



BCSIR

Available online at www.banglajol.info
Bangladesh J. Sci. Ind. Res. 49(2), 69-78, 2014

**BANGLADESH JOURNAL
OF SCIENTIFIC AND
INDUSTRIAL RESEARCH**

E-mail: bjisir07@gmail.com

Use of ^{15}N labelled urea to quantify nitrogen recovery studies and effect of salinity on rice

M. N. Mondol^{*1}, A. S. Chamon¹, R. C. Hood² and W. E. H. Blum³

¹Department of Soil, Water and Environment, University of Dhaka, Dhaka-1000, Bangladesh,

²Soil Science Unit, IAEA, Seibersdorf, Austria, and

³Dept. of forest and Soil Sciences, University of Natural Resources and Applied Life Sciences (BOKU), Vienna, Austria.

Abstract

An experiment was conducted to study the effects of salinity, and fertilisation on wetland rice production. It was a factorial design with four levels of salinity and three levels of fertilisation. Rice plants were grown in wetland system under greenhouse conditions. Fertiliser treatments were imposed at planting. Following germination salinity treatments were imposed. Plants and soils were sampled 10 weeks after planting. Plant dry weight increased with increasing inorganic fertiliser application and decreased with increasing salinity. Total shoot N increased with increasing inorganic fertiliser application but was not affected by salinity, however total root N increased with increasing inorganic fertiliser addition and decreased with increasing level of salinity (0, 50 and 100 ppm). Plant dry weight decreased with increasing salinity. Plant dry weight and N contents in shoot and root were significantly higher in all inorganic fertiliser treatments compared to the control treatments. % Ndff was in the range of 30-50 % and increased with increasing fertiliser application and salinity. Fertiliser use efficiency was in the range of 67.87-72.8 % in the inorganic fertiliser treatment.

Keywords: ^{15}N Labelling; Nitrogen recovery; Salinity; Rice

Introduction

In an agricultural soil total organic N can range between 0.5-12.5 t N ha⁻¹ of which only 20-200 kgN ha⁻¹ may breakdown/mineralize to an available form over the growing season (Cambell, 1978). After application of N as fertiliser, to determine the amount of N what comes from the mineralization and what comes from the fertiliser, isotopically labelled materials are used as tracers (e.g. nitrogen fertilisers artificially enriched or depleted in ^{15}N), however, absolute values are determined (Mondol *et al.*, 2004).

Two stable isotopes of N occur naturally in the atmospheric N₂, ^{14}N is present at an abundance of 99.6337 atom % and ^{15}N at an abundance of 0.3663 atom % ^{15}N . The advantage of using stable isotopes is that they are almost identical to the more abundant isotope in chemical and behavioral characteristics. Stable isotopes are consequently a powerful tool in elucidating nitrogen cycle in agriculture.

Nitrogen is the most limiting in rice production worldwide (Buresh and De Datta, 1991). More than 95% of the world's fertiliser-N production is based on the synthetic fixation of atmospheric N in the form of NH₄⁺; this requires a substantial amount of energy and results in higher fertiliser costs. Many developing countries have neither the energy reserves nor the means with which to purchase energy sources and, as a result, the use of N fertiliser is limited in rice developing countries, especially in under-developed and developing ones. However, there is also increasing concern over the environmental consequences of fertiliser use, including water pollution by NO₃⁻ and possible long term adverse effects on soil structure and crop productivity.

Nitrogen applied to soil in the form of inorganic fertilisers may be taken up by plants, lost from the soil plant system or immobilized by soil micro-organisms and gradually transformed into stable forms. The magnitudes of these

*Corresponding author: E-mail: mondol-bangladesh@hotmail.com

transformations vary widely according to the experimental conditions. In general, 50% of the applied fertiliser is taken up by the plants, 25% is lost by different mechanisms and 25% remains in soil in more or less stable forms (Legg and Meisinger, 1982). According to Azam *et al.*, (1985), plants (maize) derived almost equal amounts of N from different sources i.e. soil, residues (*Sesbania aculeata*) and inorganic fertiliser (ammonium sulphate). Fertiliser use efficiency is a quantitative measure of the actual uptake of fertiliser nutrient by the plant in relation to the amount of nutrient added to the soil as fertiliser. A common form of expression of fertiliser use efficiency is plant recovery or 'coefficient of utilization' of the added fertiliser. This is shown in following equation:

$$\left[\frac{\% \text{ Utilization of added fertiliser}}{\% \text{ Utilization of added fertiliser}} \right] = \frac{\text{Amount of nutrient in the plant derived from the fertiliser}}{\text{Amount of nutrient applied as fertiliser}} \times 100$$

In isotopic-aided fertiliser experiments, a labelled fertiliser is added to the soil and the amount of fertiliser nutrient that a plant has taken up is determined. In this way different fertiliser practices (placement, timing, sources, etc.) can be studied.

