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USING CARBON STABLE ISOTOPE TO EVALUATE WATER EFFICIENCY FOLLOWING SEASONAL VARIATION IN COFFEE LEAF

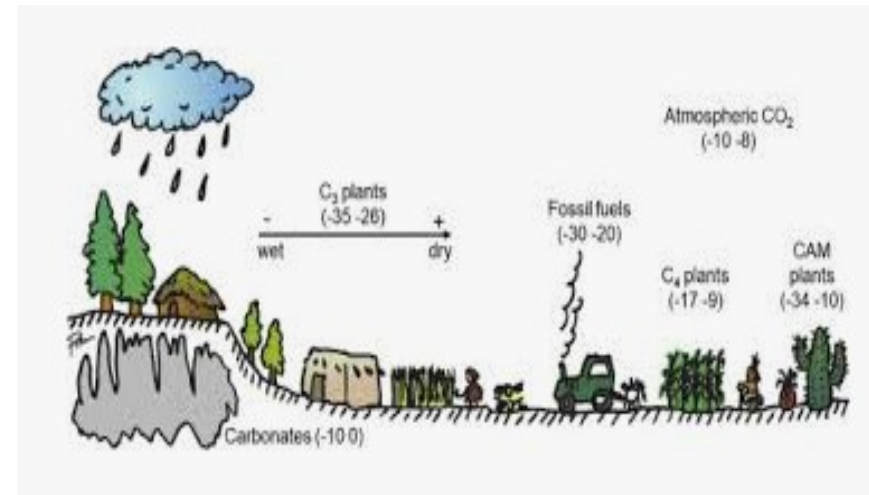
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Contents:

1. Project objectives;
2. Abstract
3. Introduction;
4. Materials and Methods;
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I. Project goals

1. Improper water management, indiscriminate water use, and climate change are the major factors reducing the global freshwater resources, and the major challenges facing many parts of the world.
2. Irrigation and agricultural purposes make up around 70% of all freshwater use, but water use efficiency (WUE) remains below 50%.
3. Developing the capacity in the analysis of stable isotopes ($\delta^{13}\text{C}$) in plant samples.
4. Improving agricultural water use can be achieved through proper soil and water conservation, minimizing soil evaporation, cultivating crops with low water demand, and improving irrigation management.

I. Project goals

5. The ability to distinguish between soil evaporation and crop transpiration can help to identify management practices that cut losses and improve water productivity (WP).
6. Enhancing capacity in the Application of nuclear techniques and related stable isotopes with multivariate statistical methods to assess the impacts of climate change related to water use efficiency in other types plants in Vietnam.
7. However, for Vietnam, this Isotope Ratio Mass Spectrometry (EA-IRMS) technique is quite new and has not been widely applied at present such as soil evaporation and transpiration under different agroclimatic and soil–plant management conditions is often unavailable.

II. Abstract

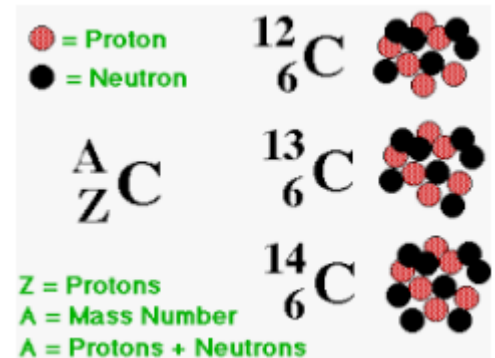
- Improper water management, indiscriminate water use, and climate change are the major factors reducing the global freshwater resources, and the major challenges facing many parts of the world.
- This study aims to determine the water use efficiency (WUE) index based on the carbon isotope discrimination ($\delta^{13}\text{C}$) to investigate the relationship between carbon absorption and water loss in coffee plants.



Measures to increase
Water Use Efficiency
in Agriculture



WATER MANAGEMENT FORUM
A Peripheral body of
The Institution of Engineers (India)



The contents:

- (1) The design of the sampling area, collecting coffee leaf samples corresponded to the design of irrigation season and regime; and measuring temperature, rainfall, and humidity parameters in the area;
- (2) Testing and optimizing the procedure for analysis of $\delta^{13}\text{C}$ stable isotope in coffee leaf samples on the EA-IRMS system at the Dalat Nuclear Research Institute;
- (3) Analyzing $\delta^{13}\text{C}$ stable isotope in coffee leaf samples at Lam Dong province (about 90 samples), in Vietnam;
- (4) Using this stable isotope data for the water use efficiency index calculation of coffee plants and the influence of weather condition factors (temperature, rainfall and humidity) on coffee plants water loss;
- (5) Evaluating the possibility of using $\delta^{13}\text{C}$ stable isotope technique in climate change research (calculation of water use efficiency index) for coffee plants.

