Agronomy & Horticulture -- Faculty Publications   Agronomy and Horticulture Department

April 2005

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Sheehy, J. E.; Mnzava, M.; Cassman, Kenneth G.; Mitchell, P. L.; Ferrer, A. B.; Robles, R. P.; and Pablico, P., "Temporal Origin of Nitrogen in the Grain of Tropical Wet-Season Rice" (2005). *Agronomy & Horticulture -- Faculty Publications*. 93.
http://digitalcommons.unl.edu/agronomyfacpub/93

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Temporal Origin of Nitrogen in the Grain of Tropical Wet-Season Rice

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ABSTRACT

The total N in the grain is the integral of the product of the total N absorbed at any instant and the fraction of that N eventually allocated to the grain. We investigated the temporal origin of N in the grain of a wet season rice crop and tested the suitability of ^{15}N nitrate ($NH_4{}^{15}NO_3$) as a label for that purpose. The total N content of rice (*Oryza sativa* L.) plants was measured by growth analysis throughout the duration of the crop and the measurements were used to calculate the rate of total N uptake. A point-placement technique was used to deliver small amounts of ^{15}N nitrate to roots of the rice plant and this enabled the eventual fate of the total N absorbed at any time to be determined. The rate at which N was acquired by the panicle exceeded that by the whole plant at 64 d after transplanting (DAT); thereafter, N was transferred from the leaves to the panicle. About 60% of N in the grain was acquired before panicle initiation and was transferred from leaves during grain filling. A comparison between the uptake and retention of labeled nitrate and urea applied separately at 35 DAT showed that 21 and 58% of the ^{15}N nitrate and ^{15}N urea, respectively, were recovered. There were no advantages of using ^{15}N nitrate as opposed to ^{15}N urea as a label in such research of irrigated rice.

E XCESSIVE N application and poor N management reduce the profit of rice farmers by increasing production costs and reducing grain yield (Peng et al., 2003). Poor resource management can also affect the emission of greenhouse gases (Matthews, 2003). In the future, farmers will have to adopt new fertilization strategies that require a determination of the potential yield of a crop in a given climatic condition and the corresponding minimum amount of N required to achieve it (Lemaire and Gastal, 1997). The N content of rice at the panicle formation stage (about 10–15 d before flowering) has been shown to be an important determinant of sink size and eventual yields (Hasegawa et al., 1994; Horie, 2001). In irrigated rice, it is common to recover 20 to 60% of the fertilizer N applied (Vlek and Byrnes, 1986; Cassman et al., 1993, 1996b). It has been suggested that to improve N-use efficiency, the crop's demand for N, and its supply from indigenous and fertilizer sources, must be synchronized through proper timing, application rate, and placement of fertilizer (De Datta and Buresh, 1989; Cassman et al., 1996a; Witt et al., 1999). However, the changing relationship between the amount of fertilizer required by the plants and the amount required in the root environment to meet those requirements is obscure. A large frac-

tion of N in the grain comes from vegetative parts (Norman et al., 1992), but little is known about when that N was absorbed during the vegetative stage of growth.

In Asia, climatic conditions vary markedly between wet and dry seasons and direct seeded and transplanted rice may require different optimum fertilizer strategies (Guindo et al., 1994), although the area of direct seeded rice still accounts for less than 22% of the total rice area (Pandey and Velasco, 2002). The average daily radiation in the wet season at IRRI is 15.9 MJ m^{-2} d^{-1}, but daily values can range from about 3 to 30 MJ m^{-2} d^{-1}.

In our previous studies, small quantities of ^{15}N urea were used as a label to trace the eventual fate of N at different times during the dry season (Sheehy et al., 2004a, 2004b). A point-placement technique was used to deliver the label. The results suggested that more than half the N in the grain was acquired before anthesis and transferred from leaves to grains during grain filling. Furthermore, there was evidence of strong competition for N between the developing panicles and tillers from mid-tillering to maturity. However, it is not known if the same patterns of N distribution occur in the wet season under conditions of reduced yield potential due to lower solar radiation and higher nighttime temperatures. Consequently, the major objective of the work described in this paper was to quantify the temporal origin of the N in the grain during the wet season. In the work of Sheehy et al. (2004a, 2004b), the residence time of the applied ^{15}N urea in the soil was about 2 wk. Consequently, daily distribution of ^{15}N in the plant could not be measured and this made the overall pattern of N use indistinct with respect to time. In this paper, we used ^{15}N nitrate in the belief that it would have a shorter residence time in the soil than ^{15}N urea, thus enabling a more detailed temporal pattern of N use by the crop to be described.

