VIRTUAL TRAINING

THE USE OF ^{15}N IN DATE PALM

Enhancing Nutrient Use Efficiency using ^{15}N Fertilizer Studies

(Part II)

Field Experimentation Techniques for the ^{15}Nitrogen Method

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Outline

- Introduction
- N Use Efficiency (NUE) Studies
- Measurement of NUE
- Measurement Methods
- ^{15}N Techniques: Direct Approach
- Questions and Discussion

Important Criteria of Isotopic Studies

- The isotopic method is the only way to solve a particular question or to obtain a particular piece of information

- Other methods are available, but the isotopic method is a quick and cost effective means to obtain the needed information

- The Golden Rule: The experiments be simple and intended to answer specific queries

Experimental Guidelines

Aspect highlighted	Action to be taken
Identify specifics of N-related problems, topics, priorities. Reflect these in the title	Done already by KISR- the Lead Centre; Revise as needed by the Cooperating Partners
Compile background information from literature etc. Define the role of isotopic technique	Done already by the CPs
The Golden Rule: Design Simple experiments with concrete and well-defined objectives	The experimental details of each CP will be worked out based on the specific needs and results of the Pilot study.
Define locations, treatments, other details like experimental designs, statistical analysis.	Pilot study to be carried out at KISR; Detailed studies at KISR and different CPs

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Experimental Guidelines (cont'd)

Aspect highlighted	Action to be taken
Estimate the amount of ^{15}N urea needed in different Centers	Pilot study and the specific needs of each CP will decide these aspects
Define sampling/harvesting times and procedures. The total number of samples to be analyzed.	Pilot study and the specific needs of each CP will decide these aspects.
Analytical methods, laboratory standards, quality control, data reporting.	-do-
Calculation of data, selection of the evaluation parameters in relation to the objectives of the study.	-do-
Compilation of information and guidelines for the experiment.	-do-

Experimental Guidelines (cont'd)

Aspect highlighted	Action to be taken
Assess the resources (physical, human and financial) needed, including a budget and sharing among institutions	Series of meetings of the participants is necessary at different CPs from time to time to contemplate on these aspects.
Revise the draft experimental guidelines (working copy) with relevant staff members.	Based on the results of the Pilot study the technical program of each CP may to be revised.
Revise the draft experimental guidelines (working copy) with relevant staff members.	-do-
Distribute the final experimental guidelines among all participating staff.	Yes

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Experimental Site

- Choose a representative site, study soil thoroughly
- Start with on farm detailed experiment
- Follow up with farmers' sites
- Transfer to beneficiaries/ end-users

Soil Series: Thyamgondlu

Classification *Typic Haplustalf*

pH: 5.9

CEC: 8.7 cmol (p^+) kg^{-1}

Available N: 224 kg ha^{-1}

Treatment and Experimental Design

- Based on the findings of Pilot study, the objectives and the specific needs of the Cooperating Partners, the details may be finalized.
- The experimental design, replications and statistical analysis (ANOVA) may be finalized appropriately.

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Plot and Field Layout

- Two types of plot are required: (i) yield (control) and (ii) isotope plots.
- The plot layout depends on the variety and the cropping system. Isotope plots are the smallest possible area (micro-plots) to obtain a representative sample for isotope enrichment measurements while reducing the amount of isotope utilized due to its cost. Micro-plot in some cases may have just one plant (e.g. a tree in the lysimeter).
- Yield (control) plots must be sufficiently large to obtain precise information on yield parameters (total biomass and economic crop yield) and for other additional observations (crop growth measurements, physiological parameters, soil water measurements, plant and soil samplings, etc.) to be made throughout the growth cycle of the crop.

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Requirements for ^{15}N labelled materials

- The amount of ^{15}N applied as fertilizer must be sufficient to be detected eventually in the plant samples collected.

- It depends both on the rate of application and the enrichment (^{15}N% atom excess) of the labelled fertilizer, the objective of the study, type of crop, duration of the experiment, and primarily the available equipment for measuring the N–isotope ratio.

- As a general guideline, fertilizer N studies (fruit tree like date palm) may mean 1000 kg N/ha dosage that may require 1, 2 or 5 atom % ^{15}N excess.

- Pilot study will take care of the suitable ^{15}N atom % excess needed as the proposed project is first of its kind study on date palm.

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Calculations of ^{15}N labelled fertilizer requirements

- The total ^{15}N labelled fertilizer requirements can be calculated from total number of lysimeters or experimental plants/ treatment units (number of trees/treatment) required for the experiment.

- With this information and number of replications in the experiment it is possible to further estimate the ^{15}N requirements and make a cost estimate based on recent bid quotations from commercial suppliers.

