

DYNAMICS OF THE ABSORPTION OF FORMS OF N BY CROP PLANTS

By

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THESIS

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Department of Agronomy

COLLEGE OF HORTICULTURE

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DECLARATION

I hereby declare that this thesis entitled "Dynamics of the Absorption of Forms of N by Crop Plants" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship of any other similar title, of any other University or Society.

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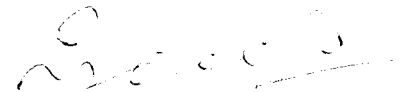
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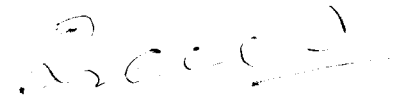
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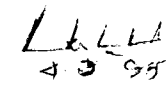


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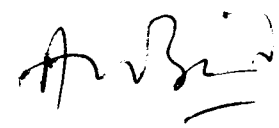
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*Dedicated to
my beloved parents*

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Introduction

1. INTRODUCTION

It is generally believed that plants absorb N in the forms of either ammonium or nitrate or both. In the case of flooded rice ammoniacal form is preferred to nitrate form primarily because nitrification is rather slow in the anaerobic soil environment. Most of the N management strategies have been formulated based on the assumption that ammoniacal and nitrate forms of N contribute significantly to N uptake by the plants.

Urea is the most popular N fertilizer used in crop cultivation. When urea is applied to soil it readily undergoes enzymatic hydrolysis leading to the production of ammonia and CO₂. Eventhough absorption of urea in the molecular form has been demonstrated by Japanese workers in early 1960s (Mitsui and Kurihara, 1962) not much attention has been given to this aspect in view of the rapid breakdown of urea in the soil. In the recent studies conducted at this laboratory employing ¹⁴C-labelled urea it was observed that urea hydrolysis is inhibited following flooding the soil (Saraswathi *et al.*, 1991) and the flooded rice plants are capable of absorbing urea in the molecular form especially when it is top dressed (Safeena

Review of Literature

2. REVIEW OF LITERATURE

The literature reviewed, with special reference to rice, in this chapter are classified under the following heads.

1. Role of nitrogen in plant growth
2. Ammonium nutrition of crop plants
3. Nitrate nutrition of crop plants
4. Absorption of molecular urea by crop plants
5. Preferential absorption of forms of nitrogen by crop plants

2.1 Role of nitrogen in plant growth

2.1.1. Nitrogen and plants

Nitrogen is a major structural constituent of the cell. The cytoplasm and cell organelles contain varying amounts of nitrogen largely in combination with carbon, hydrogen, oxygen, phosphorous and sulphur. Being a constituent of important organic compounds such as aminoacids, proteins, purines, pyrimidines, chlorophyll and many co-enzymes, N was found involved in all processes associated with protoplasm, enzyme reactions and photosynthesis (Greulach, 1973).

2.1.2. Nitrogen and rice

The intake of nitrogen by rice at successive stages of plant development was studied systematically. It was found that large parts of the carbohydrates in grains are built up by those which are photosynthesized during the period of ripening stage. Nitrogen is one of the essential nutrients to maintain the green leaf area and photosynthetic activities of leaves. So it can be inferred that more supply of nitrogen is needed during the period of ripening stage. Therefore to ensure it, comparatively a large amount of nitrogen must be taken up by the plant until the heading stage (Murayama, 1986).

2.2 Ammonium nutrition of crop plants

2.2.1 Crop plants other than rice

Plants can absorb and effectively utilize ammonium as a nitrogen source. Cox and Reisenauer (1973) demonstrated that wheat absorbed NH_4^+ more rapidly than NO_3^- , and NH_4^+ fed plants grew rapidly. Spratt (1974) observed that ammonium nutrition was the best for wheat during the early stages of growth. Leyshon et al. (1980) reported that the grain yield for wheat and barley was higher when they were supplied with NH_4^+ - N. Dibb and Welch (1976) found that ammonium as the principal form of

nitrogen increased corn yield at high K^+ rate. Smith et al. (1990) in a solution culture experiment found that pearl millet preferred ammonium for the first two weeks.

In contrast to the above results the deleterious effects of ammonium nutrition were also reported. The plant tobacco was found unable to utilize NH_4^+ satisfactorily (Morris and Gidden, 1963). Maftoun et al. (1980) demonstrated that NH_4^+ - N fertilization generally suppressed the growth of CAM plants. Caselles et al. (1987) reported that in hydroponic culture under controlled conditions, *Lycopersicon esculentum* and *Capsicum annum* were found sensitive to NH_4^+ at low concentrations. The increase in NH_4^+ (77 ppm) in the nutrient solution caused the most growth reduction for cabbage, melon and corn, with corn being least affected. Bean seemed to be well adapted to the use of NH_4^+ . The presence of (28 ppm) NH_4^+ -N in the solution also reduced the growth of cabbage, but other species were not affected (Errebhi and Wilcox, 1990). Feigin (1990) observed that under saline conditions the presence of NH_4^+ exerted a negative effect on melon plant growth.

Plants supplied with higher concentration of NH_4^+ had a lesser yield and incipient ammonium toxicity

(Cox and Reisenauer, 1973). Higher concentration of nitrogen in the plant tissues decreased the rate of ammonium uptake (Morgan and Jackson, 1988). The ammonium uptake was increased with increasing nitrogen deprivation in the plant tissues .

Ammonium uptake was greater in light than in darkness, and increased with increase in light intensity (vanEgmond, 1978). Capsicum plants were sensitive to NH_4^+ even at low concentrations (Caselles *et al.*, 1987). It was found that NH_4^+ supplied to capsicum at high light intensity was more deleterious and the plants experienced an alleviation in the toxic effect of NH_4^+ when light was reduced (Zornoza *et al.*, 1989). The decline in ammonium uptake in darkness is apparently due to the depletion of carbohydrate reserves in roots (Reisenauer, 1978).

Jackson and Volk (1967) reported that tobacco seedlings showed greater ammonium uptake with acidity control. Munn and Jackson (1978) observed that NH_4^+ uptake rate by rooted cuttings of sweet potato increased by increasing ambient pH.

Gashaw and Mugwira (1981) found that there were increases in uptake of phosphorous and iron when rate of

supply of ammonium was increased. Several workers (Cox and Reisenauer, 1973; Pill and Lambeth, 1977) reported that ammonium nutrition decreased the uptake of Ca, Mg, K, P and NO_3^- by plants. Middleton and Smith (1979) in an experiment with rye grass, reported that ammoniacal nutrition needed more oxygen. Matsumoto and Tamura (1981) also observed the same result with cucumber.

2.2.2 Rice

Several workers (Anderson *et al.*, 1946; Wahhab and Bhatti, 1962; Yoneyama, 1986) demonstrated that greater amounts of nitrogen were absorbed and better growth was observed, when rice was supplied with ammonium rather than nitrate. Tanaka *et al.* (1959) observed that ammonium nitrogen is suitable during vegetative stage of rice plants for increasing tillering capacity.

Tanaka *et al.* (1959) concluded by water culture experiment that at higher level of nitrogen (100 ppm) ammonium was inferior to nitrate. Fried *et al.* (1965) observed that ammonium could be absorbed 5 - 20 times as fast as nitrate depending on the pH of the medium. Karim and Vlamis (1962) found that the rapid uptake of ammonium lowered the pH of the culture solutions (from 5.5 to 3.8) where the acidity restricted the root activity, and plant

growth in general suffered. They obtained a better growth of rice plants in culture containing ammonium as the sole source of nitrogen with a pH buffering agent. Izawa (1961) reported that the low pH of the culture medium reduced the uptake rate of NH_4^+ .

Ta and Ohira (1981) found that in summer the NH_4^+ fed plants showed the lowest growth at any nitrogen concentration. However in autumn their growth excelled that of plants in nitrate. A similar study (Ta and Ohira, 1982) revealed that decrease of temperature had a depressive effect on the absorption of ammonium and nitrate. At low temperature (17°C) nitrate absorption was very much depressed but ammonium was absorbed significantly. Shading of rice plants also affected the absorption of NH_4^+ . Complete darkness reduced the ammonium uptake.

2.3 Nitrate nutrition of crop plants

2.3.1 Crop plants other than rice

Several experiments revealed that plants can take up and utilize NO_3^- as well. Morris and Gidden (1963) reported that crops like tobacco, tomato, cotton, corn, gram, sorghum etc absorbed significantly more nitrogen from NO_3^- than NH_4^+ . Pill and Lambeth (1977) reported that growth of tomato was greater with nitrogen supplied

as nitrate. Ashley *et al.* (1975) observed that the uptake of nitrate by intact wheat seedlings was low during the initial 3 to 6 h period and declined. Spratt (1974) demonstrated a higher grain yield in wheat when NO_3^- N was supplied after booting stage. Maftoun *et al.* (1980) reported that in CAM plants leaf area and chlorophyll concentrations were generally higher in plants supplied with NO_3^- N. Smith *et al.* (1990) reported that NO_3^- accumulation in pearl millet was highest during grain filling period.

Munn and Jackson (1978) reported that NO_3^- uptake by sweet potato was decreased by increasing ambient pH. Nitrate uptake was increased sharply with increases in the external supply of nitrate. When supply was high nitrate was absorbed in excess of the needs of plants and was accumulated internally (Blair *et al.*, 1970; Cox and Reisenauer, 1973; Gashaw and Mugwira, 1981). Increasing rate of NO_3^- uptake was associated with increase in the rate of Ca, Mg, and K intake (Cox and Reisenauer, 1973; Jackson, 1978).

Nitrate nitrogen absorbed by plant roots was found assimilated by several reductive processes into aminoacids. Nitrate reduction in plant tissues consumed

either chemical energy and organic acids, or competed for products of photolysis (Hagin *et al.*, 1990). Middleton and Smith (1979) observed that in perennial rye grass assimilation of NO_3^- form was found to need 8% more energy than ammonium. Jackson *et al.* (1976) demonstrated that the failure of root tissues to increase in soluble carbohydrates resulted in the low uptake of NO_3^- by plants.

2.3.2 Rice

Tanaka *et al.* (1959) demonstrated that at high nitrogen supply (100ppm) nitrate nitrogen was superior to rice. Nitrate nitrogen was found to promote uptake of potassium, calcium, manganese and phosphate by rice plant. Nitrate nitrogen was found to be preferred by the plants at the elongation and reproductive stage. Similarly Wahhab and Bhatti (1962) reported that NO_3^- N applied just before heading proved more beneficial to rice than that applied at the early stage of plant growth.

Karim and Vlamis (1962) demonstrated that rice grew fairly well upto maturity in nitrate culture solutions without encountering any difficulty. The growth which was initially slow became as vigorous as that of ammonium supplied plants after about a month. Yamasaki and Seino (1965) found that if NO_3^- is supplied regularly

during the early stages of plant growth, it can produce results similar to those obtained with NH_4^+ . Tanaka *et al.* (1959) and Yamasaki and Seino (1965) gave similar results indicating that NO_3^- has beneficial effects on the growth and yield of rice plants due to increased metabolic activities and development of new roots resulting in effective absorption of nutrients. But Asmunadji and Dijkshoorn (1971) reported that the rice plants accumulated carbohydrates in excess of the normal level if nitrate is consumed, and this reduced the yield. When nitrate was replaced by ammonium there was a further decrease in carbohydrate and yield increased.

The rice variety indica differed from japonica in its ability to utilize nitrate as a nitrogen source (Tanaka *et al.*, 1959; Ta and Ohira, 1981). In summer at high light intensity and temperature the growth of the indica rice varieties was better when the nitrogen was supplied as NO_3^- , whereas japonica rice varieties responded more effectively to the combination of NH_4^+ and NO_3^- , when either NH_4^+ N or NO_3^- N was supplied. There were indication of growth depression in nitrate fed rice plants, irrespective of genotype, in autumn.

Izawa (1961) reported that the low pH of culture medium reduced the uptake of NO_3^- as that of NH_4^+ . Karim and Vlamis (1962) demonstrated that nitrate uptake resulted in physiological alkalinity and due to this difficulties were created in the supply of iron and as a result chlorosis appeared. Ta *et al.* (1981) and Ta and Ohira (1981) found that the NO_3^- fed rice plants grew better at pH 4.5 than that at pH 6.0. Decrease of temperature had a depressive effect on the absorption of both ammonium and nitrate, but at low temperature the rate of nitrate uptake was much depressed than the rate of ammonium uptake (Ta and Ohira, 1982). They also reported that shading reduced the uptake of NO_3^- by rice.