MATERIALS AND METHODS

Background Theory

The theory of using ^{15}N applied with the point-placement technique is given in Sheehy et al. (2004a, 2004b). It was assumed that ^{15}N and unlabeled N were distributed in the same manner through the plant after capture. The measurements of ^{15}N were linked with measurements of total N uptake, so that a total N budget for the grain, in terms of its temporal origin, could be calculated. The N in the grain can be calculated as the sum of the daily uptake of total N multiplied by the fraction of N that is allocated to the grain. The fractional allocation of N was measured using ^{15}N as a label. Growth analysis techniques and curve fitting were used to calculate the total

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Abbreviations: DAT, days after transplanting; $E(x/y)$, the ratio of the ^{15}N content of a rice hill at maturity to the maximum amount recovered, following an application of ^{15}N; IRRI, International Rice Research Institute; N_c, the N content of a rice hill; N_g, the N content of the grain; R_c, recovery of ^{15}N by a rice hill.

uptake of N at any stage of growth. Because ^{15}N is recovered over a period of time measured in days, it is useful to write an equation describing the N content of the grain (N_g) as the outcome of a series of intervals covering the whole period of crop growth:

$$N_g = \sum_{j=1}^{n} \Delta N_{uj} f_j j \qquad [1]$$

where $f_j = {}^{15}N_{gj}/{}^{15}N_j$ and is the fraction of the ^{15}N captured in the interval j ($^{15}N_j$) that is eventually located in the grain at maturity ($^{15}N_{gj}$), ΔN_{uj} is the total amount of N accumulated in the same interval. Without much loss of accuracy, ΔN_{uj} can be calculated from values obtained from a fitted curve describing the relationship between total N and time, constructed using data from a sequence of measurements of the total net N content of the plants. If there are no losses of labeled N once taken up by the plant (as in this experiment), the gross accumulation is the same as the net accumulation of N by the plant. However, if there are losses, they can be calculated using the ^{15}N data and the curve refitted to give the gross rate of N capture (Sheehy et al., 2004b).

Nitrogen Point-Placement Technique

The N point-placement technique used in this study has been fully described in Sheehy et al. (2004a). A gelatin capsule (0.6 mL) was glued to a thin wooden handle 25 cm long so that it could be inserted at a 5-cm soil depth beneath the center of the rice hill. The wooden handles and gelatin capsules were assembled in the laboratory and taken to the field. Just before insertion, the capsule was filled with a 0.5-mL solution of nitrate ^{15}N, 99% atom ^{15}N excess ($NH_4{}^{15}NO_3$) of known concentration. The amounts supplied ranged from 0.32 to 4.48 mg ^{15}N, which represented about 30% of daily uptake of total N for the day. The ammonium nitrate was purchased in crystalline form from Icon Service, Old Kings Highway, Marion, NY 12456, USA. The gelatin dissolved within a few minutes releasing the labeled N. The quantity of labeled N was sufficiently small to ensure rapid uptake of the label and large enough to be detectable at harvest. Following uptake, the labeled N in the hill is distributed between the various plant organs depending on their demand for N during the period of uptake. Subsequent transfers of that N will occur between organs depending on their relative demands for N. The quantities of ^{15}N in the leaves, stems, roots, panicles, and grains (if present) of the exposed plants were measured at each harvest using mass spectrometry.

To determine the uptake pattern of recovery of ^{15}N as a function of time, applications of ^{15}N were made on 17 and 55 DAT and four labeled plants were harvested 1, 2, 3, 4, 7, 14, 21, and 28 d after labeling and at crop maturity. The patterns of ^{15}N recovery by the plants, following labeling, were described using the equation in Sheehy et al. (2004a, 2004b):

$$R_c = a[1 - \exp(-b\, t_{dal})] \qquad [2]$$

where a represents the maximum uptake of ^{15}N by a plant, the initial slope of the curve is ab, and t_{dal} is days after ^{15}N point-placement. The time taken for the plant to absorb 95% of the isotope (T_{95}) is derived from Eq. [1] and is $T_{95} = (1/b)$ ln(20); this is a pragmatic proxy for estimating the maximum measurable uptake because Eq. [2] has an asymptote (the value of a) when t_{dal} is at infinity.