II. Abstract

And water use efficiency index and $\delta^{13}\text{C}$ were positively correlated with temperature ($T^{\circ}\text{C}$), humidity (H%), and annual precipitation (Rmm) while carbon content was negatively correlated.

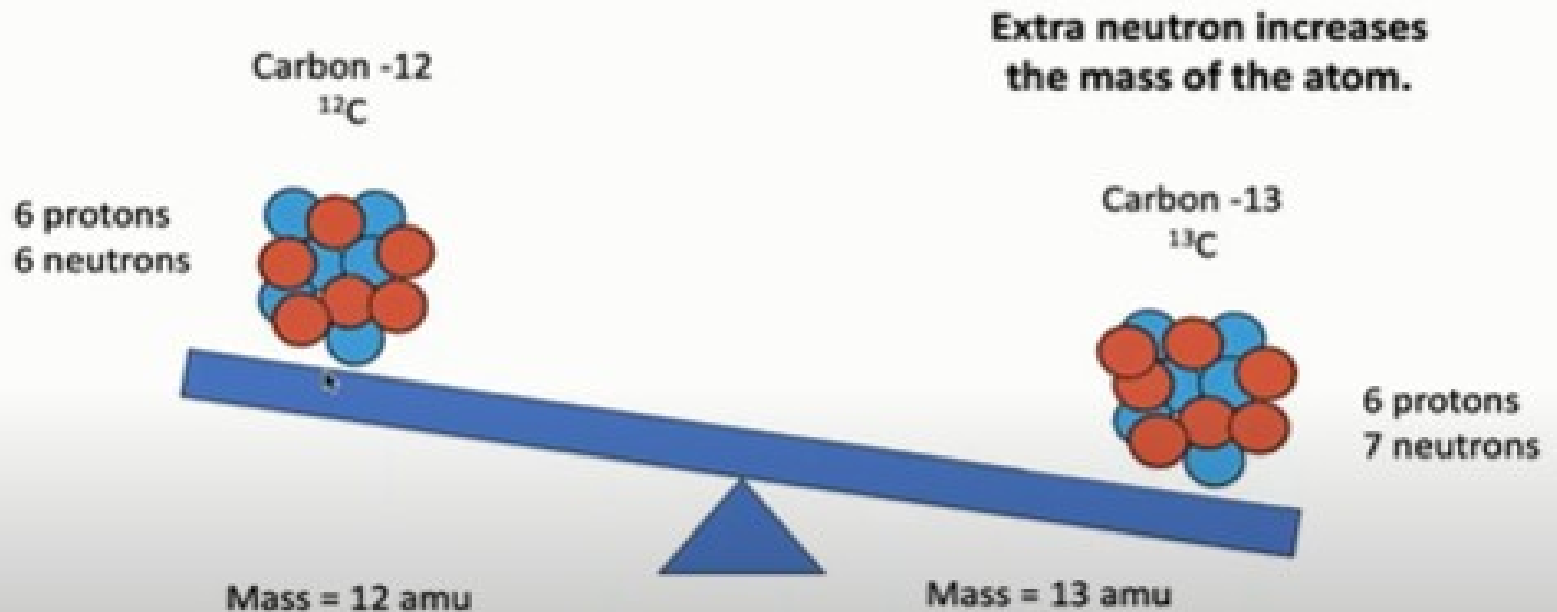
In the data observed, the results showed that in the rainy season, the water use efficiency index was nearly 14% higher than that in the dried season;

In the dry season WUE was 0.19 whereas in the rainy season, the amount of water in plants increased with the WUE index (0.22) and p-value = 0.017.

III. Introduction (1)

3.1. The carbon stable isotope composition ($\delta^{13}\text{C}$)

Isotopes are the same element, but different mass because of extra neutrons.