2.4 Absorption of molecular urea by crop plants

2.4.1 Crop plants other than rice

McCarthy (1972) studied urea uptake by different species of marine phytoplankton and found that several had a higher affinities for urea uptake. Active mechanisms for urea transport in *Aspergillus* (Pateman *et al.*, 1973) and in *Saccharomyces* (Cooper and Sumarada, 1975) were also reported. Healey (1977) reported that nitrogen deficiency increased the initial saturated rate of urea uptake by green and blue green algae.

Several workers (Frieberg and Payne, 1957; Dilley and Walker, 1961; Mitsui and Kurihara, 1962) reported that urea can enter the plants not only after its decomposition but also as a whole molecule. Frieberg and Payne (1957) noted the absorption of undecomposed urea by the leaves of banana. No activity of urease was found in the assimilatory tissues of banana.

Molecules of urea were found in the juice of the crushed leaves and in the guttation exuded by the corn grown in solution of urea, which proved the absorption of urea by corn plants (Korenkov, 1966). Absorption of urea at a rate sufficient for growth by maize and sugarbeet was demonstrated by vanBeusichem and Neetson (1982). Bradley *et al.* (1989) reported the absorption of molecular urea by wheat.

Little information is available regarding the absorption of molecular urea by the roots of plantation crops.

2.4.2 Rice

It was found that rice plants could not only absorb NH_4^+ or NO_3^- form of nitrogen, but could absorb

molecular form of urea. Urea could directly be absorbed by rice roots without prior decomposition and accumulated to a high extent in the roots and even in the shoots (Mitsui and Kurihara, 1962). Autoradiograph of rice plants fed with ^{14}C urea confirmed the molecular absorption of urea (Saraswathi et al., 1991). Quantitatively the accumulation of ^{14}C accounted to $1.8 \mu\text{g urea h}^{-1} \text{g}^{-1}$ plant tissue. It was also suggested that the molecular absorption of the applied urea could be substantial from top dressing under anaerobic situation. Safeena (1992), in a study of molecular absorption of urea by flooded rice, also obtained similar results. Absorption of nitrogen as molecular urea was much higher in flooded rice than in non-flooded rice.

2.5 Preferential absorption of forms of nitrogen by crop plants

2.5.1 Crop plants other than rice

There is much confusion in the literature on the relative effectiveness of different sources of nitrogen for plants, and it was found that this varied with species. Several workers (Berlier and Guiraud, 1967; Gashaw and Mugwira, 1981; Nishimune et al., 1980) reported that crops like rye grass, barley, wheat, sugarbeet, dwarf bean etc absorbed NH_4^+ ions preferentially compared to NO_3^- .

But several other species like tobacco, corn, pearl millet, limmabeen etc preferred NO_3^- than ammonium (Morris and Gidden, 1963; Smith et al., 1990; McElhannon and Mills, 1978). Also several other workers demonstrated that N uptake and growth of corn increased rapidly when plants were provided with nitrogen as combination of nitrate nitrogen and ammonium nitrogen than when they were supplied alone (Schrader et al., 1972; Below and Gentry, 1992; Smiciklas and Below, 1992).

Ammonium nutrition in many cases reduced the uptake of NO_3^- . Warncke and Barber (1973) observed that increase in the concentration of NH_4^+ in nutrient medium retarded the uptake of NO_3^- by corn. Munn and Jackson (1978) reported that presence of ambient ammonium at equimolar concentration decreased the rate of nitrate uptake by sweet potato. Rufty et al. (1982) demonstrated that the addition of NH_4^+ to NO_3^- medium resulted in similar inhibition of both nitrate uptake and nitrate reduction in dark grown corn. The accelerated phase of NO_3^- uptake appeared most sensitive to restriction by the addition of NH_4^+ . The severity of NH_4^+ restriction of NO_3^- uptake was moderated considerably in the presence of K^+ . Breteler and Seigerist (1984) reported that the nitrate uptake rate of dwarf bean and nitrate reductase activity

were inhibited by NH_4^+ to a maximum of 45% and 60% respectively. But Schrader *et al.* (1972) reported that the presence of NH_4^+ did not retard the uptake of NO_3^- by corn. Minotti *et al.* (1969) suggested that ammonium and high acidity adjacent to cellular boundary membranes resulting from ammonium uptake in excess of nitrate uptake resulted in alterations in membrane permeability, thereby restricting capacity for nitrate absorption.

Some workers, vanBeusichem and Neetson (1982) found that sugarbeet and maize absorbed more nitrogen in the form of ammonium rather than urea. Bradley *et al.* (1989) reported that the uptake of nitrogen by wheat from urea was less than one-third of that from NH_4NO_3 . There was reciprocal antagonism between uptake of urea and NH_4^+ . Urea inhibited both NH_4^+ and NO_3^- uptake, whereas NH_4^+ inhibited the uptake of urea, while NO_3^- enhanced the uptake of urea.

2.5.2 Rice

Most of the studies indicated that rice in general preferred ammoniacal-N to nitrate-N. (Anderson *et al.*, 1946; Wahhab and Bhatti, 1962; Fried *et al.*, 1965; Yoneyama, 1986). Ta *et al.* (1981) demonstrated that the uptake of NO_3^- was considerably repressed by the presence

of NH_4^+ in the medium, while NO_3^- exerted a minor effect on the uptake of NH_4^+ . It was noted that (Ta and Ohira, 1982) at low temperature the rate of nitrate uptake was much depressed than NH_4^+ uptake.

Tanaka et al. (1959) observed that rice preferred NH_4^+ nitrogen during vegetative stage and NO_3^- nitrogen at the elongation and reproductive stage. It was suggested that this preference for nitrogen source is related to the oxidation reduction character of the plant roots at different stages of growth. According to Ismunadji and Dijikshoorm (1971) the preference for ammonium would be related to the fact that rice plants accumulate carboxylates in excess of normal level, if nitrate is consumed, whereas ammonium lowers the production of carboxylate to a sufficient extent to prevent excessive accumulation of carboxylate so that normal level is maintained and better growth results.

Little information is available regarding the extent of molecular absorption of urea by rice in the presence of NH_4^+ and NO_3^- forms.

Materials and Methods

3. MATERIALS AND METHODS

The experiment was conducted at the Radiotracer Laboratory, College of Horticulture, Vellanikkara. The laboratory is located at $10^{\circ} 32'$ N latitude and $75^{\circ} 16'$ E longitude at an altitude of 22 m above M.S.L.

The investigation was mainly aimed to find out the preferential absorption of molecular urea by rice in the presence of ammoniacal and nitrate forms of N. It was also intended to know the possibility of molecular absorption of urea by some plantation crops.

The experiments undertaken during the course of investigation consisted of two parts.

1. Dynamics of absorption of nitrogen forms relative to their concentrations at different growth stages of rice using ^{14}C -labelled urea, ^{15}N -labelled ammonium sulphate and ^{15}N -labelled sodium nitrate.

2. Pot culture experiment using ^{14}C -labelled urea to study the possibility of absorption of molecular urea by the plantation crops.

3.1 Dynamics of the absorption of forms of nitrogen by rice at different stages of growth

To study the dynamics of the absorption of nitrogen forms relative to its concentration at different growth stages of rice, sand culture experiment was carried out in the green house of Radiotracer Laboratory, from June 1993 to August 1993.

3.1.1 Preparation of sand

Pure quartz (silica) sand of size 50 to 60 mesh obtained from M/S. Usha Minechem Industries, Bangalore was used for this experiment. The sand was first washed with running tap water and then with deionised water to get it free of nutrients.

3.1.2 Preparation of pots

Plastic buckets of 5 litre capacity and uniform size were used in this experiment. A drain hole was given at the bottom of each bucket. Through this hole PVC pipe of innerdiameter 3 mm and convenient length was inserted into the bucket and the junction was sealed tightly with 'M-seal' to prevent leakage. The inner end of the pipe was covered with porcelain cloth to prevent the entry of sand into the pipe and thereby blocking the drainage.

Also the outer end of the drainage pipe was blocked with 'T' shaped plastic stopper and was tied to the top edge of the bucket with iron wire in order to keep the outer end of the pipe above the waterlevel in the bucket, so that the minor chances for leakage could also be avoided.

The plastic buckets were filled with thoroughly washed sands. The containers were filled to capacity to give final quantity of approximately 7.5 kg sand per pot.

3.1.3 Preparation of rice seedlings

Rice variety Jaya was selected for the study. The seeds were collected from Agricultural Research Station, Mannuthy.

A plastic tray of size 45 cm x 30 cm x 10 cm filled with quartz sand washed thoroughly with demineralised water was used to raise the seedlings. The tray was first washed thoroughly in running tap water and then rinsed with deionised water. A drain whole plugged with glass wool was also provided at the bottom of the tray. The tray was filled with sand to one-third the volume prior to sowing and was levelled. The seeds were allowed to pre-soak for 24 h in a beaker of demineralised water. The pre-soaked seeds were spread uniformly over

the sand bed in the tray. The seedlings were sprinkled with deionised water in every alternate days and upto transplanting seedlings were not given any external application of the nutrients.

3.1.4 Transplanting of rice seedlings

Rice seedlings were transplanted to the experiment pots after they had attained the growth of 10 days. Healthy seedlings were transplanted at the rate of 9 seedlings per pot, in three hills of three each.

3.1.5 Standardisation of nutrient solution

Preliminary studies were conducted with standard media for rice proposed by Yoshida *et al.* (1972) to examine its suitability to grow rice in the laboratory conditions at Vellanikkara. The recommended solution was applied at the rate of 2.5 litre per pot. In view of the N deficiency observed, three levels of nitrogen viz. 40, 80, 120 ppm were tried. Of these, the nutrient solution with 120 ppm N was found to be the best as its application resulted in maximum response and survival of the plants without any supplementation up to 20 days.

From the results of these trials, the composition of the nutrient solution for rice was finalised and the same is given below.

| Element | Concentration of element in nutrient solution (ppm) |
|---------|--|
| N | 120.00 |
| P | 10.00 |
| K | 40.00 |
| Ca | 40.00 |
| Mg | 40.00 |
| Mn | 0.50 |
| Mo | 0.05 |
| B | 0.20 |
| Zn | 0.01 |
| Cu | 0.01 |
| Fe | 2.00 |
| Si | 20.00 |

3.1.6 Details of treatments

Forms of N : 3 (urea, NH_4^+ , NO_3^-)

Sources of N : 5

1. ^{14}C -labelled urea

2. ^{15}N -labelled ammonium sulphate
3. ^{15}N -labelled sodium nitrate
4. Non-labelled ammonium sulphate
5. Non-labelled sodium nitrate

Application of nitrogen:

1. ^{14}C -urea alone
2. NO_3^- alone
3. NH_4^+ alone
4. ^{14}C -urea + $^{15}\text{NO}_3^-$ (in equal proportions)
5. ^{14}C -urea + $^{15}\text{NH}_4^+$ (" ")
6. $^{15}\text{NH}_4^+$ + NO_3^- (" ")
7. ^{14}C -urea + $^{15}\text{NH}_4^+$ + NO_3^- (" ")

Intervals of sampling: 3

1. 20 days after planting
2. 40 days after planting
3. 60 days after planting

Design : Factorial CRD
 Treatments : $7 \times 3 = 21$
 Replications : 3
 Total No. of pots : $21 \times 3 = 63$

3.1.7 Preparation of ^{14}C -labelled urea stock solution

^{14}C -urea was obtained from Isotope Division, B.A.R.C., Trombay. Urea solution of the volume 700 ml was prepared having an activity of 0.9 μCi per ml containing 0.042 g of urea per ml to get nitrogen at the rate of 20 mg per ml of the stock solution.

3.1.8 Preparation of ^{15}N -labelled ammonium sulphate stock solution

^{15}N enriched ammonium sulphate was obtained from Rashtriya Chemicals and Fertilizers, Bombay, having an atom excess of 5 per cent. The stock solution was prepared by dissolving 37.71 g of ^{15}N -labelled ammonium sulphate in 400 ml of distilled water, so that it would yield 20 mg N per ml.