The first parameter to be determined when studying the fertiliser uptake by a crop by means of the isotope techniques is the fraction of the nutrient in the plant derived from the (labelled) fertiliser, i.e.: Ndff. The quantity of N derived from the fertiliser (Ndff) added was calculated according to the following equation:

$$\text{Ndff (mg)} = \frac{\% \text{ Ndff}}{100} \times \text{Total N in rice (mg)}$$

(Hood *et al.*, 1999, 2000; Mondol *et al.*, 2004).

Availability of N to plants from applied fertilisers using ^{15}N isotope has been investigated by many workers (Hood *et al.*, 1999, 2000; Azam *et al.*, 1985; Mondol *et al.*, 2004). Turnover and plant availability of N applied as inorganic fertiliser (ammonium sulphate) has also been reported (Hood *et al.*, 1999, 2000; Azam *et al.*, 1985).

Rice (*Oryza sativa* L.) is considered moderately sensitive to salinity (Ullah *et al.*, 1993). However, cultivars respond differently when subjected to salinity (De Datta, 1972). The present study deals with the transformation and plant availability of inorganic ^{15}N applied and the effect of salinity on rice.

Most plants suffer from salt injury at ECe values exceeding 4 ds m^{-1} (Karim *et al.*, 1990). The relative tolerance of 4 grain and two grass crops to increasing level of exchangeable Na^+ was studied by Pearson and Bernstein (1959), who stated the following order of tolerance,

Tall wheat grass > barley > wheat > rice > tall fescue > oat.

All rice is glycophytes lacking salt glands or other excretory structures. *Portesia coaretata* (Roxb) Tateoka, formally classified as *Oryza coaretata*, excretes excess salt by unicellular salt hairs (Bal and Dutt, 1986).

Aim

- Measurement of the effect of salinity and fertiliser source on rice yield.
- Quantification of the amount of nitrogen derived from fertiliser (Ndff) in rice.
- Quantification of the amount of nitrogen derived from soil (Ndffs) in rice.
- Quantification of the amount of residual N in soil.

Material and methods

The experiment was conducted at the FAO/IAEA Agriculture and Biotechnology Laboratories, Seibersdorf, Austria. This experiment was conducted to study the effects of salinity and fertiliser on wetland rice production. The experiment was a factorial design with four levels of salinity (0, 4, 8, 12 ds m^{-1}), one labeled fertiliser source, i.e. inorganic fertiliser (5 atom% ^{15}N exc.-labelled urea) and three levels of fertilisation (0, 50, 100ppm N). Two rice plants (variety- Pokkali) per pot were established in 2.5kg of Krumbach soil (a sandy loam soil collected from a pasture system in lower Austria; Table I) and which was supplemented with 50ppm K (applied as KCl) and 20ppm P (applied as KH_2PO_4) with three replications as a basal dose. Fertiliser treatments were done at planting. The fertiliser was mixed with the initial soils. Following germination, one week after planting, salinity treatments were imposed; this was done by watering the plants with saline solutions. Pots were harvested for above and recoverable below-ground material 10 weeks after planting and soil and plant samples were analyzed for %N and % ^{15}N atom excess.

Table I. The characteristics of Krumbach soil (Hood *et al.* 2000)

Type of analysis Granulometry	Soil Type Krumbach	Type of analysis Granulometry	Soil Type Krumbach
(Soil Particle analysis)		Total P (ppm)	285
Clay(%)	9.5	Ion exchange (Cobalt hexamine method)	
Silt (%)	12.1	exchangeable Ca (Meq/100)	7.4
Coarse sily (%)	9.7	exchangeable Mg (Meq/100)	0.95
Fine sand (%)	39.5	exchangeable K (M eq/100)	0.12
Coarse sand (%)	29.1	exchangeable Na (Meq/100)	0.09
Organic matter		exchangeable Mn (Meq/100)	0.01
Organic matter (%)	1.7	exchangeable Al (Meq/100)	0
Organic carbon (%)	0.99	exchangeable H (Meq/100)	0
total N (%)	1.11	pH cobalt	7.54
C/N-ratio	8.93	(Ca, Mg, Na, K) (Meq/100)	8.56
pH		CEC (Meq/100)	7.53
pH (H ₂ O)	7.6		
pH (KCl)	6.8		

Calculation

The percentage of nitrogen in a crop derived from N-fertiliser (%Ndff) was calculated by the following equation:

$$\%Ndff = \frac{\text{Atom}\% \text{ } ^{15}\text{N excess in the crop}}{\text{Atom}\% \text{ } ^{15}\text{N excess in the fertiliser added}} \times 100$$