$^{13}\text{CO}_2$ constitutes only **1.1%** of the CO_2 in the atmosphere

III. Introduction (1)

3.1. The carbon stable isotope composition ($\delta^{13}\text{C}$)

Light isotope details

Natural abundance

$^1\text{H} = 99.985\%$

^2H (or D) = 0.015%

$^3\text{H} =$ not stable

$^{12}\text{C} = 98.89\%$

$^{13}\text{C} = 1.11\%$

$^{14}\text{C} =$ not stable

$^{16}\text{O} = 99.759\%$

$^{17}\text{O} = 0.037\%$

$^{18}\text{O} = 0.204\%$

Measurement techniques

Physical separation of masses:
Isotope ratio mass spectrometry

Optical absorption of different
vibrational frequencies:

New laser instruments

Delta notation

$$R = \frac{\text{heavy}}{\text{light}}$$

$$\delta = \left(\frac{R_{\text{amp}}}{R_{\text{std}}} - 1 \right) \times 1000$$

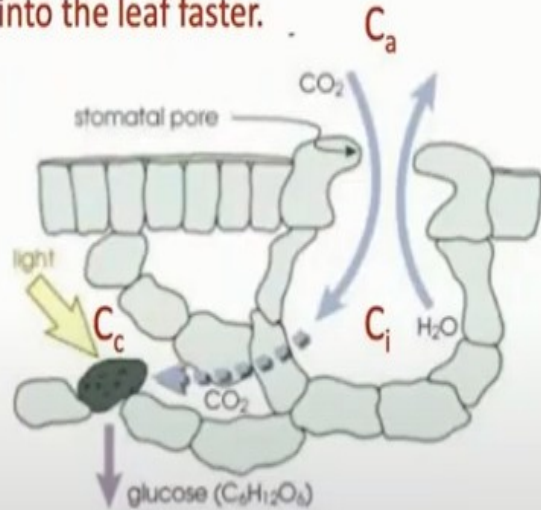
Expressed in per mil (‰)

H_2O 'types': $^1\text{H}_2^{16}\text{O}$, $^1\text{H}_2^{18}\text{O}$, $^1\text{H}^2\text{H}^{16}\text{O}$

CO_2 'types': $^{12}\text{C}^{16}\text{O}_2$, $^{13}\text{C}^{16}\text{O}_2$, $^{12}\text{C}^{18}\text{O}^{16}\text{O}$

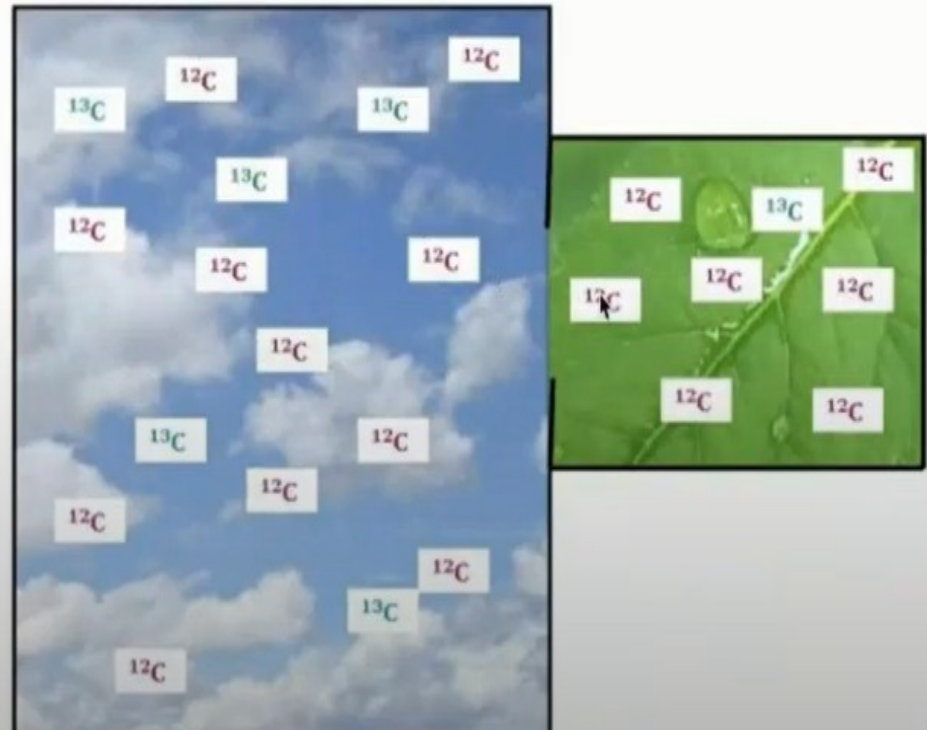
3.2. The principle and basis for the relationship between WUE (Water Use Efficiency) and $\delta^{13}\text{C}$

Lighter CO_2 diffuses into the leaf faster.



Lighter CO_2 reacts faster with Rubisco.