3.1.9 Preparation of ^{15}N -labelled sodium nitrate stock solution

^{15}N enriched sodium nitrate was obtained from Rashtriya Chemicals and Fertilizers, Bombay, having an atom excess of 5 per cent. The stock solution was prepared by dissolving 24.29 g of ^{15}N -labelled sodium nitrate in 200 ml of distilled water to get 20 mg N per ml of stock solution.

3.1.10 Preparation of non-labelled ammonium sulphate stock solution

Analytically pure chemical (AR grade) of ammonium sulphate was used for the preparation of stock solution. Stock solution was prepared by dissolving 47.14 g of ammonium sulphate in 500 ml distilled water so that it would yield N at the rate of 20 mg per ml of the solution.

3.1.11 Preparation of non-labelled sodium nitrate stock solution

Stock solution of AR grade sodium nitrate was prepared by dissolving 60.71 g of sodium nitrate in 500 ml of distilled water. One ml of this solution contained 20 mg N.

3.1.12 Preparation of nutrient solution and application of treatments.

The details of the stock solutions prepared are given in Table 1. Analytically pure chemicals (AR grade) were used for the preparation of the solutions. Stock solutions were prepared in distilled water. The stock solutions for individual macro nutrients and silicon were prepared separately. But for micro nutrients, firstly the reagents were dissolved separately, then combined with 30

Table 1. Composition of stock solution prepared for making the culture solution

| Element | Reagent | Quantity g per l | Concentration of element (ppm) |
|---------|---|---------------------|-----------------------------------|
| P | $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ | 26.161 | 5,000 |
| K | K_2SO_4 | 44.550 | 20,000 |
| Ca | CaCl_2 | 55.383 | 20,000 |
| Mg | $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ | 202.566 | 20,000 |
| Mn | $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ | 0.900 | 250 |
| Mo | H_2MoO_4 | 0.042 | 25 |
| B | H_3BO_3 | 0.572 | 100 |
| Zn | $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ | 0.022 | 5 |
| Cu | $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ | 0.020 | 5 |
| Fe | $\text{C}_6\text{H}_5\text{O}_7\text{Fe} \cdot 5\text{H}_2\text{O}$ | 5.982 | 1,000 |
| Si | $\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$ | 75.760 | 10,000 |

ml concentrated H_2SO_4 and made up the volume to one litre with distilled water.

The water for the preparation of final culture solution was filtered and demineralised by passing through an ion exchange resin column. Final nutrient solution was prepared without the nutrient nitrogen. For every pot, 5 ml of each of the stock solutions was added to 2.5 litre of water in a bucket with constant stirring. The pH of the final solution was adjusted to 5.0 by the addition of 1N NaOH or 1 N HCl by using bromocresol green - methylred mixed indicator. This was added to the pots, immediately after transplanting the rice seedlings. This brought water level just to the surface of the sand. To supplement the loss by evapotranspiration, the water level in alternate days were maintained by carefully adding deionised water without spilling over.

Citrate form of iron was used to avoid precipitation when mixed with solution containing other nutrient elements. Even then the plants showed iron deficiency at early stages of growth. For faster recovery the seedlings were sprayed with a fine mist of 0.25 per cent $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ solution of pH 4.4.

The treatments were imposed through the supplementary addition of nitrogen in the labelled and non-labelled forms. The stock solutions of nitrogen were prepared in such a way that 5 ml of the stock solution in 2.5 litre of final solution will yield 40 ppm N.

3.1.13 Sampling

Sampling was done at 20, 40 and 60 days after transplanting. Harvesting was done by cutting the plants just above the water level, carefully with scissors and rinsed the cut end in distilled water, blotted with absorbant papers, packed in labelled paper packets and were kept in hot air oven at 70 °C till obtained moisture free and constant weight. Dry weight of the samples were recorded. Then the plant samples were powdered in a mixer-cum-grinder and stored in labelled bottles.

3.1.14 Renewal of culture solution.

Renewal of the culture solution was made at an interval of 20 days. The drainage was carefully collected in separate buckets to avoid contamination of radioactivity to the surroundings. Before refilling the fresh nutrient solution, the growth media was flushed with deionised water repeatedly for five times to wash away the nutrient

residues. This drainage was also collected carefully in separate buckets.

3.1.15 ^{15}N analysis

The total nitrogen in samples was determined by kjeldahl digestion and distillation. The titrated distillate was made acidic with a few drops of 0.05 N H_2SO_4 and evaporated to dryness by keeping it in a hot air oven at not more than 50 °C. The residue was then diluted with distilled water to get a concentration of 1 mg per ml. This was then filled in capillary tubes of length 1 - 2 cm. The capillary tubes containing 9 - 11 μg N were then oven dried at 50 °C. The $^{14}\text{N} : ^{15}\text{N}$ ratio estimation was done using emission spectrometer available at Nuclear Research Laboratory, Indian Agricultural Research Institute, New Delhi.

3.1.16 Radioassay of plant samples.

The radioactivity of the plant samples was determined following biological oxidation. The powdered samples of 0.1 g each was oxidized in a biological oxidizer (OX 500 of R J Harvey Instrument corporation Hillsdale, New Jersey) and the $^{14}\text{CO}_2$ liberated during combustion was collected in specially formulated ^{14}C - 14 cock-

tail' solution supplied by the R J Harvey Instrument Corporation. The radioactivity was then determined in a microcomputer controlled liquid scintillation system (Wallac 1409 of Wallac OY, Finland).

3.1.17 Computation of parameters

a) Per cent N derived from fertilizer in plants (% Ndff)

$$= \frac{\% \text{ atom excess in the plant}}{\% \text{ atom excess in the fertilizer}}$$

b) Fertilizer N uptake = $\frac{\% \text{ Ndff} \times \text{total N uptake}}{100}$

c) Quantity of nitrogen taken up as molecular urea by plant

$$= \frac{\text{total dpm in the plant part}}{\text{specific activity of the fertilizer}} \times 0.46$$

3.2. Molecular absorption of urea by plantation crops

To study the possibility of absorption of molecular urea by the plantation crops, pot culture experiment with seedling of plantation crops was done by treating the roots with ^{14}C -labelled urea solution.

3.2.1 Planting material

Young seedling of the following plantation crops were selected for the study.

| | |
|----------|-------------------------------------|
| coconut | (<i>Cocos nucifera</i> L.) |
| banana | (<i>Musa paradisiaca</i> L.) |
| ginger | (<i>Zingiber officinale</i> Rosc.) |
| mango | (<i>Mangifera indica</i> L.) |
| turmeric | (<i>Curcuma domestica</i> Val.) |
| cashew | (<i>Anacardium occidentale</i> L.) |
| cocoa | (<i>Theobroma cacao</i> L.) |

3.2.2 Preparation of pots and planting

Plastic buckets of 5 litre capacity were used for the experiment. The buckets were first washed thoroughly under running tap water and then rinsed with demineralised water. The buckets were then filled with thoroughly washed nutrient free quartz sand one-third of the volume prior to planting the seedlings. The roots of the seedlings were washed first under running tap water and then with demineralised water. Extreme care was taken to avoid root injury during washing and transplanting. One seedling was transplanted to each pot and the container was filled to capacity with nutrient free sand.

3.2.3 Preparation and application of culture solution

The culture solution used in the experiment had the same composition of nutrients as that used for the rice. The plants were watered with 500 ml of respective nutrient solution. Also in every alternate days the plants were supplied with 50 ml of demineralised water to meet the evaporative loss.

3.2.4 Application of ^{14}C -labelled urea

^{14}C -labelled urea solution of the volume 100 ml was prepared having an activity of 0.5 μCi per ml containing 1.2857 g urea per ml.

The prepared ^{14}C -labelled urea solution was applied separately to the root zone of the plants at the rate of 5 ml per pot. The plants were allowed to absorb the nutrients for a period of 10 days.

3.2.5 Sampling

The plants that received ^{14}C -labelled urea were cut at 5 cm above the sand level and for coconut the leaves were detached from the stem.

3.2.6 Autoradiography

Immediately after harvest of the plants that received ^{14}C -labelled urea, the plant parts were arranged on separate absorbant papers in their original position and secured with the aid of adhesive tape. The specimens sandwiched between absorbant sheets were then pressed in herbarium press and allowed to dry under room temperature. After drying it was autoradiographed by placing on X-ray films in dark. The X-ray films were exposed for a period of 1 - 2 months and then developed using a commercial X-ray film developer solution.

3.2.7 Radioassay of plant samples

The radioassay of the plant samples were done in accordance with the description given under the section 3.1.16.

3.3 Statistical analysis

The statistical analysis was done according the methods suggested by Panse and Sukhatme (1985).

Results

4. RESULTS

This investigation consisted of two parts. In the first part absorption of different forms of nitrogen relative to its concentrations at different growth stages of rice was studied. In the second part an attempt was made to know the possibility of molecular absorption of urea by a few selected plantation crops.

In the first part of the study, to find out the preferential absorption of different forms of nitrogen, labelled fertilizers were applied. To know the molecular absorption of urea, ^{14}C -labelled urea was used and the presence of ^{14}C in the plant was taken as an evidence for the absorption of molecular form of urea. In the case of ammoniacal and nitrate forms of N, ^{15}N -labelled and non-labelled materials were used alternately to find out the preferential absorption.

4.1 Influence of the different forms of nitrogen on rice

4.1.1 Dry weight

The data on the dry weight of the rice plants as influenced by the application of different forms of nitro-

gen are presented in Table 2. The highest dry weight of rice plants at 20 DAP was observed when N was supplied as ammonium and nitrate in equal proportions. However this did not differ significantly from that obtained from nitrate alone and or ammonium. The second highest dry weight was observed when N was supplied as nitrate alone and this was on par with that obtained when nitrogen was supplied as ammonium alone and as urea + nitrate. There were no significant differences among the dry weights obtained following the application of urea + nitrate, urea + ammonium + nitrate, urea alone and urea + ammonium.

At 40 DAP, the highest dry weight was obtained when nitrogen was supplied as urea + nitrate and this was not significantly higher than that obtained from ammonium + nitrate. The dry weight obtained when nitrogen was supplied as ammonium + nitrate was on par with that obtained when N was supplied as nitrate alone and or ammonium. There were no significant differences among the dry weights recorded following the application of urea + ammonium, urea alone and urea + ammonium + nitrate. The lowest dry weight was observed when N was supplied as urea + ammonium + nitrate.

Table 2. Dry weight of rice plants at 20, 40 and 60 DAP following the application of different forms of nitrogen

| Treatment | Dry weight (mg per pot) | | |
|---------------------------|-------------------------|-----------------------|-----------------------|
| | 20 DAP | 40 DAP | 60 DAP |
| Urea alone | 134.667 ^{ab} | 318.000 ^{ab} | 753.333 ^a |
| Nitrate alone | 165.000 ^{cd} | 426.667 ^c | 936.667 ^a |
| Ammonium alone | 157.667 ^{bcd} | 370.667 ^{bc} | 993.333 ^a |
| Urea + nitrate | 140.667 ^{abc} | 518.000 ^d | 1013.333 ^a |
| Urea + ammonium | 127.333 ^a | 325.667 ^{ab} | 926.667 ^a |
| Ammonium + nitrate | 172.667 ^d | 438.667 ^{cd} | 1326.667 |
| Urea + ammonium + nitrate | 134.667 ^{ab} | 270.333 ^a | 1003.333 ^a |
| SEm± | 8.87 | 27.26 | 88.54 |
| CD (0.05) | 26.907 | 82.693 | 268.585 |

Values followed by the same alphabets are not significantly different

At 60 DAP, ammonium + nitrate gave the highest dry weight which was significantly higher than that obtained from all other treatments. The next highest dry weight was obtained when nitrogen was supplied as urea + nitrate. However this did not differ significantly from the dry weights obtained from other treatments.