A comparison between the uptake and retention of nitrate ^{15}N (99% atom ^{15}N excess, $NH_4{}^{15}NO_3$) and urea ^{15}N [99% atom ^{15}N excess, $CO\,({}^{15}NH_2)_2$] was made by applying them separately at 35 DAT (approximately the time of maximum N uptake). The ^{15}N content of four labeled plants in each of the urea and

nitrate treatments was measured at weekly intervals following labeling until maturity.

To investigate the temporal origin of N in the grain, ^{15}N nitrate was supplied at 2-wk intervals, on 17, 31, 45, 53, and 73 DAT and its distribution in the plant was measured 7 and 14 d after labeling and at maturity. The ratio of the ^{15}N content of a rice hill at maturity to the maximum amount recovered, following an application of ^{15}N, was calculated using the equations of Mood et al. (1974):

$$E(x/y) \approx E(x)/E(y) - C(x, y)/E(y)^2 + \\ E(x)V(y)/E(y)^3 \qquad [3]$$

and

$$V(x/y) \approx [E(x)/E(y)]^2[V(x)/E(x)^2 + \\ V(y)/E(y)^2 - 2C(x, y)/E(x)E(y)] \qquad [4]$$

where the mean value recovered at maturity is $E(x)$, the mean value of the maximum amount recovered is $E(y)$, and variances are denoted by $V(x)$ and $V(y)$, respectively; the covariance between x and y is denoted by $C(x, y)$.

Field Experimental Details

Experiments were conducted in a field under irrigated conditions during the wet season of 2001 at IRRI, Los Baños (14°11′ N lat, 121°15′ E long). The soil was an Andaqueptic Haplaquoll with pH 5.7, 20.8 g organic C kg^{-1}, and 2.38 g total N kg^{-1} in the plow layer (a depth of 20–30 cm). The plot was divided into 12 sections (10 by 8 m) by planks above the water for ease of access. The high-yielding, elite cultivar IR72 was used. Seeds were sown in plastic trays on 21 June and the seedlings were transplanted 14 d after sowing at the standard spacing of 20 by 20 cm (25 plants m^{-2}). At each planting position, known as a hill, one seedling was transplanted. The hill—for example, the single plant at a given position—was the experimental unit. The N contents of individual hills can be converted to N content m^{-2} by multiplying by 25.

One week after transplanting, the hills to be labeled with ^{15}N were selected, starting from a random position in the third row from the border row and every five hills thereafter. Each position was marked with a numbered bamboo stake. The numbered hills were randomly assigned in advance to each ^{15}N labeling and sampling date.

Management practices were employed to ensure the best possible biotic and abiotic conditions so that yields could approach the maximum achievable (yield potential). The water depth in the paddy was managed so that it was no less than 5 cm and no greater than 10 cm. A fertilizer rate of 180–90–225 kg ha^{-1} of N, P and K respectively, supplied in the form of CO $(NH_2)_2$, $Ca(H_2PO_4)_2$, and KCl and 10 kg ha^{-1} of Zn in the form of $ZnSO_4$ was used. One-third of the N and all of the P, K, and Zn were applied as basal treatments and incorporated in the soil 1 d before planting. The remainder of the N was split equally (10 kg ha^{-1}) and applied as a top-dressing at weekly intervals for 12 wk after transplanting.

Weeds were controlled by pre-emergence herbicide application of pretilachlor [2-chloro-N-(2,6-diethylphenyl)-N-(2-propoxyethyl)acetamide] + fenclorim [4,6-dichloro-2-phenyl-pyrimidine] at a rate of 0.45 kg a.i. ha^{-1} followed by spot hand-weeding 20 to 35 DAT. Snails [Pomacea canaliculata Lamarck] were controlled by application of the molluscicide niclosamide [5-chloro-N-(2-chloro-4-nitropheny)-2-hydroxy benzamide] at 0.25 kg a.i. ha^{-1} 1 wk before planting and 1 DAT followed by hand picking. Insect pests were controlled by timely application of IRRI-recommended pesticides (Sheehy et al., 2001). A lattice of strings and poles was installed before crop canopy

closure to prevent lodging. Bird scarers were employed to prevent birds from feeding on the developing and mature grains.

At maturity, the aboveground biomass and grain yield were estimated from 12 quadrats of 1 m² each, selected at random in areas of the plots where hills were not treated with ¹⁵N. Panicles were hand-threshed and the filled spikelets were separated from unfilled spikelets using a winnowing machine. Dry weights of spikelets and other plant parts were determined after oven-drying at 70°C (3–7 d) to constant weight.