The quantity of N derived from the fertiliser (Ndff) added was calculated according to the following equation:

$$Ndff \text{ (mg)} = \frac{\% Ndff}{100} \times \text{Total N in rice (mg)}$$

The amount of nitrogen in the crop as a percentage of applied N-fertiliser was calculated by the equation below (McNeill *et al.*, 1994):

$$\%N \text{ recovery} = \frac{Ndff}{\text{Amount of N applied as fertiliser}} \times 100$$

Saline water preparation

Saline water was simulated with a mixture of Na₂SO₄, CaCl₂, NaCl and MgCl₂ (10:5:4:1 on a molar basis).The

saline solutions having salt concentrations of 0 (control), 4, 8, 12 dsm⁻¹ were prepared in the laboratory by dissolving Na₂SO₄, CaCl₂, NaCl and MgCl₂ (on a molar basis). Soil water was sampled using a micro porous suction cup (Rhizon SMS moisture sampler Ejkelkamp Agriresearch Equipment NL). The salinity of the soil solution was measured in dsm⁻¹ using a conductivity meter (Accumet Portable AP50, Denver Instruments, Colorado USA).

Weeding

Weeds were controlled by uprooting. The roots of the uprooted weeds were washed with small amount of water into the respective pot, so that no nutrient loss could occur with the roots.

Control of pest and disease

According to the requirement a fungicide (Saprol) was sprayed once during the growing period.

Sampling and analysis

Rice plants were harvested after 10 weeks of transplantation. The root and shoot samples were dried at 70°C for 4 days and dry weights were recorded. After harvesting of rice pot soils were collected. Then the samples were ground and preserved for analysis.

Analysis

Soil	Plant	
	Shoot	Root
%N, ^{15}N , ^{15}N ,	DW, %N, ^{15}N , %Ndff, %Ndfs	DW, %N, %Ndff, %Ndfs

^{15}N analysis diffusion technique

The samples were prepared for ^{15}N analysis using the PTFE diffusion technique (Sørensen and Jensen, 1991). Fifty ml of KCl extract was weighed into a plastic vessel, a PTFE envelope containing a quartz filter paper disc of 5 mm diameter acidified with 10 μl of 2.5 M KHSO_4 was added to the solution, approximately 0.2 g of MgO was then added to the vessel, the vessel was closed immediately, shaken and left for 5 days in the dark at room temperature. The envelope was removed and the disc dried in desiccators containing silica gel and an acid trap. The vessels were left open over night. On the following day, an additional acidified disc was prepared and added to the vessel with 0.2 g of Devarda's alloy. The vessels were again closed, shaken and left for a further 5 days after which the disc was removed and dried. Standard solutions were also analyzed to evaluate the procedure. Plant and inorganic N samples were analyzed using continuous flow isotope ratio mass spectrometry linked to a CN analyzer

Preparation and analysis of plant samples

Oven dried plant samples were ground to a fine powder using a Retsch mill (Germany). The ground samples were weighted in the range of 10 to 20 mg in to a pre-tarred tin cup and folded into neat parcels tweezers and the weight noted. N and ^{15}N atom excess were analyzed with a carbon-nitrogen analyzer EA-1500 Carlo Erba Strumentazione (Milan, Italy) combustion unit, linked to an isotope ratio mass spectrometer (IRMS) Optima Micro mass system (Micro-mass UK, Wythanshaw).

Statistical analysis

The results of the experiment were evaluated by the LSD test. The letter was used for testing the significance between mean values. The 0.05 level of probability was chosen for these statistical techniques.

Results and discussion

*Different rates of inorganic fertiliser (^{15}N labelled urea) and effect of salinity on rice (*Oryza sativa* L.)*

Dry matter yield of rice

Significant differences ($p < 0.05$) in rice biomass production under different levels of ^{15}N urea and salinity were observed. There was no significant differences ($p < 0.05$) of shoot and root DW among the different salt treatments, where no ^{15}N urea applied (0 ppm ^{15}N urea status) (Table II). However there was a small linear decrease in shoot DW with increased salinity in the no fertiliser treatment. The gradient in decrease in the 50 and 100ppm urea level treatment was greater. Shoot and root DW was significantly higher on 50 ppm (77.6%) and 100 ppm (122.2%) ^{15}N urea treated pots compared to the control (no urea and no salinity) (Table II).

But the shoot and root DW decreased significantly ($p < 0.05$) at 12 ds m^{-1} salinity treatment compared to the other salinity treatments as well as the control, in both 50 and 100 ppm urea applied pots (Table II). Higher salinity especially 12 ds m^{-1} showed decreasing tendency in DW production and it was 23.5%, 43.9% and 51.8% less, respectively on the three treatment compared to the control (no salinity) (Table II). This showed that plant suffered more by lack of N than salt stress. Only in 50 ppm did the affect of salt stress begin to play.

