



## Nitrogen and carbon uptake by some Rice cultivars from $^{15}\text{NH}_4\text{Cl}$ and $^{13}\text{C}$ -U-glucose labeling fertilizer

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### Abstract

This experiment was conducted to determine the effects of the application of  $^{15}\text{NH}_4\text{Cl}$  (1.0 atom %) and  $^{13}\text{C}$ -U-glucose (99.8 atom %) on the nitrogen and carbon uptake by 12 different rice cultivars. Chemical nitrogen fertilizer and glucose was applied at a rate of 100 kg N ha<sup>-1</sup> and 40 mg kg<sup>-1</sup> soil, respectively. The ratio of  $^{13}\text{C}$  and  $^{15}\text{N}$  to the total C and N content of samples was calculated. The results showed that, N uptake derived by chemical fertilizer application to the rice cultivars exhibited significant differences. The rice cultivars recovered about 48.1-66.2% of applied chemical fertilizer and the amount of  $^{15}\text{N}$  taken up by rice grain was higher than that of straw and roots. A high recovery percentage of glucose-carbon was observed with cultivars Jucar, Sakha-104, and Koshihikari rice cultivars. The application of  $^{13}\text{C}$ -U-glucose permitted a rough estimation of the minimal contribution of intact glucose molecules to glucose derived  $^{13}\text{C}$  acquisition by rice cultivars. The results showed that in all cultivars, the  $^{13}\text{C}$  uptake from the glucose by the root accounted for not more than 2.06% of total C uptake by the plants, however, the contribution of the rice roots to C uptake was significantly different ( $P > 0.05$ ) among the 12 studied rice cultivars.

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## Introduction

During root exudation, a large amount of photosynthetically fixed carbon compounds released from intact live roots into the soil either as CO<sub>2</sub> in root respiration or as soluble and insoluble C compounds (Nguyen 2003). Glucose chosen as considered the standard source of exogenous organic C (Lu *et al.* 2000). The main products of anaerobic degradation of glucose were Volatile Fatty Acids (VFA's), especially acetate, propionate and butyrate Lovley and Klug (1982) and Kotsyurbenko *et al.*, (1996). These VFA's are the most common intermediate products from anaerobic degradation of organic carbon material in many aquatic ecosystems. In rice field soil, acetate is well known as the most dominant fatty acid, and it has frequently been observed to accumulate within 2 weeks after soil flooding Sugimoto and Wada (1993); Watanabe (1984). Anaerobic degradation of glucose released acetate and CO<sub>2</sub> as the main products, with acetate being release faster than CO<sub>2</sub>. In addition, Bergman *et al.*, (1999) reported that the anaerobic production of intermediate organic compounds and CO<sub>2</sub> was tightly coupled to the degradation of <sup>13</sup>C-U-glucose, but the rate of CH<sub>4</sub> production was not directly related to glucose degradation. The rate of glucose mineralization usually significantly greater in the rhizosphere soil in comparison to unplanted soil may reflect an increased microbial activity in the rhizosphere soil (Kuzyakov 2002). Consequently, (Kuzyakov and Jones 2006; Jones *et al.*, (2004a, b) reported that large amounts of C can pass directly into the soil microbial community or plant roots can recapture low molecular weight C from the rhizosphere, though intense competition from soil microorganisms may reduce the efficiency of the process. Some of previous studies have been assumed that plant roots absorb the inorganic nitrogen theory, mainly from mineralized nitrogen such as ammonium and nitrate. However, a numerous of studies have been shown that a variety of pants have a capacity to uptake organic component in the form of low molecular weight substances through plant roots (Näshlom *et al.*, 1998; Matsumoto *et al.* 2000; Persson *et al.*, 2003; Xu *et al.* 2004). Other studies

indicated that the organic component uptake by plant physiologists studying biochemical path-ways and membrane transport mechanisms in excised root systems, or uptake of pesticides in field experiments (Xia and Saglio 1988). Moreover, Ebid *et al.*, (2007); Ebid *et al.*, (2008) reported that some rice cultivars and vegetables species recovered significant amounts of C through roots using <sup>13</sup>C and <sup>15</sup>N dual labeled maize residues compost.