Whenever labeled plants were harvested during the cropping season, four unlabeled plants were also harvested. For all harvested plants, a soil core sampler 20 by 20 by 20 cm in volume was used to obtain the roots. Harvested plants were then washed with tap water and rinsed with distilled water. The plants were separated into leaves, stem (sheath + culm), roots, and panicles and oven-dried at 70°C for 3 to 7 d and their weights recorded. At every harvest, the four unlabeled plants were analyzed (Kjeldahl) for N content. In addition, the dry weights and N contents of plants at transplanting were determined. Unlabeled plants were used to determine natural abundance. To generate a total N accumulation curve for the growing season, the N content of unlabeled hills were used. Data are presented as means and standard errors unless stated otherwise.

RESULTS

Panicle initiation occurred at 40 DAT and flowering commenced at 63 DAT. During the cropping season, the average temperature was 27.9°C, the average daily rainfall, sunshine, and solar radiation were 6.3 mm, 5.2 h, and 17.3 MJ m⁻², respectively, as measured by an automatic weather station maintained by the IRRI Climate Unit.

The patterns of ¹⁵N recovery by the labeled plants for applications on 17 and 55 DAT are shown in Fig. 1a; they were described using Eq. [2] with $r^2 \geq 0.95$. The parameters a and b were significantly different for the applications on 17 and 55 DAT (Fig. 1). The time taken to recover 95% of the label applied, T_{95}, was 3 d for the application at 17 DAT and 11 d for the application at 55 DAT. As a consequence of these results, the ¹⁵N content of the plants 14 d after labeling was used as a measure of maximum recovery of the label. Figure 1b shows the ¹⁵N content of rice plants, at weekly intervals, following the application of labeled nitrate and urea at 35 DAT. The average recovery of nitrate was 23 ± 1% and of urea was 58 ± 3%; there were no significant losses of ¹⁵N from the plants following labeling. For all applications of ¹⁵N nitrate there was no significant variation during the growing season in the percentage of ¹⁵N recovered (mean = 20.7 ± 2.4) at 14 d after labeling, nor was there any significant loss of ¹⁵N between maximum uptake and maturity.

There were no significant differences in the fractional allocation of the ¹⁵N to the grain during the growing season; the average value, f_r, for the whole growing season was 0.44 ± 0.2. The pattern of total N accumulation during the growing season was described using a sigmoid curve. The equation used to relate time, t, in days after transplanting to N content in g hill⁻¹ (N_c) was

$$N_c = a/[1 + (t/b)^c] \qquad [5]$$

where a = 0.96 g N hill⁻¹, b = 37.83 d, c = −2.66, and r^2 = 0.96 (Fig. 2a). The N content of the plants at trans-
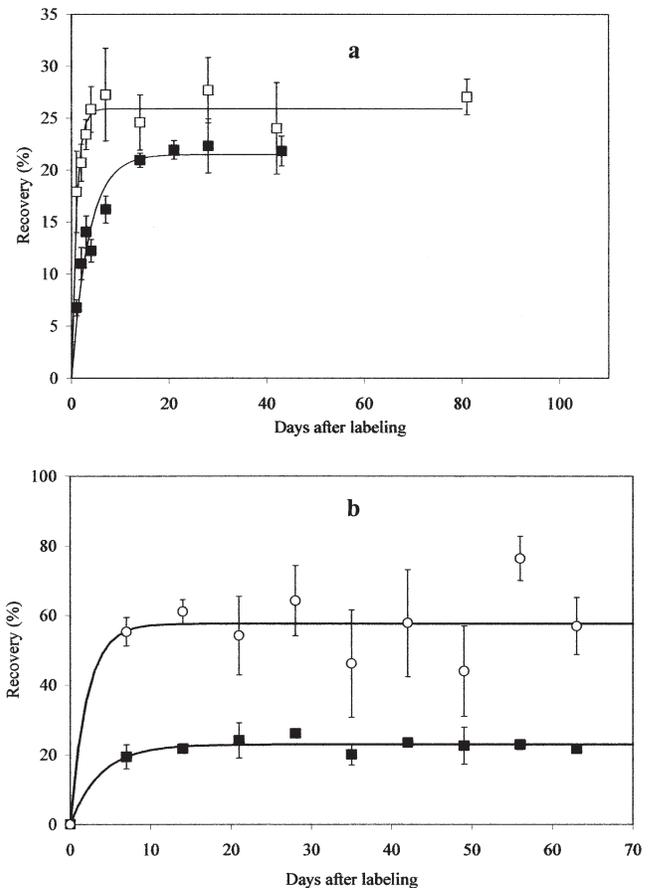
Fig. 1. (a) Time course for recovery of ¹⁵N in rice plants from an application of labeled (99% atom ¹⁵N excess) nitrate at (□) 17 DAT and (■) 55 DAT at the 5-cm soil depth in the wet season of 2001. Each point is the mean of four replicates and vertical lines indicate standard errors. The curve for 17 DAT is $R_c = 0.26[1 - \exp(-1.01t_{dal})]$, $r^2 = 0.95$ and for 55 DAT is $R_c = 0.22[1 - \exp(-0.28t_{dal})]$, $r^2 = 0.96$. (b) The recovery of ¹⁵N in rice plants at weekly intervals following an application of labeled (99% atom ¹⁵N excess) (■) nitrate and (○) urea at 35 DAT, at the 5-cm soil depth in the wet season of 2001. Each point is the mean of four replicates and vertical lines indicate standard errors. The curve for nitrate is $R_c = 0.23[1 - \exp(-0.27t_{dal})]$, $r^2 = 0.95$ and for urea is $R_c = 0.58[1 - \exp(-0.47t_{dal})]$, $r^2 = 0.80$.

