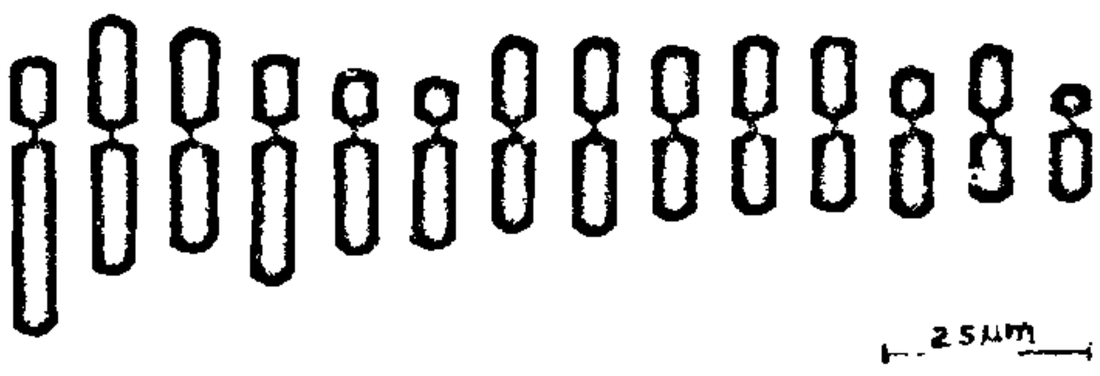


**Figure 2.** Karyotpe analysis of *C. braunii* f. *novi-mexicana.*