Thereafter, the aims of this study were: (1) to assess the N and C uptake among rice cultivars, (2) to quantify the N and C available from <sup>15</sup>NH<sub>4</sub>Cl and <sup>13</sup>C-U-glucose labeling fertilizer and (3) to elucidate the N and C distribution between soil and rice cultivars.

## Materials and methods

### *Soil samples and analysis*

Five represented soil samples were collected from rice research and training center experimental farm, Sakha, Kafr El-Sheikh, Egypt. Soil samples were air-dried at room temperature for two weeks and then sieved by 2-mm stainless steel sieve. The pH and EC of samples were measured (using 1:5 ratio with distilled water) by pH-meter and the electrical conductivity meter, respectively. Complex metric EDTA titration was employed for determining Ca<sup>++</sup> and Mg<sup>++</sup> simultaneously and individually Sparks, (1996). Sodium and potassium was determined using flame photometer. Carbonate and bicarbonate were determined by titration with H<sub>2</sub>SO<sub>4</sub> while silver nitrate was used to determine chloride (Sparks 1996). Sulphate was determined by turbidity method as described by Tabatabai (1996). Particle size distribution was analyzed according to Gee and Bauder, (1996). Some selected soil properties are shown in Table (1). About 2.50 kg soils (dry weight basis) was mixed well and transferred into Wagner pots (0.02 m<sup>2</sup>).

### *Experimental setup*

Seedlings of three weeks- rice cultivars were transplanted into each pot. Each of the 12 cultivars of rice (*Oryza sativa* L. cv.) was used with five replicates. Some selected properties of the cultivars

are presented in Table 2. After two weeks after transplanting,  $^{13}\text{C}$ -U-glucose and  $^{15}\text{NH}_4\text{Cl}$  labeling fertilizer are used.  $^{13}\text{C}$ -U-glucose (99.3 atom %) applied at rate of 100 mg pot<sup>-1</sup> (or 40 mg kg<sup>-1</sup> soil), which equivalent to 38.2, mg C pot<sup>-1</sup>. While  $^{15}\text{N}$ -labelled supplied as  $^{15}\text{NH}_4\text{Cl}$  (1.0 atom %) at rate of 100 kg N ha<sup>-1</sup>. The N fertilizer was applied in three equal splits during the cultivation period. Phosphorus as P<sub>2</sub>O<sub>5</sub> and K as KCl were applied as a basal dose to all pots at the rate of 8 g m<sup>-2</sup> in one dose just before transplanting.

### Harvesting

Rice plants were harvested from pots at maturity by cutting above the soil surface. Roots were washed over a sieve, first under running tap water and finally with distilled water to remove soil particles and plant debris. This was followed by thorough drying at 70°C for 72 hours, weighed and finally, ground into a fine powder with an electric mill for chemical analysis.

### Determination of isotopic enrichments of nitrogen and carbon

Plant samples used for N and C isotope analysis was oven-dried at 70 °C for at least 48 hours and finely ground in a ball mill to a powder. The stable isotopic composition was then determined with an automated Nitrogen and Carbon Analyzer-Mass Spectrometer (ANCA-SL, Europa Scientific Co. Ltd.). Standard samples were measured after every 10 analytical samples, for the analysis of nitrogen and carbon isotopic composition. The ratio of  $^{13}\text{C}$  and  $^{15}\text{N}$  to the total C and N content of samples was estimated.