planting was 0.03 ± 0.01 mg hill⁻¹ and this was assumed to be close enough to zero for the purposes of curve fitting. The same equation was used to describe the acquisition of N by the panicle, where a = 0.37 g hill⁻¹, b = 75.89 d, c = −13.91, and r^2 = 0.97 (Fig. 2a). At maturity, the total N content of the plants was 0.86 ± 0.06 g hill⁻¹ (215 kg N ha⁻¹). The N content of the grain at maturity was 0.33 ± 0.03 g N hill⁻¹ (82.5 kg N ha⁻¹). Using the fitted curve, the rate of total N uptake was calculated for each day from the first differential of Eq. [5] for both the whole plants and the panicle (Fig. 2b). The maximum rate of N uptake by the plants was 19.3 mg N hill⁻¹ d⁻¹ at 28 DAT and by the panicle was 17.2 mg N hill⁻¹ d⁻¹ at 75 DAT. Peng and Cassman (1998) reported absorption rates of 9.4 kg N ha⁻¹ d⁻¹ (38 mg N hill⁻¹ d⁻¹) for the 4 d following the application of 100 kg N ha⁻¹ at panicle initiation in the dry season; their reported rates slowed thereafter. This is not comparable to the results presented in our study, because the instantaneous
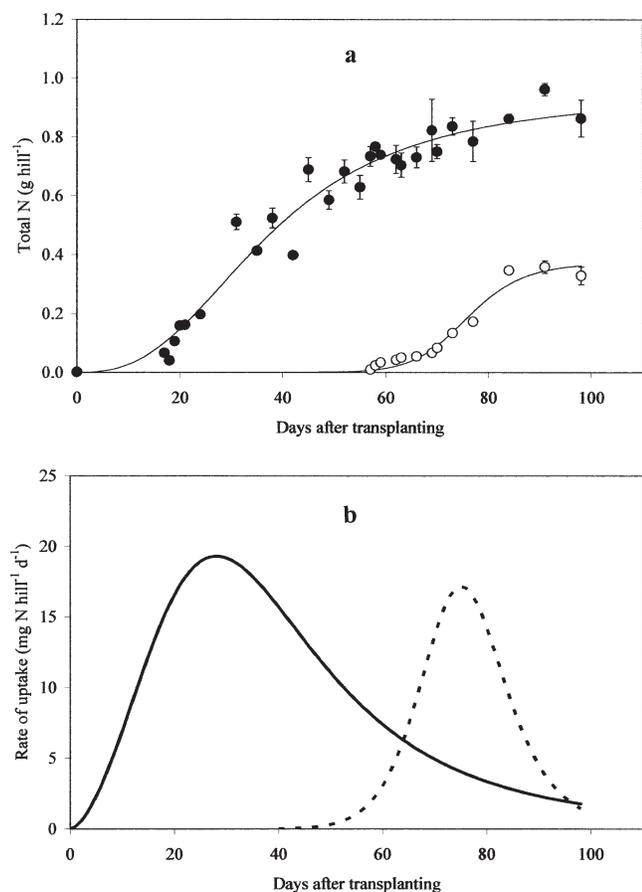
Fig. 2. (a) The accumulation of total N (g hill^{-1}) by (●) rice plants or (○) the panicles in an irrigated crop during the wet season, represented by sigmoid curves. The equations were $N_c = 0.96/[1+(t/37.83)^{-2.66}]$ for the rice plants and $N_c = 0.37/[1+(t/75.89)^{-13.91}]$ for the panicles. The points represent measurements of total net N content of the plants with standard errors ($n = 4$). (b) Rate of total N uptake by the (solid line) whole plants or the (dashed line) panicles. These curves are the first differential of Eq. [5], with parameters given in the legend to Fig. 2a.