4.1.2 Nitrogen content

The data on nitrogen content of rice as influenced by different forms of nitrogen are given in Table 3. At 20 DAP, only the mean values are presented in the table as the replications were pooled together. The pooling was necessary as the sample size was small and separate samples were required for the analysis of ^{15}N and ^{14}C employing biological oxidizer and liquid scintillation counter. From the mean values it was seen that nitrogen content was highest when nitrogen was supplied as urea + ammonium followed by that obtained when nitrogen was supplied as urea + nitrate, ammonium + nitrate, ammonium alone, urea + ammonium + nitrate, urea alone and as nitrate alone. At 40 DAP, urea + ammonium + nitrate gave the highest nitrogen content, but this was on par with that obtained when nitrogen was supplied as urea + nitrate, urea + ammonium, ammonium + nitrate and urea alone.

Table 3. Nitrogen content of rice plants at 20, 40 and 60 DAP following the application of different forms of nitrogen

| Treatment | N content (%) | | |
|---------------------------|---------------|---------------------|---------------------|
| | 20 DAP | 40 DAP | 60 DAP |
| Urea alone | 2.954 | 3.178 ^b | 3.523 ^{bc} |
| Nitrate alone | 2.632 | 2.315 | 2.618 |
| Ammonium alone | 3.122 | 2.692 ^a | 3.350 ^{ab} |
| Urea + nitrate | 3.360 | 2.959 ^{ab} | 3.225 ^a |
| Urea + ammonium | 3.780 | 3.019 ^b | 3.845 |
| Ammonium + nitrate | 3.192 | 3.066 ^b | 3.177 ^a |
| Urea + ammonium + nitrate | 3.080 | 3.215 ^b | 3.635 ^c |
| SEm± | | 0.10 | 0.06 |
| CD (0.05) | | 0.303 | 0.182 |

Values followed by the same alphabets are not significantly different

The lowest nitrogen content was observed when nitrogen was supplied as nitrate alone. At 60 DAP, urea + ammonium recorded the highest nitrogen content which was significantly higher than that obtained from all other treatments. Urea + ammonium + nitrate gave the next highest nitrogen content which was on par with urea alone, and was significantly higher than other treatments. There were no significant differences in the nitrogen content obtained following the applications of ammonium alone, urea + nitrate, and ammonium + nitrate.

4.1.3 Nitrogen uptake

The highest nitrogen uptake at 20 DAP was obtained when nitrogen was supplied as ammonium + nitrate (Table 4). However this was not significantly higher than that obtained from ammonium alone, urea + nitrate and urea + ammonium. The nitrogen uptake obtained following the application of nitrate alone, ammonium alone, urea + nitrate, urea + ammonium, and urea + ammonium + nitrate were at par. The lowest nitrogen uptake was observed when nitrogen was supplied as urea alone. At 40 DAP, urea + nitrate recorded the highest nitrogen uptake, which was not significantly higher than that obtained from urea + ammonium and ammonium + nitrate. The second highest N uptake was observed when N was supplied as ammonium +

Table 4. Nitrogen uptake by rice plants at 20, 40 and 60 DAP following the application of different forms of nitrogen

| Treatment | Nitrogen uptake (mg per pot) | | |
|---------------------------|------------------------------|----------------------|----------------------|
| | 20 DAP | 40 DAP | 60 DAP |
| Urea alone | 3.978 ^a | 10.178 ^{ab} | 26.774 ^{ab} |
| Nitrate alone | 4.343 ^{ab} | 9.890 ^{ab} | 24.385 ^a |
| Ammonium alone | 4.922 ^{bc} | 9.928 ^{ab} | 33.258 ^{bc} |
| Urea + nitrate | 4.727 ^{abc} | 15.320 ^c | 32.489 ^{bc} |
| Urea + ammonium | 4.811 ^{abc} | 13.084 ^{bc} | 35.663 ^{cd} |
| Ammonium + nitrate | 5.511 ^c | 13.440 ^{bc} | 42.026 ^d |
| Urea + ammonium + nitrate | 4.148 ^{ab} | 8.709 ^a | 36.407 ^{cd} |
| SEm± | 0.28 | 1.29 | 2.35 |
| CD | 0.849 | 3.913 | 7.129 |

Values followed by the same alphabets are not significantly different

nitrate and this was on par with that obtained from urea + ammonium, urea alone, ammonium alone and nitrate alone. At 60 DAP, ammonium + nitrate recorded the highest N uptake and this was on par with that obtained from urea + ammonium + nitrate and urea + ammonium. There were no significant differences in the N uptake recorded following the application of urea + ammonium + nitrate, Urea + ammonium, urea + nitrate and ammonium alone. The lowest nitrogen uptake was observed when N was supplied as nitrate alone and this was on par with that obtained from urea alone.

4.1.4 Uptake of urea N, ammoniacal N and nitrate N from different sources of N

To work out the preferential absorption of different forms of nitrogen, labelled fertilizer materials were used. In the case of urea ^{14}C -labelled material was used and the presence of ^{14}C in the plant was taken as evidence for the absorption of urea in the molecular form. In the case of ammoniacal and nitrate forms ^{15}N -labelled and non-labelled materials were alternately used to find out the preferential absorption. For calculating the uptake of nitrate N when it was supplied along with ammoniacal and urea N the following method was adopted. As the other two forms of N i.e. urea and ammonium were

labelled, the non-labelled part could have been assumed to have come from nitrate. However it was seen that when urea was applied alone, hydrolysis had taken place either at the root surface or within the plant and plant had absorbed nitrogen. So when the plant was supplied with ^{14}C urea, ^{15}N ammonium and nitrate, the non-labelled N in the plant could have come either from nitrate or consequent to the hydrolysis of ^{14}C -labelled urea. The probable percentage contribution from urea was calculated when urea was the sole source of nitrogen and this factor was applied to calculate the contribution of urea when it was supplied along with ammonium and nitrate. The source of ^{15}N -labelled N in the plant was ammonium. So the nitrogen other than that from urea and ammonium was assumed to have come from nitrate. The data on the uptake of different forms of N by rice are given in Table 5.

It was observed that when nitrogen was given as urea alone, it was taken up in the molecular form as evidenced by the ^{14}C content of the plant. The amount of urea absorbed was 0.7, 1.8 and 3.5 mg per pot at 20, 40, and 60 days after planting respectively (Table 5). However the plant had absorbed nitrogen in forms other than molecular urea as indicated by the total N uptake (Table 4) i.e. the total N uptake was higher than the urea N

Table 5. Uptake of urea N, ammoniacal N and nitrate N by rice from different sources of N at 20, 40 and 60 DAP

| N source | N form | Uptake of N form (mg per pot) | | |
|---------------------------|--------------|----------------------------------|--------|--------|
| | | 20 DAP | 40 DAP | 60 DAP |
| Urea alone | Urea N | 0.727 | 1.815 | 3.486 |
| | Other forms | 3.251 | 8.363 | 23.288 |
| Nitrate alone | Nitrate N | 4.343 | 9.890 | 24.385 |
| Ammonium alone | Ammoniacal N | 4.922 | 9.928 | 33.258 |
| Urea + nitrate | Urea N | 0.230 | 1.221 | 2.407 |
| | Nitrate N | 0.680 | 2.788 | 7.103 |
| | Other forms | 3.817 | 11.311 | 22.979 |
| Urea + ammonium | Urea N | 0.332 | 0.930 | 2.456 |
| | Ammoniacal N | 1.445 | 3.758 | 11.614 |
| | Other forms | 3.036 | 5.063 | 21.573 |
| Ammonium + nitrate | Ammoniacal N | 1.828 | 5.339 | 10.484 |
| | Nitrate N | 3.684 | 8.102 | 31.541 |
| Urea + ammonium + nitrate | Urea N | 0.243 | 0.720 | 1.736 |
| | Ammoniacal N | 1.079 | 2.289 | 5.798 |
| | Nitrate N | 1.741 | 2.401 | 16.984 |
| | Other forms | 1.085 | 3.299 | 12.067 |

uptake. The amount of nitrogen absorbed in other forms was found to be 3.2, 8.4, and 23.3 mg per pot (Table 5). When urea was applied along with nitrate, a reduction in the uptake of both these forms was observed. In the presence of nitrate, urea N absorbed at 20, 40, and 60 DAP were 0.2, 1.2 and 2.4 mg per pot respectively. When urea was applied with ammoniacal N, the urea N absorbed at 20, 40, 60 DAP were 0.3, 0.9 and 2.5 mg per pot respectively. Hence it was seen that, the molecular absorption of urea was reduced in the presence of either nitrate or ammonium. The urea N absorption was further reduced to 0.2, 0.7 and 1.7 mg per pot at 20, 40, and 60 DAP respectively in the presence of both ammoniacal and nitrate forms of nitrogen.

When nitrogen was supplied as nitrate alone, the amount of nitrate N absorbed were 4.3, 9.9 and 24.4 mg per pot at 20, 40 and 60 DAP respectively. In the presence of urea the amounts absorbed were 0.7, 2.8, and 7.1 and in the presence of ammonium, the amounts absorbed were 3.7, 8.1 and 31.5 mg per pot respectively at 20, 40 and 60 DAP. Hence it can be seen that nitrate nitrogen absorption was reduced in the presence of urea and was rather increased in the presence of ammonium. A reduction in nitrate N absorption was also observed when it was supplied along with ammoniacal and urea nitrogen.

When ammonium was supplied as the sole source of N the amounts of ammoniacal N absorbed were 4.9, 9.9 and 33.3 mg per pot at 20, 40 and 60 DAP respectively. In the presence of urea the amounts absorbed were 1.4, 3.8, and 11.6 and in the presence of nitrate the amounts absorbed were 1.8, 5.3 and 10.5 mg per pot respectively at 20, 40 and 60 DAP . So it can be seen that the amide and nitrate forms of nitrogen had more or less the same influence on the uptake of ammoniacal N. The amounts of ammoniacal N absorbed in the presence of both urea and nitrate N were 1.1, 2.3 and 5.8 mg per pot at 20, 40 and 60 DAP respectively.

Among the three forms of N supplied and at the different combinations, nitrate N recorded the highest uptake when present along with either urea N or ammoniacal N or both.

4.1.5 Percentage absorption of different forms of N to total N uptake

The percentage of molecular urea absorption to the total N uptake at 20, 40 and 60 DAP were 18.3, 18.2 and 13 (Table 6). The other forms accounted for 81 - 87 percentage of the total N absorption at these stages.

Table 6. Percentage absorption of urea N, ammoniacal N and nitrate N to the total N uptake by rice at 20, 40 and 60 DAP

| N source | N form | Percentage to the total N uptake | | |
|---------------------------|--------------|----------------------------------|---------|---------|
| | | 20 DAP | 40 DAP | 60 DAP |
| Urea alone | Urea N | 18.275 | 18.233 | 12.987 |
| | Other forms | 81.725 | 81.767 | 87.113 |
| Nitrate alone | Nitrate N | 100.000 | 100.000 | 100.000 |
| Ammonium alone | Ammoniacal N | 100.000 | 100.000 | 100.000 |
| Urea + nitrate | Urea N | 4.866 | 7.913 | 7.469 |
| | Nitrate N | 14.400 | 18.147 | 21.634 |
| | Other forms | 80.734 | 73.940 | 70.897 |
| Urea + ammonium | Urea N | 6.896 | 9.591 | 6.937 |
| | Ammoniacal N | 30.020 | 38.660 | 32.553 |
| | Other forms | 63.084 | 51.789 | 60.510 |
| Ammonium + nitrate | Ammoniacal N | 33.160 | 39.500 | 25.740 |
| | Nitrate N | 66.840 | 60.467 | 74.260 |
| Urea + ammonium + nitrate | Urea N | 5.847 | 8.119 | 4.767 |
| | Ammoniacal N | 26.020 | 26.240 | 15.920 |
| | Nitrate N | 41.989 | 28.462 | 46.673 |
| | Other forms | 26.144 | 37.179 | 32.640 |

When urea and nitrate N were applied together, the percentage absorption of urea N at 20, 40 and 60 DAP were 5, 8 and 7.4 and that of nitrate were 14, 18 and 21 per cent respectively. When urea N and ammoniacal N were applied together the amounts of urea N absorbed were 7, 10 and 7 per cent and that of ammoniacal N were 30, 39 and 33 per cent at 20, 40 and 60 DAP respectively. When ammoniacal and nitrate N were applied together the uptake of ammoniacal N were 33, 40 and 26 per cent and that of nitrate N were 67, 60, and 74 per cent respectively at 20, 40 and 60 DAP. When all the three forms were applied together, the percentage absorption of urea N, ammoniacal N and nitrate N at 20 DAP were 6, 26 and 42 and at 40 DAP were 8, 26 and 28 and at 60 DAP were 5, 16 and 47 respectively.