**Table 1.** Some selected soil characterization used in the study.

p H	Ec (dS m <sup>-1</sup> )	Cations (meq L <sup>-1</sup> )					Anions (meq L <sup>-1</sup> )			Particles size %			Texture Class
		Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>	Cl <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	CO <sub>3</sub> <sup>2-</sup>	SO <sub>4</sub> <sup>2-</sup>	Sand	Silt	Clay	
7.60	1.60	2.62	1.63	3.76	2.64	3.70	2.17	3.60	0.52	12.9	33.0	54.1	Clay

The N distribution to plant roots, straw, grain is shown in Table 4. The total N uptake in rice grain was two times greater than in the straw and four times greater than roots. There was a significant difference between rice cultivars. The fast N releasing characteristics of chemical N fertilizer appeared to

### Calculations

1. The atom% of  $^{15}\text{N}$  or  $^{13}\text{C}$  was recalculated by subtracting non-labeled from those of labeled fertilizer.

$$2. \text{Derived N or C (mg)} = \frac{^{15}\text{N atom\% excess of plant N}}{^{15}\text{N atom \% excess of N applied} \times \text{Total plant N or C}} \times 100$$

$$3. \text{Recovery \% of } ^{15}\text{N or } ^{13}\text{C} = \frac{^{15}\text{N or } ^{13}\text{C plant (kg /ha)}}{\text{N or C applied (kg /ha)}} \times 100$$

4. N or C uptake from soil = Total N or C uptake by plant – N or C uptake from fertilizer.

### Statistical analysis

The collected data were statistically analyzed using ANOVA at first, then the homogeneity of variances was tested using multiple comparison tests with Tukey-Kramer method at ( $p < 0.05$ ) using KyPlot software packages (Kyenslab Inc., Tokyo, Japan).

### Results and discussion

#### Dry matter production and N uptake

Table 3 shows the dry weight production of the rice cultivars. There was significant difference observed among rice cultivars in the dry weight. The rice cultivars IR-8, Keicho-2, and Sakha 104 showed the highest dry weight compared to other cultivars. Straw and grain dry weight were significantly different among rice cultivars and the highest straw dry weight was recorded by Keicho2, Takanai, Mitsuyo-23, and Keicho-2 had the highest grain dry matter production.

increase the N uptake of rice cultivars Ebid *et al.*, (2007) and Ebid *et al.*, (2008).

*N derived from fertilizer (N<sub>df</sub>), soil (N<sub>djs</sub>) and  $^{15}\text{N}$  recovery*

Table 5 shows the amount of N uptake derived from chemical fertilizer. N uptake preliminary derived from chemical fertilizer was detected in all of the rice

cultivars. N uptake derived from chemical fertilizers accumulated in the rice grain was significantly higher than that in the straw and root. No significant difference was observed between N uptakes derived from chemical fertilizer on the rice straw among rice cultivars. N derived from the soil was significantly different between the rice cultivars (Table 6). The amount of N derived from the soil treated with

chemical fertilizer was higher in grain followed by straw and root. In general cultivars IR-8 and Kieicho-2 showed the highest amount of N derived from the soil. In all studied rice cultivars, took up more N from the soil than from the chemical fertilizer. Eagle *et al.*, (2001), reported that about 50-80% of N applied used by rice plant was derived from the soil.

**Table 2.** Some rice cultivars characteristics used in the experiment.

Cultivar Name	Characteristics		
	Origin	Yield (kg m <sup>-2</sup> )	Note
Giza 178	Egypt	1.14	High yield
Sakha 101	Egypt	1.20	High yield
Sakha 104	Egypt	1.17	High yield
Koshihikari	Japan	0.63	High yield
Matsuyamamii	Japan	0.67	High yield
Takanari	Japan	0.71	Old variety
Mitsuyo-23	Korea	0.80	High yield
IR-8	Philippine	0.56	High yield
Habataki	Japan	0.73	Old variety
Keicho-2	China	0.71	High yield
Shinsei-2	China	0.58	Old variety
Jucar	Spain	0.60	High yield

**Table 3.** Dry matter yield of rice cultivars grown in soil amended with <sup>13</sup>C-U-glucose and <sup>15</sup>NH<sub>4</sub>Cl.