applied 17, 31, 45, 59, and 73 DAT, are shown in Fig. 3. It can be seen that the roots and stems contribute little N to the panicle and that before 60 DAT the leaves act as an important N reservoir for the grain, contributing more than 59% of the total N in the grain (Table 1, Fig. 3). Following flowering, the fraction contributed by the leaves to the grain falls to 3% close to maturity as the fraction of absorbed N translocated directly to the panicle rises to about 50%.

The average grain yield was 5.2 ± 0.22 t ha^{-1} (20.8 ± 0.88 g plant^{-1}, 14% moisture) with a harvest index of 0.32; 77% of the grains were filled. The N content of the grain at maturity was 1.6%; the ratio of N in the grain to N in the biomass was 0.38 ± 0.01. The dry weight of the aboveground biomass was 14.02 ± 0.21 t ha^{-1} and the N content of the straw was 1.4%. From other measurements, the distribution of the total dry weight of biomass at maturity was 38% for sheath + culm, 32% for panicle, 18% for leaves, 6% for roots, and 5% for dead matter.

DISCUSSION

The major objective of this work was to describe the temporal origin of N acquisitions by the grain and the parts where it was stored before being deposited in the grain. Consequently, a point-placement technique was used to deliver a tracer or label in the form of small amounts of ^{15}N to the roots of the rice plant at different times. The total N content of the plants was measured throughout the duration of the crop. Using ^{15}N as a tracer enabled the determination of the fate of total N absorbed at any time. There was no significant variation during the growing season in the percentage recovery of ^{15}N nitrate (20.7% ± 2.4), whereas in our previous study, ^{15}N urea as a label in the dry season was recovered between 40 and 76% (Sheehy et al., 2004b).

With the point-placement technique, it took nearly 2 wk for the complete uptake of ^{15}N urea (Sheehy et al., 2004b). A minor objective of the work described in this paper was to investigate whether the complete uptake of ^{15}N nitrate, applied with the point placement technique, would be faster, in part owing to rapid loss through denitrification. However, it took 3 d to recover 95% of the label applied at 17 DAT and nearly 11 d for that at 55 DAT (Fig. 1). The reason for the difference is obscure. The slow uptake of nitrate N at 55 DAT was surprising and seems to contradict the results of kinetic studies with ^{13}N (Kronzucker et al., 2000), where it was shown that rice roots are very efficient in absorbing nitrate and that a mixed ammonium-nitrate nutrition may be desirable for rice. Issues such as what parts of the root system are exposed to what form of N addition and the efflux of absorbed N from roots may be important in explaining the observation. Also, there may have been

uptake of N is usually a function of quantity as well as rate coefficient. The daily rate of N absorption by the panicle exceeded that of the whole plant after 64 DAT (Fig. 2b); thereafter N was transferred from other plant parts to the panicle.

Because the fractional allocation to the grain (f_r) was observed to be constant (0.44 ± 0.2), the amount of N (captured on any day) that is located in the grain at maturity is given by $f_r(\Delta N/\Delta t)_{hill}$; that amount expressed as a fraction of the total N in the grain is given by $f_r(\Delta N/\Delta t)_{hill}/N_g$. That equation was used to calculate the total amount of N accumulated in the grain in different intervals (Table 1); its temporal origin. Using that approach, the amount at maturity was overestimated by 19%.

The maximum amount of label (%) in each of the plant fractions and the amount remaining at maturity, for label

Table 1. The cumulative percentage contribution of the different intervals to the total N accumulated in the grain during the wet season (2001); the figures in parentheses are scaled to 100%.