4.1.6 Uptake of different forms of N during the three stages of growth

The amounts of urea N, ammoniacal N and nitrate N absorbed during 0 - 20 days, 20 - 40 days and 40 - 60 days were worked out and are presented in Table 7. The amounts of urea N absorbed in the molecular form during 0 - 20, 20 - 40 and 40 - 60 DAP were 0.7, 1.1 and 1.7 mg per pot respectively. When the nitrate was the sole source, the amounts absorbed during these three stages were 4.3, 5.5 and 14.5 mg per pot. The corresponding values for

Table 7. Uptake of urea N, ammoniacal N and nitrate N at different growth stages of rice

| N source | N form | Uptake of N form (mg per pot) | | |
|---------------------------|--------------|----------------------------------|-----------|-----------|
| | | 0-20 DAP | 20-40 DAP | 40-60 DAP |
| Urea alone | Urea N | 0.727 | 1.088 | 1.671 |
| | Other forms | 3.251 | 5.112 | 14.925 |
| Nitrate alone | Nitrate N | 4.343 | 5.547 | 14.495 |
| Ammonium alone | Ammoniacal N | 4.922 | 5.006 | 23.330 |
| Urea + nitrate | Urea N | 0.230 | 0.991 | 1.186 |
| | Nitrate N | 0.680 | 2.108 | 4.315 |
| | Other forms | 3.817 | 7.494 | 11.668 |
| Urea + ammonium | Urea N | 0.332 | 0.598 | 1.526 |
| | Ammoniacal N | 1.445 | 2.313 | 7.856 |
| | Other forms | 3.036 | 2.027 | 16.510 |
| Ammonium + nitrate | Ammoniacal N | 1.828 | 3.540 | 5.145 |
| | Nitrate N | 3.684 | 4.418 | 23.439 |
| Urea + ammonium + nitrate | Urea N | 0.243 | 0.477 | 1.016 |
| | Ammoniacal N | 1.079 | 1.210 | 3.509 |
| | Nitrate N | 1.741 | 0.660 | 14.583 |
| | Other forms | 1.085 | 2.214 | 8.985 |

ammoniacal N were 4.9, 5.0 and 23.3 mg per pot. When these three forms were applied in different combinations also, the maximum uptake of all the forms were observed at the third stage i.e. 40 - 60 DAP. When ammonium and nitrate were applied together, while the ammonium uptake was only 5.1 mg per pot, the nitrate N uptake was 23.4 mg per pot. In the presence of all the three forms also, the uptake of nitrate N was much higher than that of other two forms during 40 - 60 DAP.

4.2 Molecular absorption of urea by plantation crops

To know the possibility of molecular absorption of urea by the plantation crops, a pot culture experiment with seedling of plantation crops were done. ^{14}C -labelled urea was applied to the seedlings of coconut, banana, ginger, mango, cashew, pepper, turmeric and cocoa and the plants were allowed to absorb the nutrients for a period of 10 days. The plants were harvested and autoradiographs of these plants were taken. Eventhough the X-ray films were exposed for a period of 1 - 2 months, imprints of the plant parts on the film could not be obtained. These plants were then subjected to the radio-assay employing liquid scintillation technique. The ^{14}C content of the plants were estimated and based on these values the molecular urea uptake was worked out. The data

Table 8. Amounts of molecular urea absorbed by a few selected plantation crops

| Crop | Uptake of urea (μg per g dry weight) |
|----------|--|
| Coconut | 0.530 |
| Cashew | 0.450 |
| Banana | 0.720 |
| Pepper | 3.270 |
| Ginger | 0.930 |
| Turmeric | 2.430 |
| Mango | 1.200 |
| Cocoa | 1.011 |

are presented in Table 8. It was observed that the quantity of urea absorbed by the different crops was negligible and ranged from 0.5 - 3.3 μg per g of dry weight. Among the different crops, black pepper and turmeric recorded the highest absorption of molecular urea.



Discussion

5. DISCUSSION

This investigation forms the third part of a study on molecular absorption of urea. In the first part, it was observed that urea hydrolysis was inhibited following flooding the soil (Saraswathy et al., 1991). The second part of the study revealed that flooded rice plants were capable of absorbing urea in the molecular form, especially when it was top dressed (Safeena et al., 1994). In the present investigation a pot culture study was undertaken to know the absorption of molecular urea in the presence of other forms of nitrogen i.e. ammoniacal and nitrate nitrogen. In this study ^{14}C -labelled urea, ^{15}N -labelled ammonium sulphate and ^{15}N -labelled sodium nitrate were used in different combinations to know the preferential absorption.

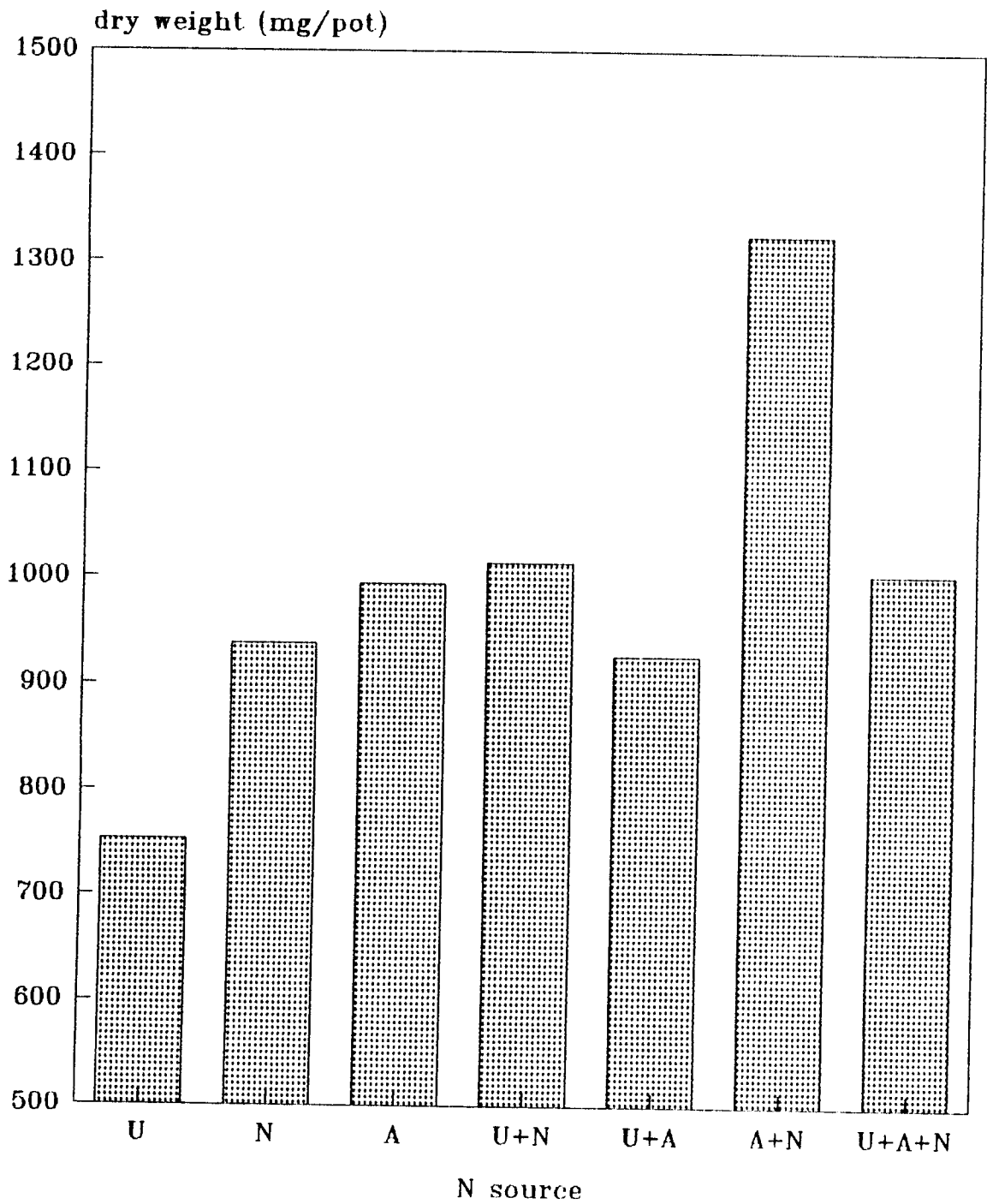
An attempt was also made in this investigation to know the possibility of molecular absorption of urea by a few selected plantation crops.

5.1 Influence of different forms of nitrogen on rice

5.1.1 Dry weight

It was observed that the dry weight of the plants was higher when they were given combinations of

Fig.1 Influence of different forms of N on dry weight of rice at 60 DAP



[U = Urea ; N = Nitrate ; A = Ammonium]

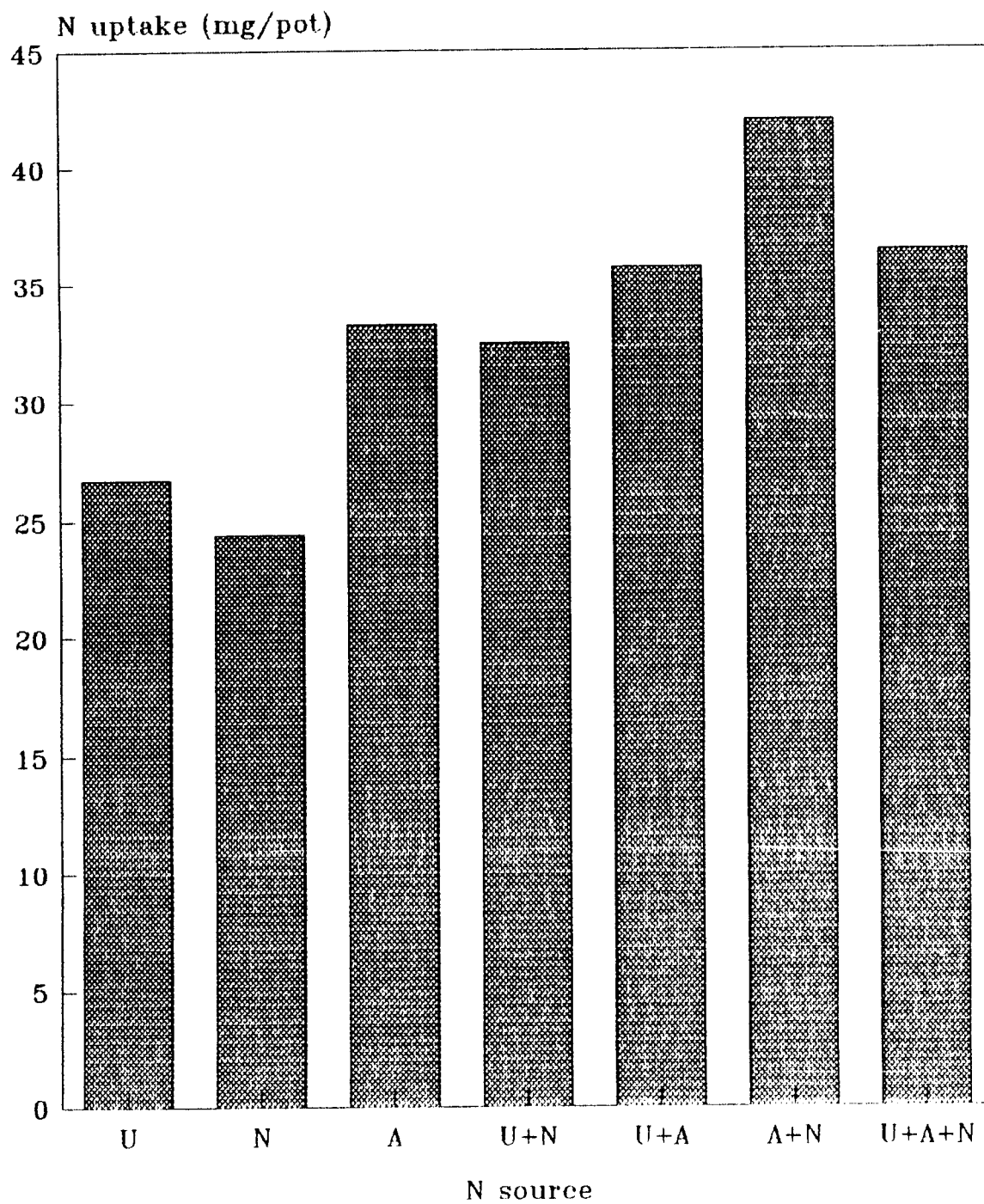
different forms of nitrogen than a single form (Table 2; Fig.1). Among the different combinations, ammonium + nitrate resulted in the maximum dry weight (Fig. 1). Many other workers have also reported that fresh weight and dry weight per plant generally increased more rapidly when plants were supplied with a combination of nitrate and ammonium N than when either nitrate N or ammonium N was supplied alone (Schrader *et al.*, 1972; Gashaw and Mugwira, 1981). Comparative studies of nitrate and ammonium as the source of nitrogen for the rice plant began way back from 1884 with the work of Kellner. In his solution culture studies Kellner (1884) observed that during the first three weeks the nitrate supplied plants appeared sickly and ammonium supplied plants were healthy and during the later stages nitrate supplied plants yielded more than the ammonium supplied plants. His results also showed that the best growth throughout the experimental period was obtained by supplying both nitrate and ammonium nitrogen. However these conclusions were strongly challenged by several other early workers like Kelly (1911); Nagaoka (1904) who were of the opinion that application of nitrate usually fails to bring out a significant increase in yield. In another elaborate study, Ta and Ohira (1981) found that the growth of Indica and Japonica rice plants fed with ammonium was very poor in summer compared to