^{15}N atom excess

^{15}N atom excess in both shoots and roots under different levels of salinity were significantly ($p < 0.05$) different (Table II). With increasing rate of urea application ^{15}N were increased significantly ($p < 0.05$) on both shoots and roots (Table II). With increasing salinity and urea treatment also produced significant differences on ^{15}N enrichment in shoots and roots.

Root:shoot ratio

Root:Shoot ratios declined linearly with increasing urea level from 0 to 100ppm but there was no significant effect of different levels of salinity treatments (Table II).

Table II. Effect of different levels of urea and salinity on dry matter production, %¹⁵N atom excess of rice

¹⁵ N Urea status	Salinity level (ds m ⁻¹)	DW-Shoot (g pot ⁻¹)		DW-Root (g pot ⁻¹)		% ¹⁵ N excess in Shoots		% ¹⁵ N excess in Roots		root:shoot ratio	
		mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
0 ppm	0	13.30 ab	1.36	5.99 ab	0.386	0.014 a	0.003	0.010 c	0.002	0.467	0.082
	4	11.85 ab	0.69	5.65 ab	0.61	0.012 a	0.002	0.008 a	0.002	0.474	0.03
	8	11.37 ab	0.6	5.51 ab	0.159	0.011 a	0.002	0.007 a	0.001	0.489	0.041
	12	10.17 a	0.26	4.65 a	0.234	0.010 a	0.001	0.007 a	0	0.456	0.012
50 ppm	0	23.62 d	0.93	8.58 d	1.012	1.909 b	0.057	1.175 ac	0.034	0.363	0.039
	4	19.7 c	0.99	7.82 cd	0.816	2.005 bc	0.031	1.346 b	0.038	0.395	0.024
	8	17.01 c	0.94	6.7 bc	0.706	1.993 bc	0.004	1.386 b	0.016	0.392	0.23
	12	13.26 ab	0.48	5.58 ab	0.497	2.025 c	0.041	1.436 b	0.024	0.420	0.24
100 ppm	0	29.55 e	3.16	10.54 e	0.411	2.628 d	0.08	1.804 bd	0.031	0.369	0.055
	4	29.02 e	0.47	10.91 e	0.549	2.757 e	0.064	1.905 e	0.023	0.376	0.021
	8	20.90 cd	1.13	6.89 bc	0.322	2.892 f	0.024	2.085 de	0.055	0.333	0.032
	12	14.24 b	0.94	4.75 a	0.414	2.946 f	0.027	2.157 e	0.046	0.335	0.028

Mean values with the same letter (s) are not significantly different ($p < 0.05$)

Nitrogen contents in rice shoots and roots

The amount of N in shoots and roots of rice differed significantly ($p < 0.05$) under different treatments. With increasing urea level i.e. at 50 and 100ppm urea, N contents in shoots and roots were significantly ($p < 0.05$) higher than the control (no urea) (Table III). On 50 ppm and 100 ppm, ¹⁵N urea treated pots, the amount of N in shoots under 8 and 12 ds m⁻¹ salinity levels were lower than the control (no salt) and 4 ds m⁻¹ salinity treatment. In case of roots there was a significant ($p < 0.05$) linear decreasing effect of salt treatment at 8 ds m⁻¹. With increasing the level of ¹⁵N urea N contents in shoots also increased linearly and significantly ($p < 0.05$) compared to the control (Table III).

Total amount of N (shoot + root) in rice

Total amount of N in rice increased linearly and significantly ($p < 0.05$) with increasing levels of ¹⁵N urea compared to the

control. There was no significant ($p < 0.05$) difference in total amount of N among the 4 salinity treatments (0, 4, 8 & 12 ds m⁻¹) where no residue applied. 56.3% more N was in 100 ppm ¹⁵N urea (no salinity) treated pots compared to the control (no urea) without salinity (Table III). With increasing salinity from 0 to 12 ds m⁻¹ on 100 ppm ¹⁵N urea applied pots the total amount of N in rice increased significantly ($p < 0.05$). But in the 50 ppm ¹⁵N urea treatment significantly ($p < 0.05$) lower (240.4 mg pot⁻¹) N was observed on 12 ds m⁻¹ salinity compared to 0 salinity (Table III). The highest value (368.07 mg pot⁻¹) was in 100 ppm ¹⁵N urea treated pots compared to the control (no ¹⁵N urea). However ¹⁵N urea treated pots 12 ds m⁻¹ salinity treatment decreased significantly ($p < 0.05$) (12.1% in 50 ppm and 13.8% in 100 ppm ¹⁵N urea treatment, respectively) the N content in rice compared to the control (no salinity). Total N content in rice decreased because total DW decreased with salt treatment (Table III).