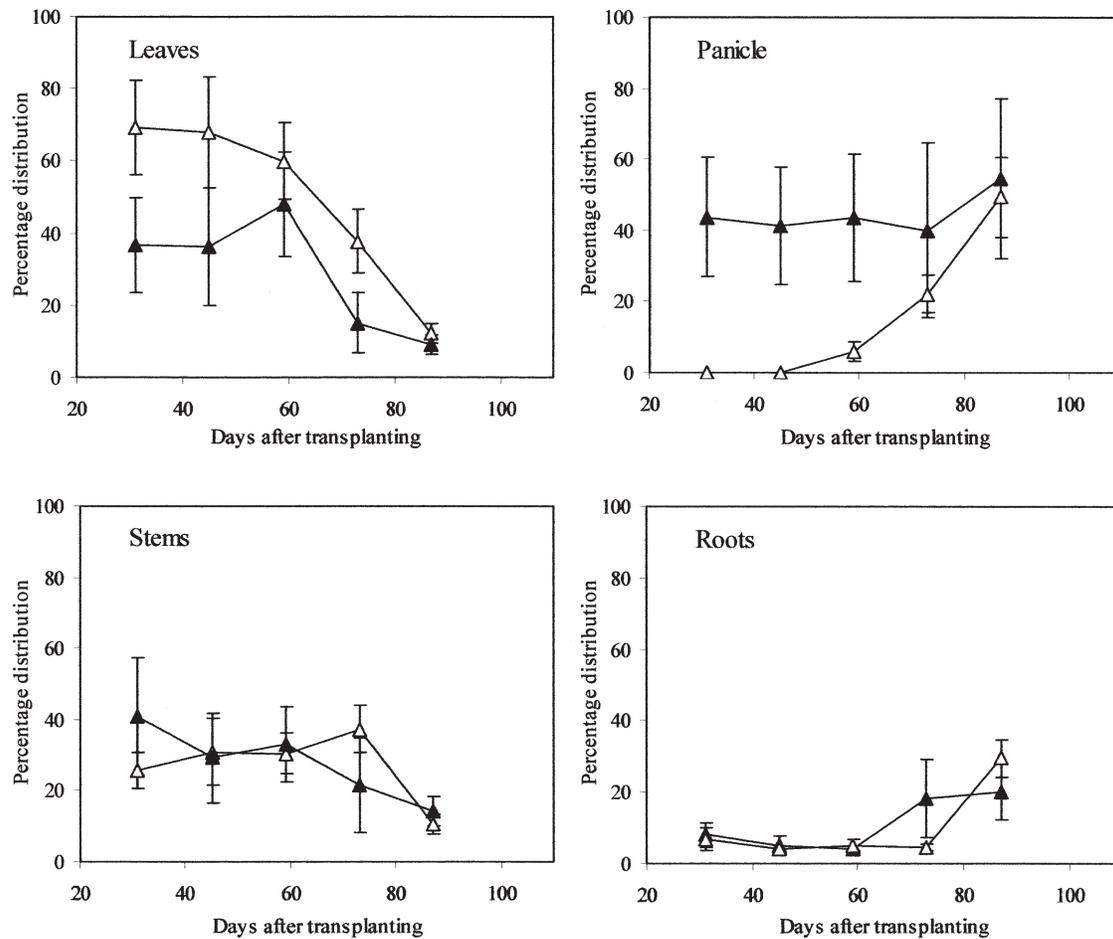| Interval (DAT)†	0–20 d	0–40 d	0–60 d	0–80 d	0–100 d
Cumulative amount of total N in grain (%)	21 (18)	70 (59)	99 (83)	117 (98)	119 (100)

† Days after transplanting.

Fig. 3. The distribution of ^{15}N (applied on 17, 31, 45, 59, and 73 DAT) to different plant parts (△) 14 d after application and the reallocation of that N (none is lost from the plant) (▲) at maturity. Standard errors are shown ($n = 8$) and each point is calculated independently of the others on any given day.

nitrate recycling around roots rather than a one-directional nitrate flow into the plant, which could have caused delays. However, such investigations were beyond the scope of these experiments. The observations made on ^{15}N urea and nitrate absorptions, when applied at 35 DAT, showed that urea uptake was two and a half times greater than nitrate. Thus, the use of nitrate does not offer an advantage over urea in point-placement labeling experiments in irrigated rice.

The detailed patterns of ^{15}N recovery by the labeled plants for applications on 17 and 55 DAT (Fig. 1) supported the underlying assumptions made in Sheehy et al. (2004a) concerning the use of first-order kinetics to describe the uptake and loss of labeled N applied using the point-placement technique. However, the use of eight replicates as described in Sheehy et al. (2004b), instead of the four replicates used in this study, would have increased the accuracy of estimation of ^{15}N distribution. Losses of labeled N acquired by the plants were not significant, suggesting that there was no large loss of N through volatilization, once the N had been incorporated to the plant biomass (Fig. 1a and 1b). The 19% error in the estimation of grain N (Table 1) was unlikely to have been the result of an error in any one part of the computation, but was probably the results of the cumu-

lative effects of small errors arising from all parts of the calculation (note the variation of data points around the fitted curves). The errors observed in Sheehy et al. (2004b) using urea were 4%, and this suggests that urea is a more suitable label than nitrate for studying the fate of N absorbed at any time during the growing season.