those fed with nitrate or ammonium + nitrate. They also observed that the plants fed with ammonium appeared to be sickly with tip burning on their lower leaves and with development of a poor root system, especially during the summer season. They have attributed the harmful effect of ammonium on the growth of rice plants, to a rapid decrease in the medium pH caused by higher uptake of ammonium. In the present investigation it was found that the dry weight of rice plants did not differ significantly when ammonium or nitrate was supplied alone (Table 2). However a significant increase in dry weight was observed when these two forms were supplied together (Table 2; Fig. 1). It could be possible that the presence of nitrate enhanced the nitrogen assimilation and mitigated the toxicity of ammonium. Tanaka *et al.* (1959) and Yamasaki and Seino (1965) obtained similar results indicating that nitrate had beneficial effects on the growth and yield of rice plants due to increased metabolic activities and the development of new roots resulting in effective absorption.

5.1.2 Nitrogen uptake

The rice plants in all the stages recorded a higher uptake of nitrogen when supplied with combination

Fig. 2 Influence of different forms of N on N uptake by rice at 60 DAP



[U = Urea ; N = Nitrate ; A = Ammonium]

of nitrogen forms than a single source. The rice plants fed with both ammonium + nitrate recorded in general, the highest uptake (Table 4; Fig.2). There were several reports suggesting that uptake of nitrate was considerably repressed by the presence of ammonium in the medium while nitrate exerted a minor effect on the uptake of ammonium (Mengel and Viro, 1978; Lycklama, 1963, and Minotti et al., 1969). One of the reasons attributed to this inhibitory effect was the adverse effect of ammonium on nitrate reductase activity. The inhibition of nitrate reductase activity can cause the accumulation of nitrate in the plant which in turn can reduce the uptake of nitrate. In the present study no such inhibitory effect of ammonium on nitrate uptake was observed. Schrader et al. (1972) also observed that when both nitrate N and ammonium N were supplied to corn the uptake of nitrate was not retarded by the presence of ammonium. The results of the present investigation did not fully agree with findings of several other workers, (Kelley, 1911 and Nagaoka, 1904) who in soil culture experiments showed that nitrate nitrogen failed to increase yield of rice and also ammonium nitrogen was indispensable for rice growth. When nitrate nitrogen is available to plants there is no reason why it should not increase the yield. In their experiment with soil possibly leaching and denitrification of nitrate

reduced the availability of nitrate nitrogen to the plant leading to its lower efficiency.

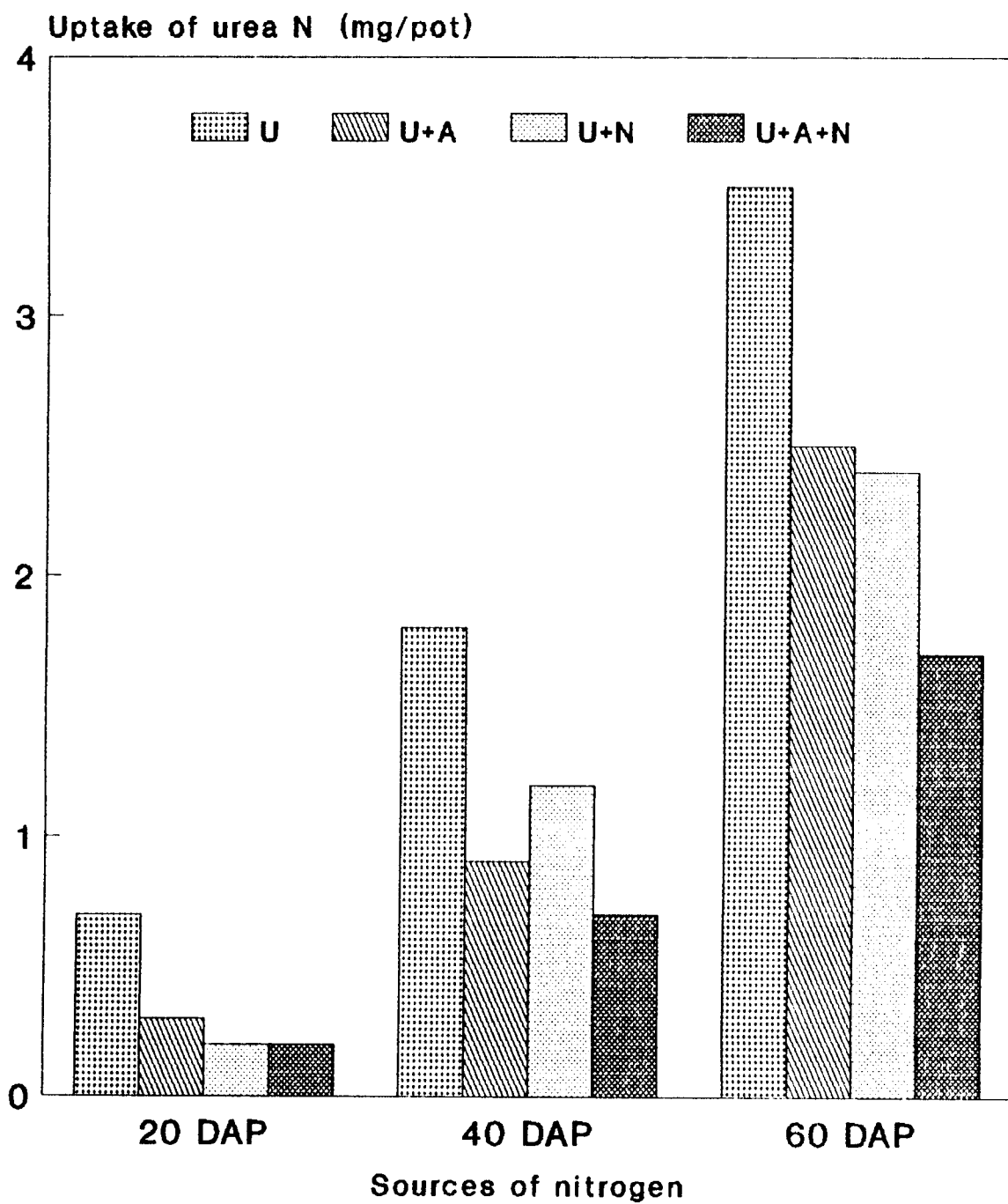
5.1.3 Uptake of urea N, ammoniacal N and nitrate N from different sources of N

It was evident from the data that rice plants could absorb molecular urea and the amount of urea N absorbed varied from 0.7 to 3.5 mg per pot at the different stages (Table 5) and was approximately 12 - 18 per cent of the total N uptake (Table 6). It was also observed that in the presence of either nitrate or ammonium or both, rice plants absorbed molecular form of urea. Even though it has been reported that rice plants are capable of absorbing molecular urea (Mitsui and Kurihara 1962; Saraswathi et al., 1991 and Safeena et al., 1994), it is observed for the first time that rice plants absorbed urea even in the presence of other readily available forms of nitrogen. In the presence of either nitrate or ammonium the urea N absorption varied from 5 - 10 per cent and in the presence of both these forms urea N absorption was 4 - 8 per cent of the total N uptake. The pathways of urea after its uptake needs a detailed investigation.

In the present investigation when urea was applied alone, the urea absorbed does not account for the

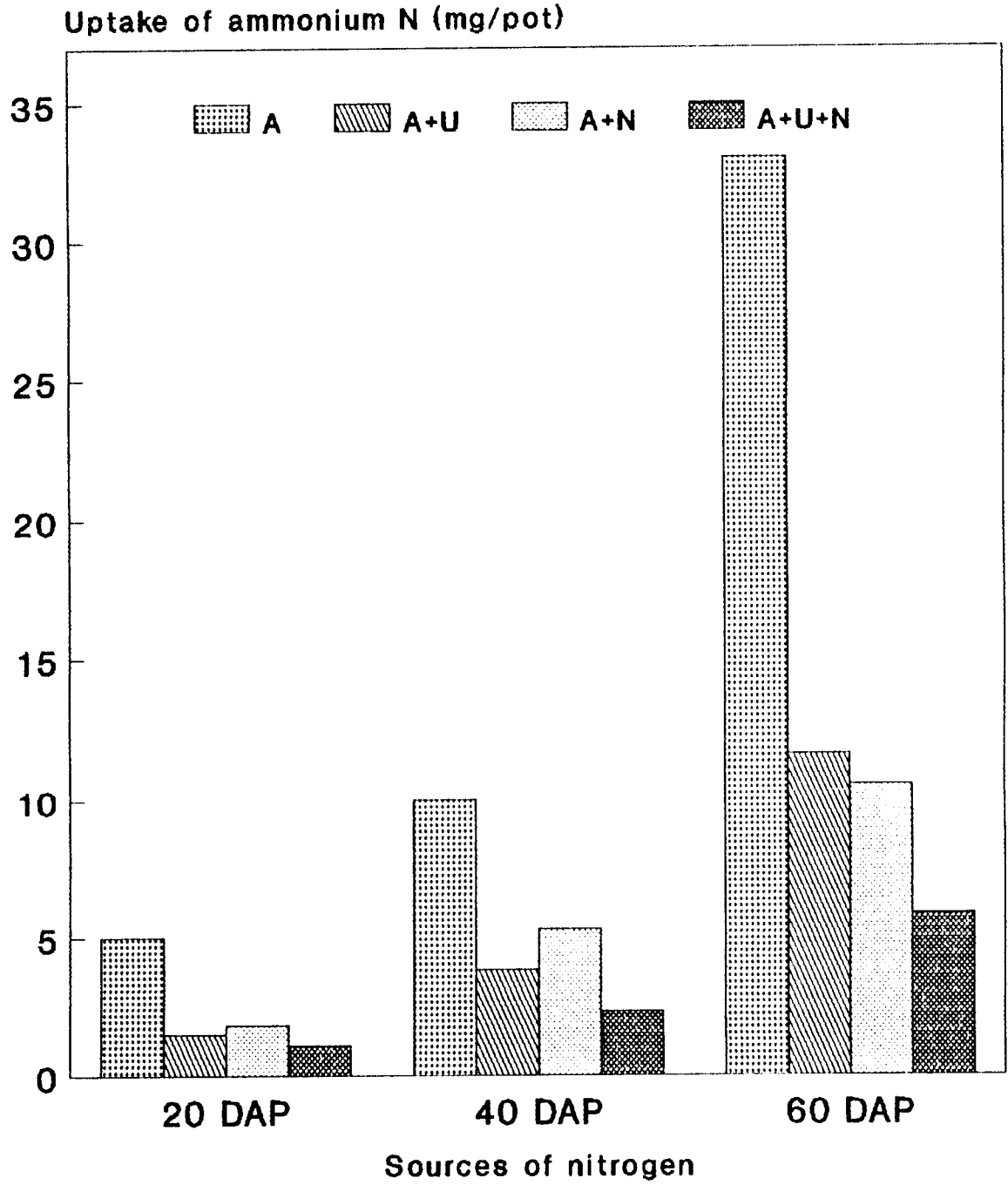
total N uptake of the plants, thus indicating presence of other forms of N also. These forms accounted for almost 80 - 87 percentage (Table 6) of the total N uptake. The presence of N in forms other than urea may be explained as follows. As the rice plants were grown in inert quartz sand there was little chance for the presence of urease enzyme in the media. Hence the hydrolysis of urea must have taken place either at the root surface or after its translocation to the shoots. According to Hartel (1977), urea can be taken up directly by the roots and can be rapidly hydrolysed by the enzyme urease either within the roots or after its translocation to the shoots. In a water culture experiment vanBeuschem and Neetson (1982) observed that xylem exudates of both ammonium and urea supplied to maize plants were found to be almost free from these N sources leading to the conclusion that urea and ammonium are almost quantitatively metabolized in the roots. They have also suggested at least two pathways for assimilation of urea, the first being the hydrolytic decomposition catalysed by urease followed by incorporation of ammonium and second being a direct incorporation of urea via the reversal of the ornithine cycle. Findings of the present investigation is contradictory to the second hypothesis of the direct incorporation of urea. Had there been a direct incorporation of urea, there would