Application of the ^{15}N labelled materials

- Ideally, the ^{15}N should be applied in a form that reflects the standard practice to be tested (e.g. solid fertilizer). It should be fertilizer should be chemically (carrier) and physically (form) identical to the commercial fertilizer.

- This is obviated since subsurface fertigation is contemplated to address N fertilization in conjunction with need based irrigation

- Other application practices are left up to the research team for the development of fertilizer management practices.

Field Observations

- Field visits should be made regularly, to follow the development of the crop and any differences among treatments.

- The experimental field book should be kept up to date, with detailed records of experimental designs and procedures, crop observations, planting, cultural practices and applications, weed and pest control, crop growth, changes in climatic conditions, etc.

- Details of harvesting and sampling procedures should also be recorded.

Harvesting and Sampling

Laborious and time consuming but is critical to the validity of the ^{15}N recovery data.

Quantitative estimates of plant recovery of fertilizer require estimation of biomass produced and the total amount of nutrient taken up in the experiment.

The harvesting procedure consists of gathering all above ground plant material in the lysimeter or treatment plant/s of the isotope plot.

Avoid contamination of plant samples with labelled soil very carefully.

Some practical considerations

(i) Plan detailed harvesting/sampling operations including preparation bags, labels and a field book to record in advance

(ii) Label and organize samples carefully

(iii) Collect all samples before leaving the field and verify

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Sample Preparation

- Size of sample may be very small (10-150mg)
- Bulky samples need chopping and quartering
- Stepwise sampling and sub-sampling procedures may involve :

- Sample (e.g. plant material)
- Separation into different plant parts
- Total fresh weight per plant harvested (TFW)
- Cutting 1-3cm
- Mixing; Quartering;
- Sub-sample
- Sub-sample fresh weight (SFW)
- Drying at 60 C
- Sub-sample fresh weight (SDW)
- Grinding sub-sample and
- Analysis

Nitrogen Analyses

- Estimate Total N by Kjeldahl method
- Estimate ^{15}N:^{14}N ratio using MS

Calculations: Basic Primary Data

- Dry matter of different parts and whole plant for component and total N uptake
- Total N content
- Plant % ^{15}N abundance of parts and whole plant
- Fertilizer % ^{15}N abundance
- ^{15}N labelled fertilizer
- N rate applied

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Quantification of FNUE

- % Ndff and % Ndfs
- Biomass produced per hectare (kg/ha)
- Total N uptake or N yield (kg/ha)
- Fertilizer N uptake or yield (FNU)
- Fertilizer N use efficiency (FNUE)

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Quantification of FNUE : Isotopic parameters

Nitrogen derived from the fertilizer (Ndff)

$$\% \, Ndff = \frac{\text{Atom \% } ^{15}N \text{ excess plant}}{\text{Atom \% } ^{15}N \text{ excess in Fertilizer}} \times 100$$

Nitrogen derived from the soil (Ndfs)

$$Ndfs = 100 - \% \, Ndff$$

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FNUE : Isotopic parameters (cont'd)

Biomass produced
(dry matter yield per hectare (kg/ha*)

$$\text{Dry matter yield} = \frac{[FW(kg) \times 10{,}000 m^{2*}]}{\text{Harvested area } (m^2) \times [SDW/SFW]}$$

where:

FW is the fresh weight of the harvested area and

SFW and SDW are the subsample fresh and dry weights (in kg)

FNUE : Isotopic parameters (cont'd)

Total N uptake or N yield (kg/ha)

$$\text{N yield} = \frac{(\text{Dry matter yield (kg/ha)} \times \%\ \text{total N})}{100}$$

Fertilizer N uptake or fertilizer N yield (kg/ha)

$$\text{(FNU)} = \frac{[\text{N yield (kg/ha)} \times \%\ \text{Ndff}]}{100}$$

FNUE : Isotopic parameters (cont'd)

Fertilizer N use efficiency/

Fertilizer N recovery/

Real coefficient of utilization (FNUE)

$$\% \text{ FNUE} = \frac{\text{Fertilizer} - \text{N yield}}{\text{Applied N rate}} \times 100$$

Some Sample Calculations: Example 1

Given:

In pots containing 2 kg soil, 100 mg N/kg as ammonium sulphate (1.39% ^{15}N abundance) applied to flooded rice.

At harvesting, the plant dry matter yield per pot was 14 g; Plant samples had 0.70% ^{15}N abundance and 2.2% total N.

Questions

- What fraction of N in the plant was derived from the fertilizer?
- What fraction of N in the plant was derived from the soil?
- What was the total N uptake or yield of the crop?
- What was the fertilizer N uptake?
- What was the FNUE or recovery by the crop?