Table III. Effect of different levels of urea and salinity on total N contents in rice

^{15}N Urea status	Salinity level (ds m^{-1})	N in Shoot (mg pot^{-1})		N in Root (mg pot^{-1})		Total amount of N shoot + root (mg pot^{-1})	
		mean	SE	mean	SE	mean	SE
0 ppm	0	93.67 a	5.93	56.67 bc	2.91	150.45 a	5.56
	4	94.33 a	2.91	49.67 ab	3.53	144.06 a	5.95
	8	96.00 a	3.06	48.00 ab	1	143.77 a	2.68
	12	102.67 a	1.77	44.33 a	3.72	146.83 a	4.9
50 ppm	0	192.67 b	6.97	74.67 e	7.43	267.51 c	14.13
	4	179.33 b	8.22	69.00 de	4.17	247.80 bc	4.63
	8	183.00 b	6.81	57.33 bcd	5.18	240.40 b	11.02
	12	184.33 b	2.73	50.67 abc	2.97	235.15 b	5.91
100 ppm	0	265.33 cd	13.7	91.00 f	6.57	356.20 e	11.26
	4	273.33 d	3.93	94.67 f	2.34	368.07 e	2.1
	8	248.67 c	7.81	62.00 cd	2.31	310.76 d	8.73
	12	256.00 cd	9.55	51.33 abc	2.97	306.97 d	7.72

Mean values with the same letter (s) are not significantly different ($p < 0.05$)

Percentage nitrogen recovery from fertiliser (%Ndff)

Shoot: There was no significant difference on %Ndff in shoots among different salinity treatment on 50 ppm ^{15}N urea applied pots. However, %Ndff in shoots of different salinity treatment differed significantly ($p < 0.05$) in 100 ppm ^{15}N urea treated pots (Table IV). The highest %Ndff was in 12 ds m^{-1} and the lowest was on no salinity on 100 ppm ^{15}N urea treated pots. %Ndff increased significantly ($p < 0.05$) with increasing ^{15}N urea as well as salinity from 0 to 12 ds m^{-1} (Table IV).

Root: Also in the roots of rice %Ndff increased significantly ($p < 0.05$) with increasing levels of ^{15}N urea and salinity. 53.6% more %Ndff was observed on 100 ppm ^{15}N urea (no salinity), compared to 50 ppm ^{15}N urea (no salinity). On 50 ppm ^{15}N urea, the salinity treated pots differed significantly ($p < 0.05$) than the control as well as on 100 ppm ^{15}N urea except 4 ds m^{-1} salinity which was not significantly different from the control (Table IV). The highest value (43.2%) was in 12 ds m^{-1} salinity treatment in 100 ppm ^{15}N urea and the lowest value was in the control (no salinity) (23.5%) 50 ppm ^{15}N urea (Table IV)

Nitrogen recovery from fertiliser in rice (%Ndff, weighed average value)

%Ndff in rice (weighed average) increased significantly ($p < 0.05$) with increasing urea level as well salinity treatment on rice. On 50 ppm ^{15}N urea, salinity treated pots were not significantly different in %Ndff among themselves but significantly ($p < 0.05$) different from the control (Table IV). On the other hand on 100 ppm ^{15}N urea, the different salinity treated pots differed significantly. The highest (55.9%) value was in 12 ds m^{-1} salinity and the lowest (48.3%) in the control (Table IV).

Nitrogen recovery from soil (%Ndfs)

Nitrogen derived from soil was the highest in 50 ppm ^{15}N urea (low) without salinity (65.9%). With increasing urea level, %Ndfs decreased significantly ($p < 0.05$) (Table IV). There was no significant difference in %Ndfs among the treatments from 4 to 12 ds m^{-1} salinity at 50 ppm ^{15}N urea treatment in rice but all the salinity treatment differed significantly ($p < 0.05$) than the control. However, on 100 ppm ^{15}N urea treated pots in all

Fertiliser use efficiency (%)

Total recoveries of labelled fertiliser by rice are presented in table IV. The estimated values of fertiliser N recovery using labelled ^{15}N urea under different salinity treatment were not significantly different among themselves. On the whole, it appeared that fertiliser use efficiencies remained more or less constant and were not affected by the rates of N application (Table IV). The percentage of ^{15}N fertiliser recovered (%FUE) by rice plants ranged from 68.7% to 72.8%, respectively for the treatments of 100 and 50 ppm N (Table IV).

Residual N in soil (%)

After harvesting of rice, pot soils were analyzed. There was no significant difference in %N content of soil with increasing rate of N application and salinity level. Only on 100 ppm ^{15}N urea treatment 8 and 12 ds m^{-1} salinity treated pots contained significantly ($p < 0.05$) higher %N compared to the control (no salinity) of the same N rate. However, ^{15}N atom excess in soil increased significantly with increasing N rate and salinity compared to the control (no ^{15}N urea) (Table V).