Fig.3 Uptake of urea N by rice from different sources of N at 20, 40 & 60 DAP



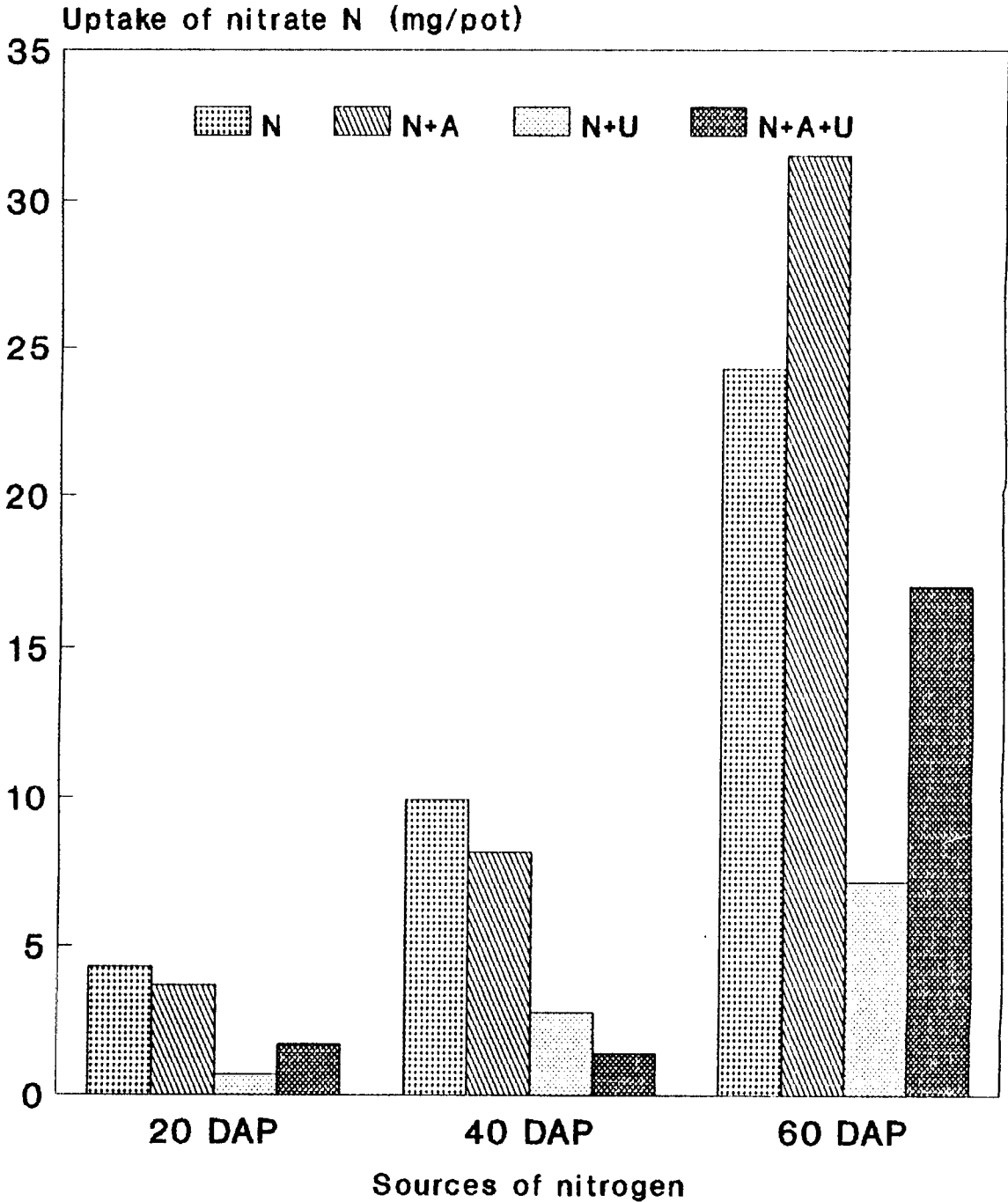
[U = Urea , A = Ammonium , N = Nitrate]

Fig.4 Uptake of ammonium N by rice from different sources of N at 20, 40 & 60 DAP



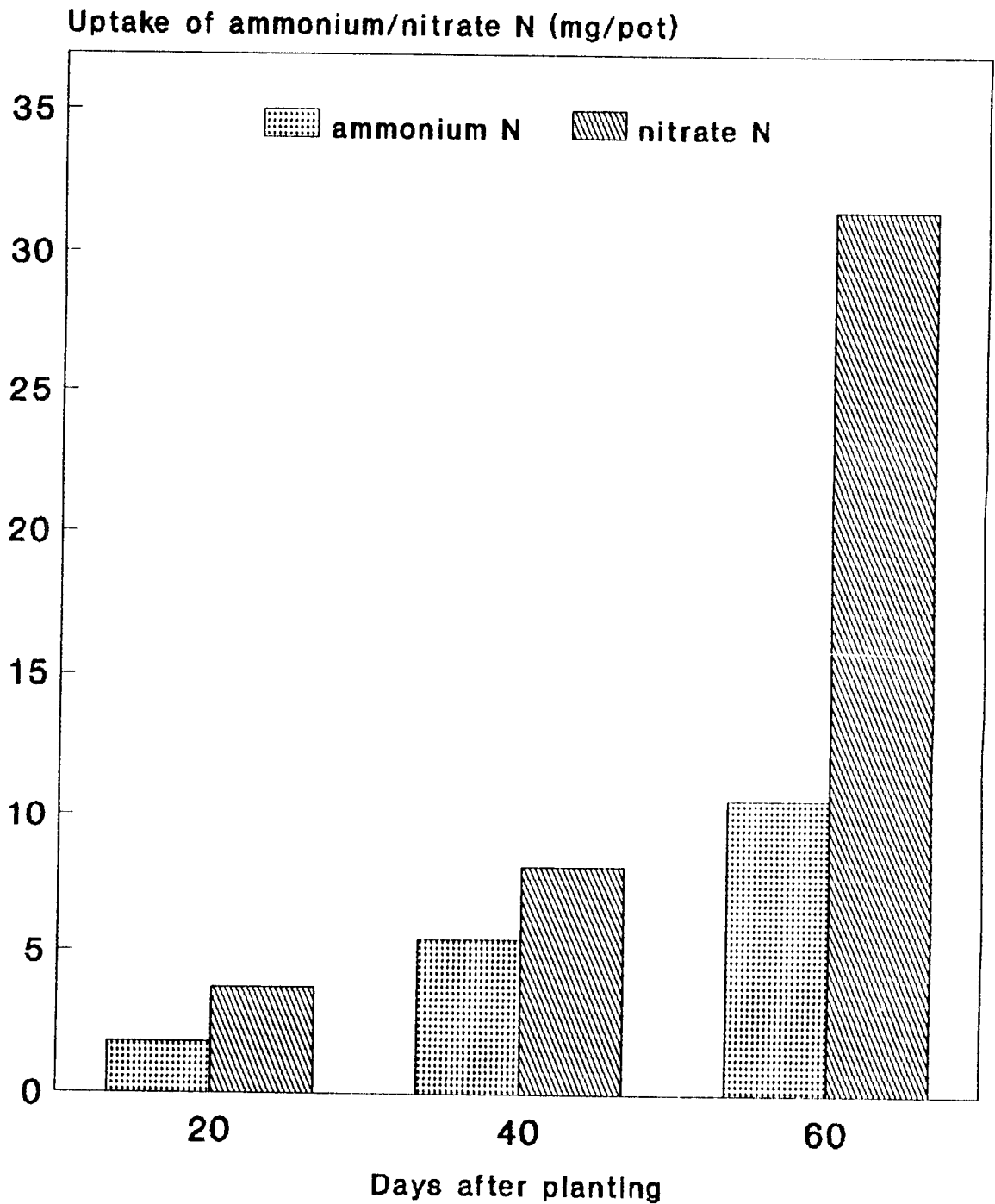
[U = Urea ; A = Ammonium ; N = Nitrate]

Fig.5 Uptake of nitrate N by rice from different sources of N at 20, 40 & 60 DAP



[U = Urea ; A = Ammonium ; N = Nitrate]

Fig.6 Uptake of ammonium N and nitrate N by rice following the combined supply of ammonium & nitrate at 20, 40 & 60 DAP



Summary

6. SUMMARY

An investigation was conducted at the Radio-tracer Laboratory, College of Horticulture, Vellanikkara in the year 1993. The following experiments were undertaken during the course of this investigation.

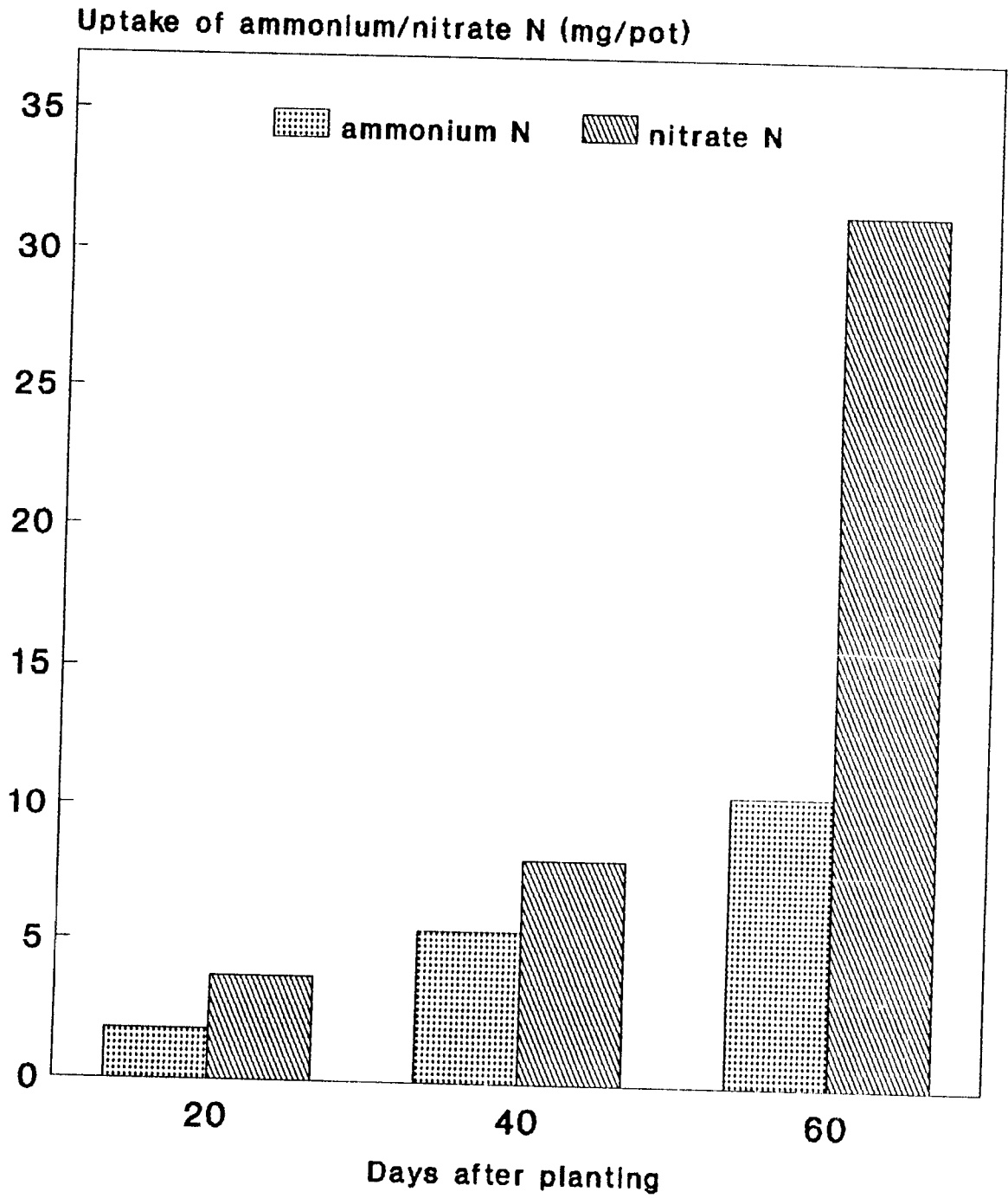
1. Dynamics of the absorption of nitrogen forms relative to its concentration at different growth stages of rice using ^{14}C -labelled urea, ^{15}N -labelled ammonium sulfate and ^{15}N -labelled sodium nitrate.