Cultivar	Dry Weight (g pot <sup>-1</sup> )			
	Root	Straw	Grain	Total
Giza 178	5.3cd	14.5bc	22.1b	41.9b
Sakha 101	4.2cd	15.4bc	16.3cd	35.9b
Sakha 104	7.9bc	16.9bc	17.7cd	42.5b
Koshihikari	4.8cd	15.9bc	15.6cd	36.3b
Matsuyamamii	3.2d	17.3bc	15.4cd	35.9b
Takanari	4.9cd	19.0ab	25.6ab	49.5b
Mitsuyo-23	5.8cd	19.1ab	26.7ab	51.6ab
IR-8	11.4a	14.5bc	24.1b	50.0ab
Habataki	5.4cd	12.4c	19.5c	36.4b
Keicho-2	9.2ab	25.2a	31.1a	65.5a
Shinsei-2	2.9d	11.5c	15.4cd	29.8b
Jucar	3.2d	16.2bc	13.3d	32.7b

The mean values within a column followed by the same letter (S) are not significantly different (Tukey-Kramer test;  $P < 0.05$ ),  $n = 5$ .

**Table 4.** Total N uptake by rice cultivars grown in soil amended with <sup>13</sup>C-U-glucose and <sup>15</sup>NH<sub>4</sub>Cl.

Cultivar	Total N uptake (mg pot <sup>-1</sup> )			
	Root	Straw	Grain	Total
Giza 178	32.5cd	127.7a	267.1b	427.3bc
Sakha 101	38.5b	126.7a	164.1c	329.3d
Sakha 104	64.9ab	124.3a	211.1c	400.3c
Koshihikari	35.6bc	128.5a	209.9c	374.0cd
Matsuyamamii	18.4d	109.9ab	183.2c	311.5d
Takanari	24.6d	117.9ab	327.3ab	469.8ab
Mitsuyo-23	30.2cd	134.3a	336.7a	501.2a
IR-8	56.8a	103.8ab	340.2a	500.8a
Habataki	31.4c	86.3b	248.5bc	366.2c
Keicho-2	74.9a	156.5a	287.3b	518.7a
Shinsei-2	30.6cd	69.1b	196.3c	296.5d
Jucar	42.7b	97.5b	173.3c	313.5d

The mean values within a column followed by the same letter (S) are not significantly different (Tukey-Kramer test;  $P < 0.05$ ),  $n = 5$ .

The percentage of N recovery by rice cultivars is shown in Table 7. The results indicated that, the N recovery ranged from 48.1 % to 64.0 of applied chemical N fertilizer with a higher amount of plant available N quickly stimulated rice growth in

agreement with Ghoneim *et al.*, (2008); Ghoneim *et al.*, (2012). The obtained higher apparent recovery of applied chemical fertilizer in this study could be attributed due to the higher initial soil N level.

**Table 5.** Nitrogen uptake derived from  $^{15}\text{NH}_4\text{Cl}$  ( $\text{N}_{\text{dff}}$ ).

Cultivar	N derived from fertilizer ( $\text{N}_{\text{dff}}$ mg pot <sup>-1</sup> )			
	Root	Straw	Grain	Total
Giza 178	6.4b	25.3a	91.2a	122.9a
Sakha 101	8.1b	32.8a	61.4b	102.3b
Sakha 104	14.4a	31.6a	73.6b	119.6a
Koshihikari	8.8b	30.5a	64.1b	103.4b
Matsuyamamii	5.6b	31.4a	59.7b	96.7b
Takanari	6.2b	24.2a	97.5a	127.9a
Mitsuyo-23	6.8b	25.5a	81.8ab	114.1ab
IR-8	15.3a	24.0a	93.1a	132.4a
Habataki	7.7b	16.6a	85.5a	109.8b
Keicho-2	7.8b	26.0a	72.0b	105.8b
Shinsei-2	4.3b	19.2a	78.6b	102.1b
Jucar	6.2b	30.1a	65.6b	101.9b

The mean values within a column followed by the same letter (S) are not significantly different (Tukey-Kramer test;  $P < 0.05$ ),  $n = 5$ .