Table IV. Effect of different levels of urea and salinity on N recovery and N fertiliser use efficiency in rice

^{15}N Urea status	Salinity level (ds m^{-1})	Ndff in shoots %		Ndff in roots %		%Ndff in rice (Weighed average)		Ndfs in rice %		Fertiliser use efficiency (%)	
		mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
0 ppm	0										
	4										
	8										
	12										
50 ppm	0	38.18 a	1.143	23.49 a	0.68	34.10 a	1.13	65.90 e	1.13	72.8 ab	2.12
	4	40.11 a	0.618	26.91 b	0.77	36.40 b	0.66	63.60 d	0.66	72.27 ab	1.87
	8	39.85 a	0.074	27.71 b	0.33	36.97 b	0.09	63.03 d	0.09	70.93 ab	3.01
	12	40.50 a	0.821	28.72 b	0.48	37.97 b	0.74	62.03 d	0.74	71.20 ab	0.92
100 ppm	0	52.55 b	1.604	36.08 c	0.62	48.31 c	1.02	51.69 c	1.02	68.67 a	0.88
	4	55.14 bc	1.286	38.11 c	0.46	50.75 d	0.92	49.25 b	0.92	68.67 a	0.96
	8	57.84 cd	0.481	41.71 d	1.09	54.62 e	0.54	45.38 a	0.54	67.87 a	1.31
	12	58.92 d	0.549	43.15 d	0.91	55.96 e	0.15	44.04 a	0.15	69.07 a	2.37

Mean values with the same letter (s) are not significantly different ($p < 0.05$)

salinity treatments were significantly ($p < 0.05$) different compared to the control which was the highest in %Ndfs among the treatment and the lowest was in 12 ds m^{-1} salinity treated pots (Table IV).

Discussion

From rice DM yield data it was observed that maximum shoot and root DW was in soil treated with ^{15}N urea and minimum where no urea was applied (no salinity) (Table II). Application

of fertiliser to the field crops and nitrogen studies on the fertiliser have frequently been reported (Legg and Meisinger, 1982). Azam *et al.*, (1985) also found that maize shoot DW was also the highest in soil treated with labelled inorganic fertiliser (ammonium sulphate) and the lowest where no fertilizer was applied. With increasing rate of inorganic fertiliser the rice shoot and root DW increased significantly compared to the control. The highest DW of rice on the highest rate of urea (100 ppm N) may have been due to the ready availability of urea was also reported (Rahman and Parsons, 1999 and Mondol *et al.*, 2004). With increasing rate of fertilizer application increased the yield of wheat reported by Mohannad *et al.*, (2012).

decreased with increasing salinity level. An excess of specific ion present under saturated condition in saline environment may be toxic to various plant physiological processes (Breesler *et al.*, 1982). A significant decline in dry matter yields of tomato plants by increasing salinity was reported by many investigators (Al-Rawahy *et al.*, 1990 and Subba Rao *et al.*, 1987). Variation in dry matter production was also dependent on salt types among the soluble salts, NaCl is the most detrimental to plant growth and nutrient uptake (Al-Rawahy *et al.*, 1990; Ullah *et al.*, 1993). Previous research has found that increasing soil salinity resulted in a decreased photosynthesis in the plant (Qui *et al.*, 2011) might be a result of decline in DW of shoot and root on urea treated pots (Table II).

Table V. Effect of different levels of urea and salinity on residual N in soil (%)

¹⁵ N Urea status	Salinity levels (ds/m)	Total %N in soil	% ¹⁵ N excess in soil
0 ppm	0	0.087 ab	0.001 a
	4	0.087 ab	0.002 a
	8	0.087 ab	0.002 a
	12	0.083 ab	0.002 a
50 ppm	0	0.083 ab	0.067 b
	4	0.083 ab	0.067 b
	8	0.080 a	0.063 b
	12	0.083 ab	0.064 b
100 ppm	0	0.80 a	0.125 d
	4	0.83 ab	0.107 c
	4	0.90 b	0.123 d
	12	0.90 b	0.130 d

Mean values with same letter (s) are not significantly different ($p < 0.05$)

Salinity significantly decreased DW of shoot and root on urea treated pots. 12 ds m⁻¹ salinity decreased 23.5%, 43.9%, and 51.8% DW of shoot (Table II) on different urea treated pots respectively compared to the control (no salinity). Rice (*Oryza sativa* L.) is considered moderately sensitive to salinity (Maas and Hoffman, 1977). However, cultivars respond differently when subjected to salinity (De Datta, 1972). Salinity lowers the water potential in the soil, consequently lowering the water potential gradient from the soil to the plant cells. As a result, water availability, turgor pressure and growth rate reduced (Meiri and Poljakoff-Mayber, 1970). A significant decline in dry matter yield of tomato plants by increasing salinity was reported by many investigators (Al Rawahy *et al.*, 1990 and Subba Rao *et al.*, 1987); while Ullah *et al.*, (1993) reported an increase in dry matter production in tomato plants due to salinity. In this study total N decreased because total DW