2. Pot culture experiment using ^{14}C -labelled urea to study the possibility of absorption of molecular urea by the plantation crops.

The results of the investigation are summarised below:

It was observed that the dry weight of rice plants was higher when they were given combinations of different forms of nitrogen than a single form. Among the different combinations ammonium + nitrate resulted in the maximum dry weight. When nitrogen was supplied in a

Fig.6 Uptake of ammonium N and nitrate N by rice following the combined supply of ammonium & nitrate at 20, 40 & 60 DAP



data that the uptake of urea was reduced in the presence of either ammonium and or nitrate (Fig. 3). Bradley *et al.* (1989) observed that in wheat seedlings urea inhibited both ammonium and nitrate uptake, whereas ammonium inhibited the uptake of urea, while nitrate enhanced the uptake of urea.

At 60 DAP, when nitrate alone was applied at a concentration of 120 ppm N the nitrate N uptake was 24 mg per pot (Table. 5). When the concentration of nitrate was reduced to half i.e. 60 ppm and when applied along with ammonium at the same concentration the nitrate uptake was as high as 32 mg per pot. A comparison between the uptake of ammoniacal N and nitrate N, when these two forms were present together indicated that rice preferred nitrate form of N to ammoniacal form at the different growth stages studied (Table 5; Fig.6). This is in contrast to the findings of Ta and Ohira (1981) who observed uptake of nitrate was considerably repressed by the presence of ammonium in the medium. The data in the present investigation clearly indicate that though urea had an inhibitory effect on nitrate uptake, ammonium had a highly favourable influence on the uptake of nitrate (Fig. 5). With respect to ammonium uptake, it was seen that urea and nitrate had more or less similar influence on its uptake

(Fig. 4). The uptake of ammoniacal N was slightly better in the presence of urea than in the presence of nitrate. These observations are in line with the findings of Morgan and Jackson (1988) who observed that net ammonium was low in plants grown continuously in nitrate.

The data on the uptake of different forms of nitrogen, during the different growth stages (Table 7) indicated that uptake of all the different forms of N was maximum during the third stage of observation i.e. 40 - 60 DAP. It was also seen that when ammonium + nitrate was applied, though there was not much difference in the uptake pattern of ammonium and nitrate in the initial two stages (0 - 20 DAP and 20 - 40 DAP) a substantial increase in nitrate uptake was observed during the later stage of growth (40 - 60 DAP). This indicates the preference of rice plants for nitrate N over ammoniacal N during the later stages of growth. According to Karim and Vlamis (1962), during the initial stages of rice growth there could be rapid uptake of ammonium, lowering the pH of the culture solution and the resulting acidity may restrict the root activity. According to Tanaka *et al.* (1959) at higher levels of nitrogen, nitrate was superior to ammonium as the latter gave restricted root growth and nitrogen metabolism was disturbed resulting in accumulation of nonprotein nitrogen.

were supplied with a combination of N forms than a single source. Among the different combinations ammonium and nitrate applied in equal proportions resulted in the best growth of the plant and maximum N uptake. Though the presence of urea had a repressing effect on the uptake of nitrate, ammonium enhanced the uptake of nitrate. It was also observed from the study that, the plantation crops did not prefer molecular urea.

single form though nitrate recorded the highest dry weight, it did not differ significantly from the dry weight of the plants supplied with ammonium. The lowest dry weight was obtained when nitrogen was supplied as urea alone.

The highest nitrogen content in plants was observed when nitrogen was supplied as urea + ammonium. Among the plants supplied with nitrogen in a single form, those received urea recorded the highest N content.

The nitrogen uptake by rice plants was highest when supplied with ammonium + nitrate. When the plants were fed with only one form of nitrogen, ammonium treated plants recorded a higher N uptake than the nitrate or urea treated plants.

The molecular absorption of urea by the rice plant was found 12 - 18 percentage of the total N uptake when urea was supplied as the sole source of nitrogen. A reduction in urea uptake was observed when urea was supplied along with either ammonium or nitrate or both. When urea was supplied along with nitrate a reduction in the uptake of both these forms was observed.

With respect to ammonium uptake, it was seen that urea and nitrate had more or less similar influence on it. The uptake of ammoniacal N was slightly better in the presence of urea than in the presence of nitrate.

Among the three forms of nitrogen supplied at different combinations, nitrate N recorded the highest uptake when present along with urea N or ammoniacal N or both. Though a reduction in nitrate N absorption was observed in the presence of urea, it was rather increased in the presence of ammonium. The uptake of nitrate N was more in the presence of ammonium than in its absence.

During the three stages (0 - 20, 20 - 40, 40 - 60 DAP) of observation it was found that rice absorbed more amount of N during the period 40 - 60 DAP . In all the three stages in the presence of all the three forms, the uptake of nitrate N was much higher than that of other two forms.

Among the three forms of N and different combinations, ammonium + nitrate was found to be the best for the greater N uptake and better growth.

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*Original not seen

Appendices

Appendix 1

Weather data (weekly average) for the experimental period
(from 4-6-1993 to 26-8-1994)

| Stan- dard Weed No. | Month and Date | Total rain- fall (mm) | No. of rainy days | Temperature | | Relative humidity | | Sun shine hours | Evapo- ration mm/day |
|------------------------------|----------------------|--------------------------------|----------------------------|-------------|------------|----------------------|-----------------|-----------------------|----------------------------|
| | | | | Max. °C | Min. °C | Fore- noon% | After- noon% | | |
| 23 | Jun 4-10 | 236.6 | 6 | 29.6 | 23.3 | 95 | 80 | 1.8 | 3.5 |
| 24 | Jun 11-17 | 237.9 | 7 | 29.2 | 23.8 | 95 | 80 | 1.8 | 3.5 |
| 25 | Jun 18-24 | 85.5 | 4 | 30.4 | 24.5 | 94 | 73 | 4.4 | 3.8 |
| 26 | Jun 25-Jul 2 | 186.4 | 5 | 29.2 | 23.6 | 94 | 82 | 2.9 | 3.3 |
| 27 | Jul 2-8 | 188.9 | 6 | 28.6 | 22.7 | 95 | 78 | 2.0 | 3.1 |
| 28 | Jul 9-15 | 167.8 | 7 | 28.7 | 22.6 | 92 | 83 | 1.8 | 3.1 |
| 29 | Jul 16-22 | 128.1 | 6 | 28.9 | 22.9 | 94 | 76 | 2.8 | 2.9 |
| 30 | Jul 23-29 | 101.0 | 6 | 28.0 | 23.1 | 94 | 80 | 2.9 | 3.1 |
| 31 | Jul 30-Aug 5 | 96.4 | 6 | 29.1 | 23.7 | 95 | 76 | 3.6 | 3.8 |
| 32 | Aug 6-12 | 54.9 | 4 | 29.9 | 23.5 | 95 | 75 | 4.6 | 3.9 |
| 33 | Aug 13-19 | 66.3 | 6 | 29.2 | 23.1 | 93 | 78 | 3.3 | 3.7 |
| 34 | Aug 20-26 | 61.9 | 4 | 29.8 | 23.2 | 96 | 74 | 5.6 | 4.0 |

Appendix 2

Abstract of Anova

Effect of different N sources on dry weight of rice at 20, 40 and 60 DAP.

| Source | df | Mean Square | | |
|-----------|----|-------------|------------|------------|
| | | 1 | 2 | 3 |
| | | 20 DAP | 40 DAP | 60 DAP |
| N Sources | 6 | 912.873* | 21782.206* | 88433.333* |
| Error | 14 | 236.286 | 2228.524 | 23519.048 |

* Significant at 5%

Appendix-3

Abstract of Anova

Effect of different N source on nitrogen content in rice at 40 and 60 DAP

| Source | df | Mean square | |
|-----------|----|-------------|--------|
| | | 1 | 2 |
| | | 40 DAP | 60 DAP |
| N Sources | 6 | 0.303* | 0.469* |
| Error | 14 | 0.028 | 0.012 |

* Significant at 5%

Appendix-4

Abstract of Anova

Effect of different N sources on nitrogen uptake by rice at 20,40 and 60 DAP.

| Source | df | Mean square | | |
|-----------|----|-------------|---------|----------|
| | | 1 | 2 | 3 |
| | | 20 DAP | 40 DAP | 60 DAP |
| N sources | 6 | 0.822* | 17.735* | 106.736* |
| Error | 14 | 0.228 | 4.977 | 16.547 |

* Significant at 5%

DYNAMICS OF THE ABSORPTION OF FORMS OF N BY CROP PLANTS

By

P. K. RETHEESH

ABSTRACT OF A THESIS

Submitted in partial fulfilment of the
requirements for the degree of

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Faculty of Agriculture

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COLLEGE OF HORTICULTURE

Vellanikkara - Thrissur

Kerala

1995

ABSTRACT

An investigation was conducted at the Radio-tracer Laboratory, College of Horticulture, Vellanik-kara in the year, 1993 which consisted of two parts. In the first part absorption of different forms of nitrogen relative to its concentrations at different growth stages of rice was studied using labelled and non-labelled fertilizers. To know the molecular absorption of urea ^{14}C -labelled urea fertilizer was used and the presence of ^{14}C in the plant was taken as an evidence for the absorption of molecular form of urea. In the case of ammoniacal and nitrate forms of N, ^{15}N -labelled and non-labelled materials were used alternately to find out the preferential absorption. In the second part of the investigation an attempt was made to know the possibility of molecular absorption of urea by a few selected plantation crops.

The uptake of N and dry matter production were higher when rice plants were supplied with a combination of ammonium and nitrate in equal proportions. Among the three forms of nitrogen supplied at different combinations, nitrate N recorded the highest

uptake when present along with urea N or ammoniacal N or both. Though the presence of urea had a repressing effect on the uptake of nitrate, ammonium enhanced the nitrate uptake. The uptake of nitrate N was more in the presence of ammonium than in its absence. With respect to ammonium uptake it was found that urea and nitrate had more or less similar influence on it.

It was seen that rice plants absorbed molecular form of urea even in the presence of other readily available forms of nitrogen. The molecular absorption of urea by rice plant was found to vary from 12 - 18 percentage of the total N uptake. When urea was supplied along with ammonium and or nitrate the uptake of urea was found reduced. The quantity of urea absorbed by the plantation crops was found negligible and ranged from 0.5 - 3.3 μg per g of the plant dry weight.

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