In the wet season, about 60% of the N in the grain was absorbed by the crop before panicle initiation (40 DAT), but at the same stage only 32% had been acquired in the dry season (Sheehy et al., 2004b). In the early stages of growth, temperatures are higher in the wet season than in the dry season. The higher temperatures probably result in the higher early rates of N uptake observed in the wet season. Another difference between the dry and wet seasons was that N acquired early in the dry season was lost through the death and detachment of old leaves and this did not occur in the wet season to any considerable extent. However, a more precise explanation of such differences would require further investigation. For high rice yields in the tropics, Sheehy et al. (1998) showed that half of the total N at maturity had to be acquired by the time crops attained 25% of their final biomass, by about 35 d after transplanting. Similarly, the results in this paper suggest that at least half of the N in the grain is acquired by the plants before

40 DAT (Fig. 2 and Table 1); in the wet season, early acquisition of N appears to be important for the grain (Wilson et al., 1989). These experiments were conducted with N applications calculated to enable the achievement of yields close to yield potential and the results may differ from those obtained at rates currently used by poor farmers. Linear upward trends in crop yields have been characteristic across decades, countries, and crops (Sheehy, 2001). The factors influencing the rate of increase (genetic 29%, N fertilizer 48%, and others 24%) have been described by Bell et al. (1995). It is clear that poor farmers increase their inputs of fertilizer as they take advantage of new genotypes and the research in this paper is aimed at understanding the future requirements of those poor farmers. Before panicle initiation, the leaves are the most important location for N; thereafter, the panicle becomes increasingly dominant (Fig. 3). Close to maturity, an increasing fraction of the ^{15}N was allocated to the roots (29%, Fig. 3). The reasons for this are not clear, but in the dry season the sheath and culm fraction increased, probably to support new tiller growth (Sheehy et al., 2004b). Rice is a weak perennial and does produce ratoon tillers toward the end of grain filling.

In contrast to results obtained in the dry season experiments (Sheehy et al., 2004b), the results in this paper suggest that there was no significant variation over the growing season in the fractional amount of ^{15}N captured or transferred to the grain. Consequently, the distribution of total N in the grain followed the absolute rate of total N uptake during the growing season. The N content of the grain was 16 g kg^{-1} and the N content of the residual biomass at maturity was about 14 g kg^{-1}, and this gave rise to the low N harvest index (0.38). The limit to the amount of N extracted from vegetative tissues comes when the concentration in the vegetative portions falls to the concentrations probably associated with the structural tissues, which is approximately 0.8% in rice (Makarim et al., 1994). The results in this paper suggest that N did not limit yield. For example, at a harvest index of 0.5 and a radiation use efficiency of 2.2 g DW MJ^{-1} the yield would have been about 6.8 t ha^{-1} at 14% moisture content (Mitchell et al., 1998). The reason for the low harvest index in this study (0.32) is unlikely to have been weather related (Horie et al., 1997) and may have been the result of a small amount of sheath blight that was observed but not quantified, apart from the low percentage of grain filling (77%).

CONCLUSIONS

The results in this paper showed that the daily demand for N by the panicle exceeded the supply after early flowering and demonstrated the importance of the leaves as a reservoir of N (Simpson et al., 1983; Jamieson and Semenov, 2000). Furthermore, the results showed that the pattern of N absorption by the grain depended on the early acquisition of N by the leaves. The processes described here were similar to those reported for the dry season (Sheehy et al., 2004b), increasing our confidence that these results are typical of rice. The development

of the panicle triggers a demand for energy to be used for synthetic and maintenance purposes of that rapidly developing organ. Consequently, it is likely that roots are deprived of energy during this period and this leads to a decline in daily N uptake by the roots and withdrawal of N from the leaves. For the various reasons outlined in this paper, urea rather than nitrate should be used in point-placement labeling experiments in irrigated rice. Finally, the results from this experiment and that of Sheehy et al. (2004b) will be used in the construction of a model to investigate different strategies of fertilizer use in rice.

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