**Table 6.** Nitrogen uptake derived from the soil ( $\text{N}_{\text{dfs}}$ ).

Cultivar	N derived from soil ( $\text{N}_{\text{dfs}}$ mg pot <sup>-1</sup> )			
	Root	Straw	Grain	Total
Giza 178	26.2b	99.4b	175.9b	301.5b
Sakha 101	30.4b	93.9b	102.7c	227.0c
Sakha 104	50.6a	92.7b	137.5b	280.8b
Koshihikari	26.8b	98.0b	145.9b	270.7b
Matsuyamamii	13.4c	78.4bc	123.5bc	215.3c
Takanari	18.4c	93.7b	229.8a	341.9ab
Mitsuyo-23	23.4bc	108.8b	254.9a	387.1a
IR-8	41.5b	79.8bc	247.1a	368.4a
Habataki	23.7b	69.7c	163.0b	256.4bc
Keicho-2	67.2a	130.5a	215.3ab	413.0a
Shinsei-2	26.4b	49.9c	117.7c	193.0c
Jucar	36.5b	67.4c	107.7c	211.6c

The mean values within a column followed by the same letter (S) are not significantly different (Tukey-Kramer test;  $P < 0.05$ ),  $n = 5$ .

**Table 7.** Nitrogen recovered (% NRc) by rice cultivars.

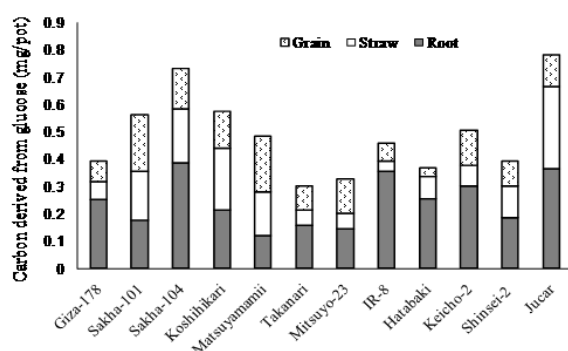
Cultivar	N recovery (%NRc)			
	Root	Straw	Grain	Total
Giza 178	3.2b	12.7a	45.6a	61.5a
Sakha 101	4.0b	16.4a	30.7b	51.1b
Sakha 104	7.2a	15.8a	36.8b	59.8a
Koshihikari	4.4b	15.3a	32.0b	51.7b
Matsuyamamii	2.5b	15.7a	29.9b	48.1b
Takanari	3.1b	12.1a	48.7a	64.0a
Mitsuyo-23	3.4b	12.8a	40.9ab	57.1ab
IR-8	7.7a	12.0a	46.5a	66.2a
Habataki	3.9b	8.3a	42.7a	54.9b
Keicho-2	3.9b	13.0a	34.0b	50.9b
Shinsei-2	2.1b	9.6a	39.3b	51.0b
Jucar	3.1b	15.0a	32.8bc	51.9b

The mean values within a column followed by the same letter (S) are not significantly different (Tukey-Kramer test;  $P < 0.05$ ),  $n = 5$ .

**Table 8.**  $^{13}\text{C}$  recovered in rice parts expressed as a % of initial carbon content of  $^{13}\text{C}$ -U-glucose.

Cultivar	$^{13}\text{C}$ recovery (%)			
	Root	Straw	Grain	Total
Giza 178	0.66b	0.18b	0.19a	1.03b
Sakha 101	0.46bc	0.47ab	0.55a	1.48ab
Sakha 104	1.01a	0.52a	0.38a	1.91a
Koshihikari	0.56bc	0.59a	0.35a	1.50ab
Matsuyamamii	0.31c	0.42a	0.54a	1.27ab
Takanari	0.42b	0.14b	0.23a	0.79b
Mitsuyo-23	0.38c	0.15b	0.33a	0.86b
IR-8	0.93ab	0.10b	0.17a	1.20ab
Habataki	0.67b	0.21b	0.09a	0.97ab
Keicho-2	0.79b	0.20b	0.34a	1.33ab
Shinsei-2	0.49bc	0.31b	0.24a	1.04b
Jucar	0.96ab	0.79a	0.31a	2.06a

The mean values within a column followed by the same letter (S) are not significantly different (Tukey-Kramer test;  $P < 0.05$ ),  $n = 5$ .



