INTRODUCTION
Nitrogen fixation by legumes growing in natural habitats plays a substantial part in nitrogen economy in nature. This ability, almost unique to the Leguminosae, to draw on atmospheric nitrogen depends on the presence in the soil of bacteria grouped in the family Rhizobiaceae Conn. (Breed, Murrey and Hitchens, 1948). These bacteria stimulate legume roots under suitable conditions (Nutman, 1958; Allen and Allen, 1958; Vincent, 1962; Raggio and Raggio, 1962) to form tumour-like nodules. Growth and multiplication of the rhizobia within the nodule and the ensuing symbiosis normally results in the conversion of atmospheric nitrogen into utilizable forms - a process which neither the plant nor the microorganism is capable of carrying on, on its own. The extent of this association between species of legumes and rhizobia, the specificity of infection and nodulation, the variation in the efficiency with which symbiotic systems fix nitrogen pose some of the most fascinating problems in the biology of legume-rhizobia relationships.

The existing evidence shows that symbiosis between the rhizobia and the legume host depends on several factors governing the host and bacteria (Nutman, 1958; Hallsworth, 1958; Allen and Allen, 1958; Raggio and Raggio, 1962; Jordan, 1962). Among these, the inherent plant factors (Nutman, 1959), the adaptation of rhizobia to particular legume hosts (Nutman, 1958), the variation in effectiveness of the bacteria in
nitrogen fixation (Bjalfve, 1935; Thornton, 1939; Chen, 1941; Bond, 1942; Burton and Briggeman, 1948; Virtanen, 1949; Kleczkowska, 1950; Jordan and Garrard, 1951; Parker and Allen, 1952; Erdman, 1953; Vincent, 1954), the influence of host physiology (Thornton, 1930, 1952; Nutman, 1956, 1958; Pate, 1958a) and the effects of the environment (van Schreven, 1958; Pate, 1958b) are of particular importance.

The rhizobia by themselves are aerobic and heterotrophic and exist in the free living state in the soil. However, when they associate with the root cells of a compatible legume host, they induce the formation of a nodule and participate in the fixation of atmospheric nitrogen. Depending on their capacity for nitrogen fixation the rhizobia could be regarded as effective or ineffective and accordingly nodules are known as effective or ineffective nodules.

In this symbiotic association, in so far as the bacteria are concerned, Allen and Allen (1958) have pointed out that the effectiveness of strains may not be correlated with their

1) cultural and physiological characteristics (Almon and Fred, 1933; Burton and Lochhead, 1952; Fred, 1932; Jordan, 1952b)
2) their antigenic structure (Kleczkowski and Thornton, 1944; Vincent, 1944), 3) colonial mutation (Jordan, 1952a; Kleczkowski, 1950), 4) phage sensitivity (Almon and Wilson, 1933; Brush and Allen, 1955; Kleczkowski, 1945b, Laird, 1952)
and 5) sensitivity to antibiotic action. Bacterial strain
effectiveness could, however, undergo a change through
prolonged culture in unfavourable media (Wolf and Baldwin,
1940), through storage in sterilized soil (Mutman, 1946a;
Reid et al., 1935; Thornton, 1949), by ultrasonic vibrations,
phage action and mutagenic agents (Allen and Baldwin, 1931b,

The nitrogen fixing capacity of a Rhizobium strain could
be established only in its symbiotic state in a defined
host-strain partnership. The ultimate response and nodule
nitrogen fixation, therefore, depend on the combined influence
exerted by both the plant and rhizobia. Indeed, it has been
demonstrated that the formation of bacteroids - the form in
which bacteria occur within the nodule cells is dependent not
solely on either bacterium or host but upon a complex inter-
action between the two entities (Bergersen and Mutman, 1957).

There is a great deal of literature emphasizing the
importance of the rhizobia-legume association in temperate
countries. However, on the basis of the general picture that
emerges from an examination of this literature, Morris (1958)
suggested that one must refrain from applying to tropical
species, mainly because they too are "legumes", treatments
that have been worked out on members of the Viciae and
Trifolieae so far examined in temperate situations. The reason
for this were firstly, tropical species are inherently
different in their ability to obtain their nutrients from
the soil. Secondly, tropical soils are different in their
ability to hold and supply nutrients. Thirdly, rhizobia
examined so far in the tropics and grouped under the
"Cowpea-miscellany" (Morris, 1956, 1959b) do not show the
host-rhizobium specificity most common in temperate legumes.
Records on root nodules in the tropics confirm this (Bewen,
1956a; Masefield, 1952, 1957, 1958; Bonnier, 1957b;
Allen and Allen, 1939, 1940; Rajagopalan, 1938; Saric, 1956).
Inoculation with an effective cowpea strain of rhizobium was
also shown in this laboratory to lead to effective nodulation
in species of Arachis, Dolichos, Phaseolus, Clitoria,
Centrosema, Vigna, Psophocarpus, Cyamopsis, Cajanus, Gliricidia,
and Crotalaria of the sub family Papilionaceae (Rajagopalan,
1964a, 1964b; Rajagopalan and Sadasivan, 1964).