With increasing rate of urea %N in shoot and also total N content in rice increased significantly compared to the control (no urea,) (Table III). DW, straw and grain total N increased with the rate of fertiliser N was also reported by many authors (Hood *et al.*, 1999 and 2000). There was no significant difference on %N content in roots on fertiliser added pots. Total nitrogen content in rice was higher on 50 and 100 ppm urea added pots compared to control (0 ppm ¹⁵N urea status); these results may have been due to the ready availability of urea-N. Experiments with ¹⁵N labelled fertilisers often (but not always) show that the addition of fertiliser N increases the uptake of soil N by the crop, an effect sometimes called a 'priming' effect (increase in uptake of soil N in treatments where N was applied) and perhaps better described as an 'added nitrogen interaction' (Jenkinson *et al.*, 1985; Valiente *et al.*, 2000).

Inorganic fertilisers increase the mineralization of soil thus making more N available to plants (Azam *et al.*, 1985). The inorganic source (fertiliser) was greatest in all pools of N measured over time. %Ndff in rice (weighed average) was higher on high rate of fertiliser added pots. %Ndffs in rice on ¹⁵N urea added pots were significantly higher on low rate of urea i.e. on 50 ppm urea treated pots compared to 100 ppm treatment. There was no significant difference on % fertiliser use efficiency on 50 ppm and 100 ppm urea treatment. Fertiliser use efficiencies were not influenced by the addition of different rates of fertiliser this was also reported by Ngoran *et al.*, (1998).

The total ¹⁵N recoveries from soil to rice in the urea treated pots were more. These results confirm the immediate availability of urea-N compared to control. The percentage of ¹⁵N fertiliser recovered (%FUE) by rice plants ranged from 68.7% to 72.8%, respectively for the treatments of 100 and 50 ppm N (Table IV). The unaccounted N could be mainly the result of losses through denitrification or ammonification. Application of fertiliser N to the field crops and nitrogen balance studies on the fertiliser have frequently been reported by many authors (Legg and Meisinger, 1982; Mondol *et al.*, 2004). Combining N fertilization has the potential to change N transformations through a negative interactive effect on mineral N reported by Gentile *et al.*, (2009). Salinity affected significantly on %Ndff (increasing) and %Ndffs (decreasing) in rice on both rate of urea, compared to the control (no salinity) (Table IV). However, salinity did not affect any significant difference in %fertiliser use efficiency on rice.

The reduction in nitrogen content due to increased salinity might be a result of specific ion effects of Cl⁻. As for specific ion effects, it might involve in exerting direct toxicity of Cl⁻ and/or Na⁺ and antagonistic effects of Cl⁻ on NO₃⁻ uptake (Al-Rawahy *et al.*, 1990). Such reduction in nitrogen content was reported by many authors (Ullah *et al.*, 1993). Most plants suffer from salt injury at ECe values exceeding 4 ds m⁻¹ (Karim *et al.*, 1990). All rice's are glycophytes lacking salt glands or other excretory structures. Decreasing osmotic pressure of the culture solution reduced the dry matter production, N absorption and water content in plants as reported by Pessarakli *et al.*, (1988) and Mondol *et al.*, (2004). High level of salinity negatively affected seed yield and N accumulation in tissue of faba bean was also reported (Gadalla *et al.*, 2007).

On 100 ppm ¹⁵N urea treatment 8 and 12 ds m⁻¹ salinity treated pots contained significantly (p<0.05) higher residual %N compared to the control (no salinity) of the same N rate. However, %¹⁵N atom excess in residual soil increased significantly with increasing N rate and salinity compared to

the control (no ¹⁵N urea) (Table V). Soils on lower slopes and near saline seeps have higher ¹⁵N values than well-drained soils (Young *et al.*, 2011), perhaps because the greater denitrification in more boggy areas results in heavy residual nitrate.

Conclusion

% Ndff was increased with increasing rates of fertilizer application and salinity. % Ndffs was in the range of 44-66%. Fertilizer use efficiency was in the range of 67-72%. Nitrogen remained in the soil after harvest of rice was the highest in the highest rate (100 ppm) of ¹⁵N urea applied pots.

Acknowledgements

The first author would like to thank the Afro-Asiatisches Institute (AAI), Vienna, Austria, for supporting his successful studies in Austria and to FAO/IAEA Agricultural and Biotechnology Laboratory, Seibersdorf, Austria for providing him with library, laboratory and all other research facilities as and when required.