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Example 1: Greenhouse Experiment

Calculations and results

- at. % ^{15}N excess plant = 0.70 – 0.37 = 0.33
- at. % ^{15}N excess fertilizer = 1.39 – 0.37 = 1.02

(1) Ndff = 0.33/1.02 = 0.324 or % Ndff = 32.4

(2) Ndfs = 1 – 0.324 = 0.676 or % Ndfs = 67.6

(3) Total N uptake or N yield of the crop =

(14g/pot × 2.2% N)/100= 0.308g

(4) Fertilizer N uptake by the crop =

(0.308 × 32.4) / 100 = 0.1 g or 100 mg

(5) FNUE or % recovery by the crop =

(100 / 200) × 100 = 50%

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Example 2: Field Experiment

In a field experiment, 80 kg N/ha was applied as labelled urea (1.37% ^{15}N abundance) to a maize crop. Plants were harvested at tasseling. Dry matter yield was 4000 kg/ha, and the plant samples had 0.67% ^{15}N abundance and 3% total N.

Questions

(1) What fraction of N in the plant was derived from the fertilizer?

(2) What fraction of N in the plant was derived from the soil?

(3) What was the total N uptake or yield of the crop?

(4) What was the fertilizer N uptake?

(5) What was the FNUE or recovery by the crop?

Example 2: Calculations and results

- At. % ^{15}N excess plant = 0.67 – 0.37 = 0.30

- At. % ^{15}N excess fertilizer = 1.37 – 0.37 = 1.00

(1) % N derived from the fertilizer:

$$\% \text{ Ndff} = 0.30/1.00 \times 100 = 30$$

(2) % N derived from the soil:

$$\% \text{ Ndfs} = 100 - \%$$

$$100 - 30 = 70\%$$

Example 2: Calculations and results (cont'd)

- (3) N yield of the crop: The total amount of N contained in the crop during the experimental period is obtained by recording the dry matter yield and multiplying it by the % total N in the crop as follows:

  $$(4000 \times 100) / 3 = 120 \text{ kg N/ha}$$

- (4) Fertilizer N uptake by the crop: The amount of fertilizer N taken up by the crop is calculated by multiplying the total N yield by the fraction of Ndff:

  $$120 \times (100 / 30) = 36 \text{ kg N/ha}$$

- (5) Fertilizer N use efficiency or recovery by the crop: The fraction of the fertilizer nutrient taken up by the plant in relation to the rate of fertilizer nutrient applied is commonly expressed as a percentage:

  $$\text{FNUE} = (36 /80) \times 100 = 45\%$$

Some Sample Calculations: Example 3

In a field experiment, 60 kg N/ha as ^{15}N labelled ammonium sulphate was applied to hybrid sorghum. The ^{15}N treated plots were harvested at the milk stage of grain development. The harvest consisted of gathering all above ground material in the harvesting area of the isotope plots and separating it into shoots and panicles. Fresh weights of both components were recorded. Adequate subsamples were taken, and chemical and isotopic analyses were performed on each subsample separately.

Question

What was the fertilizer N utilization of sorghum?

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Example 3: Calculations and results

CALCULATION SHEET FOR FERTILIZER NITROGEN USE EFFICIENCY

Plant part	Dry matter yield (t/ha)	Total N (%)	N yield or uptake (kg/ha)	Ndff (%)	Fertilizer N Yield (kg/ha)
Shoots	5.0	1.2	60	27	16.4
Panicles	2.2	2.1	46	20	9.10
Total	7.7		106		25.5

$\%\ Ndff = 25.5 / 106 \times 100 = 24\%$

$\%\ FNUE = (25.5 / 60) \times 100 = 42.5\%$

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Measures to Enhance FNUE

- Apply optimal/ recommended rates
- Timed split applications to crop need and development stage
- Follow fertigation at proper depth
- Adjust fertilization plan to avoid unexpected losses or deviation from crop development stage
- Follow balanced nutrition of secondary and micro-nutrients.

References

IAEA Publications

- Use of Isotope and Radiation Methods in Soil and Water Management and Crop Nutrition, *Training Course Series No. 14* (2001).

- (1) Zapata, F. Introduction to Nitrogen Management in agricultural systems, pp. 1-18. and

  (2) van Cleemput, O., Zapata, F. and Vanlauwe, B. Use of tracer technology in mineral fertilizer mmanagement. pp. 19-125.

  In. Guidelines on Nitrogen Management in Agricultural Systems, *Training Course Series* 29 (2008).

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Summary

- Experimental guidelines of ^{15}N studies
- Experimental procedures of ^{15}N studies
- Calculations for experiments with ^{15}N
- Some examples
- References

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Thank you!