The atmospheric $^{13}\text{C}/^{12}\text{C}$ ratio is higher than the plant $^{13}\text{C}/^{12}\text{C}$ ratio



III. Introduction (1)

3.3. The carbon stable isotope composition ($\delta^{13}\text{C}$) is measured on EA_IRMS (elemental analyzer-isotope ratio mass spectrometry)



3.4. WUE (Water Use Efficiency)

- The WUE index was a concept, which was introduced 100 years ago by Briggs and Shantz (1913) and showed the relationship between crop yield and water use. Due to the changes of CO₂, water and temperature regimes were the most obviously seen at the leaf level.

What is the relationship between WUE and ¹³C?

$$\Delta^{13}\text{C} = 4.4 + 22.6 \left[\frac{{}^{13}\text{CO}_2(i)}{{}^{13}\text{CO}_2(e)} \right]$$

How are ¹³C data expressed?

- $\Delta^{13}\text{C} = \left(\frac{{}^{13}\text{CO}_2: {}^{12}\text{CO}_2}{\text{air}} \right) / \left(\frac{{}^{13}\text{CO}_2: {}^{12}\text{CO}_2}{\text{plant}} \right)$
 - Inversely proportional to WUE
- $\delta_{\text{V}}^{13}\text{C} = \left(\frac{{}^{13}\text{CO}_2: {}^{12}\text{CO}_2}{\text{plant}} \right) / \left(\frac{{}^{13}\text{CO}_2: {}^{12}\text{CO}_2}{\text{standard}} \right)$
 - Directly proportional to WUE

What is the relationship between WUE and ^{13}C ?

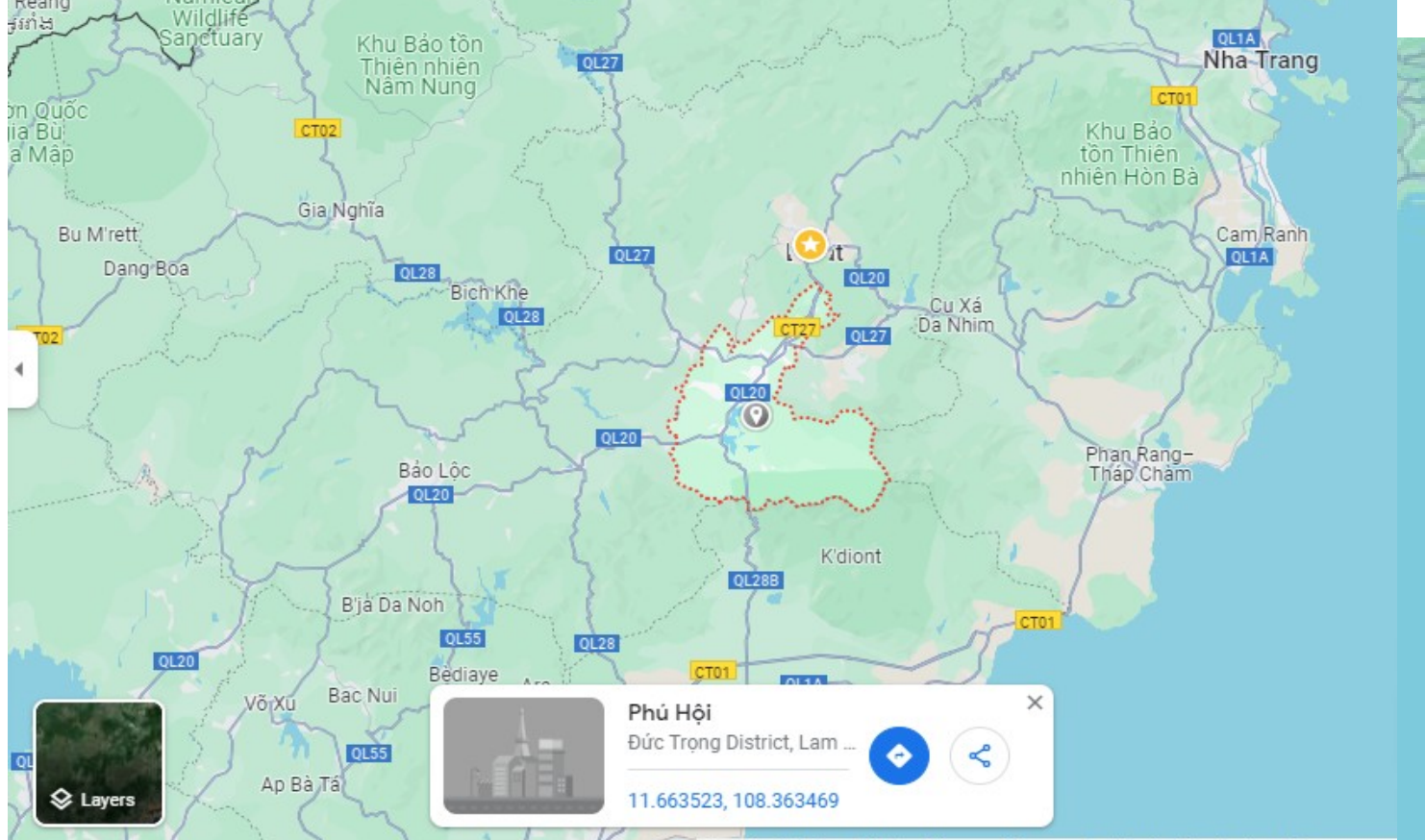
$$\Delta^{13}\text{C} = 4.4 + 22.6 \frac{^{13}\text{CO}_{2(i)}}{^{13}\text{CO}_{2(e)}}$$