Again as pointed out by Morris (1958), while most
temperate crops prefer fertile soils with large amounts of
calcium and phosphorus and show little tolerance for acid
soils, the majority of tropical legumes and their associated
rhizobia exist under poor soil conditions of low nutrient
availability, base-exchange capacity and acidity. It may not,
therefore, be expected that requirements of temperate legumes
could be shared by tropical species as well. In this regard,
less is known about the contribution of legumes to soil
fertility particularly in India.
Fig. 42  Effect of potassium deficiency on the free amino acid content and composition of root nodules of *A. hypogaea*.

The figures in the histogram represent:

1. Aspartic acid
2. Glutamic acid
3. Serine
4. Glycine
5. Asparagine
6. Arginine
7. Histidine
8. r-methylene glutamic acid
9. Glutamine
10. Threonine
11. Alanine
12. Valine
13. Leucine
in so far as the transport of carbohydrates from shoots are concerned. It is likely that at the lower level of potassium (40 ppm) carbohydrates are locked up in the shoots, which is reflected in the high values recorded for the shoot and the corresponding low values for nodules.

**Effect of potassium deficiency on the free amino acid content and composition of root nodules.** (Table 33).

From what has been described above on the differential response of shoots and nodules in terms of their dry weight, carbohydrate and nitrogen content and C/N ratios, it was thought worthwhile to investigate the free amino acid content and composition of nodules with reference to sufficient and deficient levels of potassium. The composition of the nutrient solution and other details were the same as outlined in previous experiment. The free amino acids were analysed by the dimensional paper chromatography and quantitatively spectrophotometrically as outlined earlier (*vide*, Material and Methods).

The free amino acid content and composition with decreasing levels of potassium are presented in Fig. 42 along with the results obtained for magnesium for comparison.

The differential response to potassium levels is again evident in the free amino acids content as well as its composition. Decreasing potassium levels from 480 to 96 ppm
TABLE 33

THE EFFECT OF POTASSIUM ON THE COMPOSITION OF FREE AMINO ACIDS AND AMIDES (mg/g. NODULES) IN NODULES OF *A. HYPOGAEA* (VAR. TNV 2).

<table>
<thead>
<tr>
<th>Amino acid or amide</th>
<th>Treatment level of potassium (ppm)</th>
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<tbody>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>1. Aspartic acid</td>
<td>55.9</td>
</tr>
<tr>
<td>2. Glutamic acid</td>
<td>15.2</td>
</tr>
<tr>
<td>3. Serine</td>
<td>7.0</td>
</tr>
<tr>
<td>4. Glycine</td>
<td>-</td>
</tr>
<tr>
<td>5. Asparagine</td>
<td>188.7</td>
</tr>
<tr>
<td>6. Arginine</td>
<td>8.8</td>
</tr>
<tr>
<td>7. Histidine</td>
<td>27.0</td>
</tr>
<tr>
<td>8. γ-methylene glutamic acid</td>
<td>-</td>
</tr>
<tr>
<td>9. Glutamine</td>
<td>11.1</td>
</tr>
<tr>
<td>10. Threonine</td>
<td>8.3</td>
</tr>
<tr>
<td>11. Alanine</td>
<td>8.3</td>
</tr>
<tr>
<td>12. Valine</td>
<td>7.5</td>
</tr>
<tr>
<td>13. Leucine</td>
<td>27.6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>365.0</td>
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</tbody>
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increased the amino acid content, which decreased with further reductions of potassium levels up to 8 ppm. However, the free amino acid content again markedly increased when the potassium concentration was reduced from 8 to 4 ppm. These results are in contrast to the effects of magnesium where the free amino acid content progressively increased with decreasing magnesium levels.

As compared to magnesium, potassium deficiency increased the number of free amino acids. The relative proportions of free amino acids to the amide asparagine varied strikingly with the degree of potassium deficiency. Thus, decreasing the potassium level from 480 to 48 ppm (moderate deficiency) increased the number of free amino acids. The amide asparagine accumulated to as much as 79 percent with a further decrease in potassium to 8 ppm (extreme deficiency). The concomitant decrease in the number of amino acids may also be noted. With the lowest level of potassium, 4 ppm (near total deficiency) the number of free amino acids again increased with a reduction in the asparagine content. Thus, the effect of 4 ppm of potassium closely resembled that of 48 to 240 ppm of the element.