References

- Al-Rawahy S. A., Stroehlein J. L. and Pessarakli M. (1990). Effect of salt stress on dry matter production and nitrogen uptake by tomatoes. *J. Plant Nutrition*. **13(5)**: 567-577.
- Azam F., Simmons F. W. and Mulvaney R. L. (1993). Mineralization of N from plant residues and its interaction with native soil N. *Soil Biol. Biochem*. **25**: 1787-1792.
- Bal A. R. and Dutt, S. K. (1986). *Plant and soil*. **92**: 399-404.
- Bresler E., McNeal B. L. and Carth B. L. (1982). Saline and sodic soils. Principles Dynamics-Modeling. Springer-Verlag, Berlin, Heidelberg, New York. 167-181.
- Buresh R. J. and De Datta S. K. (1990). Denitrification losses from puddle rice soils in the tropics. *Biol. Fertil. Soils* **9**: 1-13.
- Cambell C. A. (1978). Soil organic carbon, nitrogen and fertility. In: Developments in Soil Science 8. Soil organic matter (M Schnitzer and S.U. Khan eds), 174-271, Elsevier, Amsterdam.
- De Datta S. (1972). A study of salt tolerance of twelve varieties of rice. *Curr. Sci*. **41**: 456-457.

- Gadalla A. M., Hamdy A. and Galal Y. G. 2007. Use of saline irrigation water for production of some legume and tuber plants. In: Lamaddalena N (ed), Bogliotti C. (ed), Todorovic M. (ed), Scardigno A. (ed). Water saving in Mediterranean agriculture and future research needs (vol. 2). *Bari:CHEM*: 85-97. Proceeding of the International Conference WASAMED Project. Valenzano (Italy).
- Gentile R., Vanlauwe B., Van Kassel C. and Six J. 2009. Managing N availability and losses by combining fertilizer N with different quality residues in Kenya. *Agric. Ecosystems and Environ.* **131**: 308-314.
- Hood R. C., Merckx R., Jensen E. S., Powlson D., Matijevic M. and Hardarson G. (2000). Estimating crop N uptake from organic residues using a new approach to the ¹⁵N isotope dilution technique. *Plant and Soil* **223**: 33-44.
- Karim Z., Hussain S. Z. and Ahmed M. (1990). Salinity problems and crop intensification in the coastal regions of Bangladesh. *BARC Publication No.* **33**: 12-14.
- Mondol M. N., A. S. Chamon, R. C. Hood and W. E. H. Blum. 2004. Effect of different levels of ¹⁵N labeled residue and salinity on growth, yield and nitrogen contents in rice. *Bangladesh J. Environ. Sci.* **10**: 341-345.
- McNeill A. M., Hood R. C. and Wood M. (1994). Direct measurement of nitrogen fixation by *Trifolium repens* L. and *Alnus glutinosa* L. using ¹⁵N₂. *J. Exp. Bot.* **75**: 749-755.
- Meiri A. and Poljakoff-Mayber A. (1970). Effect of various salinity regimes on growth, leaf expansion and transpiration rate of bean plants. *Soil Science*. **109**: 26-31.
- Mohammad W., S.M. Shah, Shehzadi and S. A. Shah. 2012. Effect of tillage, rotation and crop residues on wheat crop productivity, fertilizer N and water use efficiency and soil org. carbon status in dry area (rainfed) of north-west Pakistan. *J. Soil Sci. and plant Nutrition*. **12(4)**: 715-727.
- Ngoran K., Zapata F. and Sanginga N. (1998). Availability of N from Casuarina residues and inorganic N to maize, using ¹⁵N isotope technique. *Biol Fertil Soils*. **28**: 95-100.
- Pearson G. A. and Berstein L. (1959). Salinity effects at several growth stages of rice. *Agron, J.* **5**: 654-657.
- Qui YongQin, JianYing M. A. and Wei Sun. 2011. Application of stable isotope technique to the study of soil salinization. *J. Arid Land*. **3(4)**: 285-291.
- SØrensen P. and Jensen E. S. (1991). Sequential diffusion of ammonium and nitrate from soil extracts to a polytetrafluoroethylene trap for ¹⁵N diffusion. *Analytica Chimica Acta* **252**: 201-203.
- Ullah S. M., Soja G. and Gerzabek M. H. (1993). Ion uptake, osmoregulation and plant water relations in faba beans (*Vicia faba* L.) under salt-stress. *Die Bodenkultur*. **44(4)**: 291-301.
- Valiente F. E., Ucha, A., Quesada A., Leganes F. and Carreres R. (2000). Contribution of N₂ fixing cyanobacteria to rice production: availability of nitrogen from ¹⁵N labeled cyanobacteria and ammonium sulphate to rice. *Plant and Soil*. **221**: 107-112.
- Young M.B., Harter T., Kendall C., Silva S.R., Esser B. K., Singleton M.J., Holstege D., Lockhart K., Applegate O. 2011. Stable isotopes as indicators of sources and processes influencing nitrate distributions in dairy monitoring wells and domestic supply wells in the Central Valley, California. *American. Geophysical Union, Fall Meeting: abstract: #H53H-1508*

Received: 2 May 2013; Revised: 12 March 2014

Accepted: 3 June 2014.