How are ^{13}C data expressed?

- $\Delta^{13}\text{C} = (^{13}\text{CO}_2 : ^{12}\text{CO}_2)_{\text{air}} / (^{13}\text{CO}_2 : ^{12}\text{CO}_2)_{\text{plant}}$
 - Inversely proportional to WUE
- $\delta^{13}\text{C} = (^{13}\text{CO}_2 : ^{12}\text{CO}_2)_{\text{plant}} / (^{13}\text{CO}_2 : ^{12}\text{CO}_2)_{\text{standard}}$
 - Directly proportional to WUE

IV. Materials and Methods

(1) The design of the sampling area, collecting coffee leaf samples corresponded to the design of irrigation season and regime; and measuring temperature, rainfall, and humidity parameters in the area;



IV. Materials and Methods

(2) Testing and optimizing the procedure for analysis of $\delta^{13}\text{C}$ stable isotope in coffee leaf samples on the EA-IRMS system at the Dalat Nuclear Research Institute;

United States Geological Survey

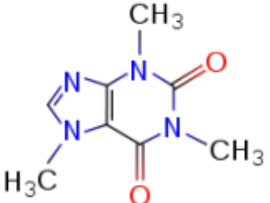
Reston Stable Isotope Laboratory

Report of Stable Isotopic Composition

Reference Materials USGS61, USGS62, and USGS63

(Hydrogen, Carbon, and Nitrogen Isotopes in Caffeine)

Recommended values: Stable hydrogen isotopic compositions are expressed herein as delta values [1] relative to VSMOW (Vienna Standard Mean Ocean Water) on a scale normalized such that the $\delta^2\text{H}$ value of SLAP (Standard Light Antarctic Precipitation) is -428‰ [2,3]. Stable carbon isotopic compositions are expressed herein as delta values relative to VPDB (Vienna Peedee belemnite) on a scale normalized such that the $\delta^{13}\text{C}$ values of NBS 19 calcium carbonate and LSVEC lithium carbonate are $+1.95\text{‰}$ and -46.6‰ , respectively [4]. Stable nitrogen isotopic compositions are expressed relative to atmospheric nitrogen, which is isotopically homogenous [5]. On this scale, the $\delta^{15}\text{N}_{\text{AIR-N}_2}$ value of USGS32 KNO_3 is $+180\text{‰}$ exactly. Stable hydrogen-, carbon-, and nitrogen-isotope delta values of USGS61, USGS62, and USGS63 caffeines with combined standard uncertainties are:

Reference	Structure	$\delta^2\text{H}_{\text{VSMOW-SLAP}}$	$\delta^{13}\text{C}_{\text{VPDB-LSVEC}}$	$\delta^{15}\text{N}_{\text{AIR-N}_2}$	Data source
USGS61		$+96.9 \pm 0.9\text{‰}$	$-35.05 \pm 0.04\text{‰}$	$-2.87 \pm 0.04\text{‰}$	[6]
USGS62		$-156.1 \pm 2.1\text{‰}$	$-14.79 \pm 0.04\text{‰}$	$+20.17 \pm 0.06\text{‰}$	[6]
USGS63		$+174.5 \pm 0.9\text{‰}$	$-1.17 \pm 0.04\text{‰}$	$+37.83 \pm 0.06\text{‰}$	[6]

IV. Materials and Methods

(3) Analyzing $\delta^{13}\text{C}$ stable isotope in coffee leaf samples in Lam Dong (about 90 samples);