**Fig. 1.** Distribution of carbon uptake by rice cultivars derived from  $^{13}\text{C}$ -U-glucose. Values are the means of five replicates.

#### Glucose-carbon uptake and distribution

Carbon uptake derived from glucose was detected in all the rice cultivars (Figure 1). The glucose-carbon uptake rate varied among the rice cultivars and plant parts. The amount of  $^{13}\text{C}$  taken up by rice roots derived from the glucose was significantly higher than that taken up by rice straw or grain. The highest amount of  $^{13}\text{C}$  was found in Jucar followed by Sakha 104 rice varieties. Among rice cultivars,  $^{13}\text{C}$  allocation in the root was significantly higher than the amount of  $^{13}\text{C}$  originated from the glucose in straw and grain. Kuzyakov and Jones (2006) reported that the amounts of  $^{13}\text{C}$  recovered in the maize shoot were about 3-9 times less than total amount of  $^{14}\text{C}$  recovered in the root suggested that the decreases

associated with two processes: respiration of the glucose or its labeled transformation products by shoots, or its re-translocation back to the roots with the main photosynthetic stream. Table 8 shows the final recovery rate of  $^{13}\text{C}$  uptake derived from glucose by rice cultivars. The final recovery rate of  $^{13}\text{C}$  by rice plants tended to be higher in the rice roots compared with rice straw or grain. The total recovery of  $^{13}\text{C}$  recovery ranged from 0.79 to 2.06% in the whole plant. In another study, with  $^{13}\text{C}$  and  $^{15}\text{N}$ -labeled maize residue compost we reported that about 3.70% of carbon was recovered by rice plants (Ebid *et al.*, 2007). Similarly with  $^{13}\text{C}$  labeled rice straw. Jones (1999); Jones and Kielland (2002) reported that 1.0 % of the C applied was recovered by rice plants. The high yield varieties such as Shakha 101, Shakha 104, Jucar, Koshihikari, Matsuyamami, and Keicho took up high amount of  $^{13}\text{C}$  compared to old varieties. Rice cultivars roots have the capacity to recapture low molecular weight C from soil although this is in direct competition with soil microorganisms (Kuzyakov and Jones (2006); Jones *et al.*, (2004a, b). A high recovery percentage of glucose-carbon was recorded by rice cultivars Jucar, Sakha-104, and Koshihikari. Yamamuro *et al.*, (2002) reported that  $^{13}\text{C}$  concentration was much higher in the roots than in the plant top, suggesting that the organic C taken up preferentially accumulated in the roots. Values for C

uptake by root reported in this study probably represent maximum estimate since the interstitial dissolved inorganic carbon contribution to which the plants were submitted were two to four times higher than in the root. Since, plants discriminate against  $^{13}\text{C}$ , causing the carbon isotopic composition to become more negative (lower % of  $^{13}\text{C}$  than the standard Pee Dee Belemnite [PDB]) in the plant tissues than in the atmosphere.

### Conclusion

The results of the present study revealed that  $^{13}\text{C}$  uptake by rice cultivars from applied glucose was slightly low compared to inorganic C (such as  $\text{HCO}_3^-$ ) form probably due to the fraction of  $^{13}\text{C}$  isotopes taken up by rice roots that is subsequently lost via plant respiration and the fast turnover and breakdown of glucose in soils. However, the C uptake by roots varied among rice cultivars species. Further experiments are necessary to testing and clarify the direct C uptake through rice roots.

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