supply as in Merremia and Jacquemontia (Fig. D). Further evolution is marked by complete fusion of the nectary and absence of any vascular supply as in Cuscuta (Fig. E). Discussing the evolution and phylogeny of nectaries in angiosperms, Fahn (1953) has suggested "migration of the nectary from the perianth acrocentrically to the ovary". Accordingly the nectary in Cuscuta may be considered as more advanced.

Department of Botany,
University of Rajasthan,

C. M. Govil

1. Fahn, A., Phytomorphology, 1953, 3, 424.

CHANGES IN TOTAL AND SOLUBLE NITROGEN CONTENTS OF JUTE PLANTS FOLLOWING INFECTION BY MACROPHOMINA PHASEOLI (MAUBL. ASHY)

Considerable enhancement of total nitrogen content in host-pathogen complex in the early stages has been reported in a number of virus diseases as well as in potato infected with Synchytrium1 and several other hosts infected with rusts and powdery mildews2. Such increase may be due to an increase in the protein synthesis by the pathogen3. Many fungal infections also stimulate greater protein synthesis in the host than can be accounted for, by the pathogen themselves. As regards changes in soluble nitrogen, as a result of disease development, conflicting views (both increase and decrease) are reported4-6. In the majority of the cases, however, at very late stages of disease, the total nitrogen content of the fungus infected plant organs, usually decreases6. This decrease is associated with the decomposition of proteins, mainly due to the breakdown of the cell structure of infected tissues and correlated with an increased activity of the proteolytic enzymes. The present investigation has been undertaken with a view to estimating quantitatively the total and soluble nitrogen contents of the stems (2nd to 5th internode) of healthy and Macrophomina-infected jute plants (Corchorus capsularis L.) of different ages.

In analysing total nitrogen the method described by Vogel1 (1961) and modified by Gupta5 (1970) was mainly followed. 15 mg of dry tissue was digested with 2 ml of analar sulphuric acid for 45 min in a micro-Kjeldahl flask. About 0.8-1 ml of hydrogen peroxide (30%) was added to decolourise the digested material and the final volume, after decolorisation, was made up to 100 ml with distilled water. To 1 ml of the above aliquot 1 ml of a mixture of 10% sodium silicate and 10% sodium hydroxide (1 : 1) and 5 ml of Nessler's reagent were added. After 10 to 15 min the intensity of the yellowish brown colour was measured at 430 mp in a 'Hilger' pattern biochemical absorbptiometer'. A blank test was maintained with 1 ml of analar sulphuric acid treated in an identical manner. The total nitrogen content was expressed as mg/gm dry weight of tissue, by comparing the observed values.
with standard curve made from analar ammonium sulphate.

For estimating soluble nitrogen, 500 mg of fresh tissue was homogenised with 5-8 ml of distilled water and it was kept overnight in ice chamber after the addition of 1 ml of 50% TCA. It was centrifuged and the volume of the supernatant was made up to 10 ml with distilled water. Then it was digested with analar sulphuric acid and similar procedure was followed as in the case of determination of total nitrogen.

**Table 1**

<table>
<thead>
<tr>
<th>Age of the plants (in month)</th>
<th>Total nitrogen content in mg per gm of dry tissue</th>
<th>Soluble nitrogen content in mg per gm of dry tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>Infected</td>
<td>Gain (+) or Loss (−)</td>
</tr>
<tr>
<td>1:0</td>
<td>7-1 10-2</td>
<td>+3-1</td>
</tr>
<tr>
<td>1-5</td>
<td>8-4 11-8</td>
<td>+3-4</td>
</tr>
<tr>
<td>2-0</td>
<td>10-1 14-2</td>
<td>+4-1</td>
</tr>
<tr>
<td>2-5</td>
<td>13-1 18-2</td>
<td>+5-1</td>
</tr>
<tr>
<td>3-0</td>
<td>15-7 21-8</td>
<td>+6-1</td>
</tr>
<tr>
<td>3-5</td>
<td>15-9 17-4</td>
<td>+1-5</td>
</tr>
<tr>
<td>4-0</td>
<td>15-9 13-5</td>
<td>−2-4</td>
</tr>
</tbody>
</table>

The results show that the total nitrogen content of the tissues of healthy jute plant increases gradually with age up to three months after which it more or less remains uniform. Infected tissues, however, show considerable increase in total nitrogen even in one month old plants. With further aging, the infected plants also show gradual increase in total nitrogen up to three months, and then it suddenly decreases. Soluble nitrogen content gradually increases in healthy plant tissues with age up to three months. With further aging the amount of soluble nitrogen decreases slightly. In the infected plants, however, the tissues show a decrease in soluble nitrogen content from those of healthy plants at all stages of development. The loss of soluble nitrogen gradually rises with age and reaches maximum in the three month old plants. The gradual decrease in soluble nitrogen content, simultaneously with increase in total nitrogen content may possibly be due to utilization of the former by the pathogen, while enhancement of the latter may be accounted for the stimulated protein synthesis of the host or pathogen or both.

Botany Department, Burdwan University, Burdwan (W.B.), January 7, 1975.

Debdas Mukhopadhyay, B. Nandi.


**GENETIC TRANSFER OF NITROGEN FIXATION GENE (nif+*) FROM AZOTOBACTER CHROOCOCCUM TO RHIZOBIUM TRIFOLII**

**INTRA-STRAIN** genetic transfer of nitrogen fixation genes (nif−) has been accomplished by transduction1 and conjugation2. This was followed by a report on intergeneric conjugal transfer of nif+ from *Klebsiella pneumoniae* to *Escherichia coli*3. The present report deals with an intergeneric transfer of nitrogen fixation gene (nif−) by DNA transformation from the bacterium *Azotobacter chroococcum* to *Rhizobium trifolii*, a species which does not naturally fix nitrogen in the free living state.

The donor *Azotobacter chroococcum* B3 is a non-symbiotic nitrogen fixer, isolated from the rhizosphere of barley plants. The recipient *Rhizobium trifolii* RT/3 is an ultraviolet-induced streptomycin resistant mutant (30 μg/1) of *R. trifolii* T 54. The transforming DNA was isolated from the donor according to the method of Marmur and after isopropanol precipitation was stored in saline citrate over a layer of chloroform at 0°C.

Transformation was carried out by adding 0.5 ml of the donor DNA to 0.5 ml of the competent recipient cells (10⁶ cells) to make the final volume to 1 ml (final DNA concentration 20 μg) and incubating the mixture for 30 min at 30 ± 1°C. After stopping the reaction, the mixture was plated on nitrogen free mannitol agar (20 g mannitol, 5 g CaCO₃, 0.1 g K₂SO₄, 0.2 g KH₂PO₄, 0.2 g MgSO₄·7 H₂O, 0.2 g NaCl, 15 g agar, 1000 ml distilled water; pH 7.3) and the colonies were replicated on a series of silica plates fortified with the same nitrogen free medium. Controls included plating the recipient culture on silica plates without DNA treatment, recipient treated with the recipient DNA, plating the donor DNA alone, addition of DNase (50 μg/ml) before adding DNA and also on yeast extract mannitol agar for total counts.