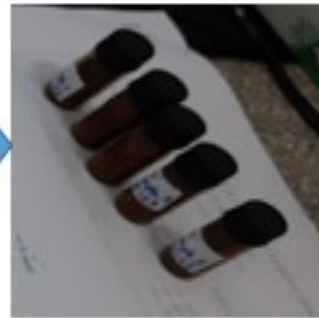
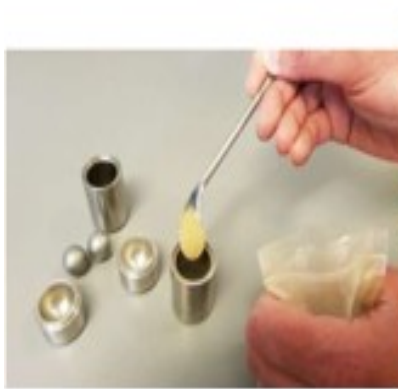


Vườn trồng cà phê thực nghiệm và chủng 102002 tại Bắc Tĩnh, Lâm Đồng

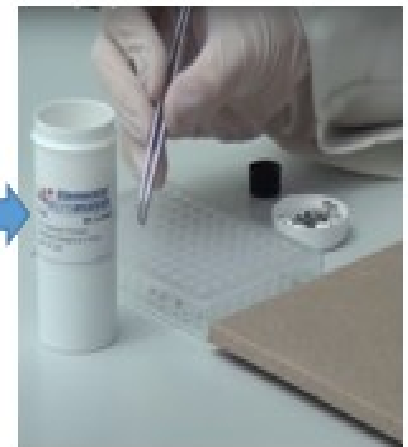
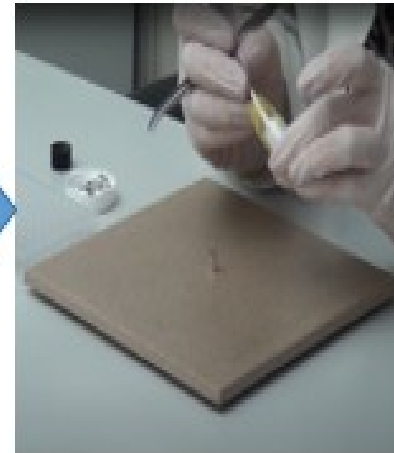


Kiểm soát lượng nước tưới tại các cây cà phê được chọn nghiên cứu

Procedure for handling and preparing samples for leaves according to instructions (IAEA-TECDOC-1870). A) Sample processing



WEIGHING SAMPLES AND PREPARING SAMPLE FOR EA-IRMS ANALYSIS AT THE LABORATORY Procedure for handling and preparing samples for leaves according to instructions (IAEA-TECDOC-1870). B) Prepare measurement samples





Chuẩn bị mẫu phân tích thành phẩm đồng vị ^{13}C

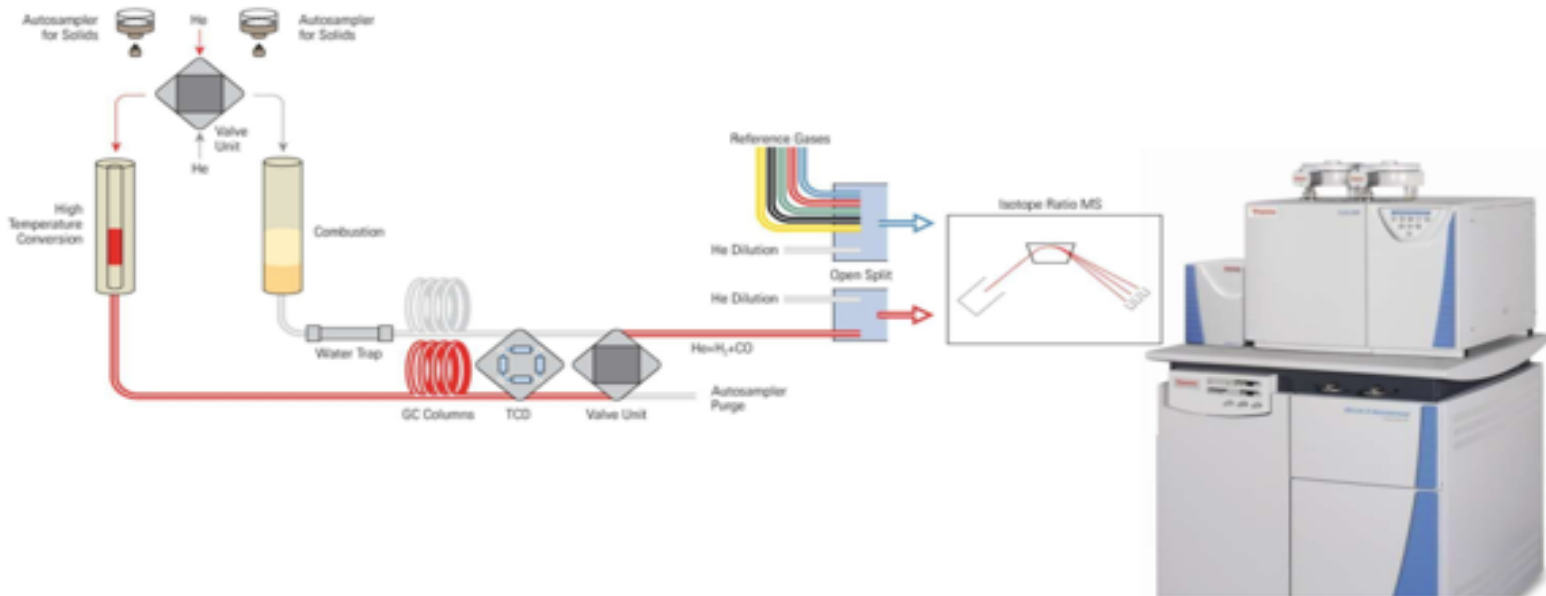


Hệ thiết bị phân tích đồng vị ^{13}C tại Viện Nghiên cứu thực phẩm

Elemental Analyser Isotope Ratio Mass Spectrometry (EA-IRMS)

Diagram of the EA-IRMS stable isotope ratio analysis measurement system at the Da Lat Nuclear Research Institute

Elemental Analyzer (EA) coupled to an Isotope Ratio Mass Spectrometer (IRMS)



Hình 1.11. Sơ đồ nguyên lý hệ đo phân tích tỷ số đồng vị bền EA-IRMS tại Viện Nghiên cứu hạt nhân Đà Lạt.

IV. Materials and Methods

Using this stable isotope data for the water use efficiency index calculation of coffee plants and the influence of weather condition factors (temperature, rainfall and humidity) on coffee plants water loss;

$$WUE = \frac{b - \Delta^{13}C}{1.6(b - a)}$$

Where a, b are constants according to the environmental conditions of the area; $\Delta^{13}C$ is carbon isotope discrimination;

IV. Materials and Methods

(5) Evaluating the possibility of using $\delta^{13}\text{C}$ stable isotope technique in climate change research (calculation of water use efficiency index) for coffee plants following 2 seasons (dry and rainy season).

V. Results & Discussion :

1. The procedure for the analysis of $\delta^{13}\text{C}$ in coffee leaf samples in particular and applying to the other plant samples in general on the EA-IRMS system was studied and established at the Dalat Nuclear Research Institute.
2. The analytical procedure involves the sample treatment and preservation, preparation of measured sample, the analysis of prepared samples, and the results calculation and evaluation. Various technical parameters for the sample running method on the measuring system were optimized including oven temperature, carrier gas speed, analysis run time, and dilution of standard gas. This analytical procedure was written in the form of Vietnamese Standards and was approved by the scientific council of the Dalat Nuclear Research Institute.
3. The quality control process was based on isotope standard ratio samples USGS61, USGS62, USGS63, and content standards sample Sulfanilamide.

Table 1. Results of evaluating standard samples USGS61, USGS62 and USGS63 on EA-IRMS in Dalat Nuclear Research Institute.

Name	Sample type	N	%C (%)	SD (%)	%C (Cert)	$\delta^{13}\text{C}$ (‰)	SD (‰)	$\delta^{13}\text{C}$ (Cert)	Z-score (%C)	Z-Score ($\delta^{13}\text{C}$)
Sulphanilamide	Content standard	5	41,78	0,94	41,81				-0,03	
USGS 61	Isotope standard	18				-34,999	0,063	-35,05		0,80
USGS 62	Isotope standard	24				-14,765	0,058	-14,79		0,42
USGS 63	Isotope standard	6				-1,207	0,160	-1,17		-0,23

V. Results & Discussion :

7. To apply the WUE index assessment, the project collected and analyzed $\delta^{13}\text{C}$ and carbon content values of 90 coffee leaf samples in the experimental garden in Duc Trong District, Lam Dong Province, corresponding to different irrigation regimes (100, 200, 300, 400 liters and no irrigation) and seasons (Dry season: January, February, March and April 2023; Rainy season: July, and October 2023).
8. The $\delta^{13}\text{C}$ value changed from -29 to -26.5 ‰;
9. Carbon content values were from 42 – 48%.

For C_3 plants (coffee plants), there was a range of $\delta^{13}\text{C}$ value published in literatura from -34 to -21 ‰;

According to Sayak Basu (2015), the results values of present study are fit well.

Table 2. Correlation processing results between the variables Temperature (T), humidity (H), precipitation (R) of C and WUE

	T (°C)	H (%)	R (mm)	C (%)	$\delta^{13}\text{C}$ (‰)	WUE
T (°C)	1					
H (%)	0.299	1				
R (mm)	0.415	0.790	1			
C (%)	-0.067	-0.277	-0.482	1		
$\delta^{13}\text{C}$ (‰)	0.413	0.417	0.395	-0.080	1	
WUE	0.413	0.417	0.395	-0.080	1.000	1

V. Results & Discussion :

- Based on meteorological data (temperature, humidity and precipitation) and the $\delta^{13}\text{C}$ and carbon content data of 90 coffee leaf samples the calculation of $\Delta^{13}\text{C}$ and WUE at the investigated location was carried out, the statistical test results were given to determine the correlation of $\delta^{13}\text{C}$, carbon content, WUE with weather index values.
- Water use efficiency index and $\delta^{13}\text{C}$ were positively correlated with temperature ($T^{\circ}\text{C}$), humidity (H%), and annual precipitation (Rmm) while carbon content was negatively correlated.

- **The results:** The methods to calculate water use efficiency index for crops corresponding to the growing season were developed (in below Figure). The results showed that in the rainy season, the water use efficiency index was nearly 14% higher than that in the dried season; In the dry season WUE was 0.19 whereas in the rainy season, the amount of water in plants increased with the WUE index (0.22) and p-value = 0.017.

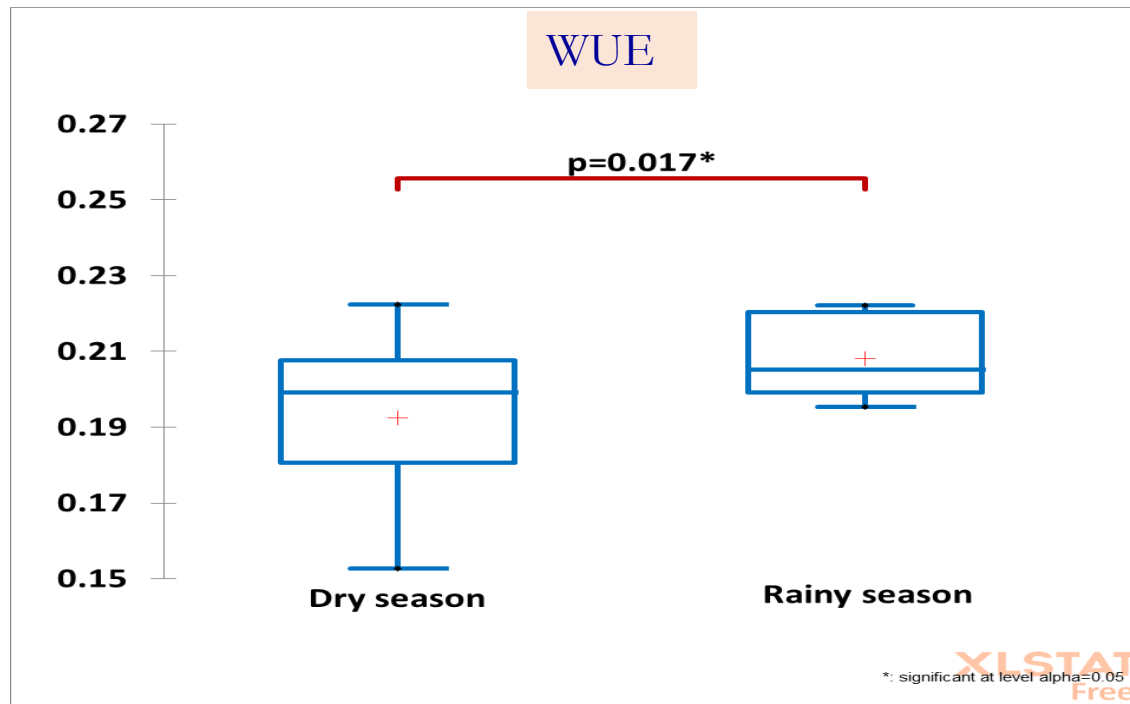


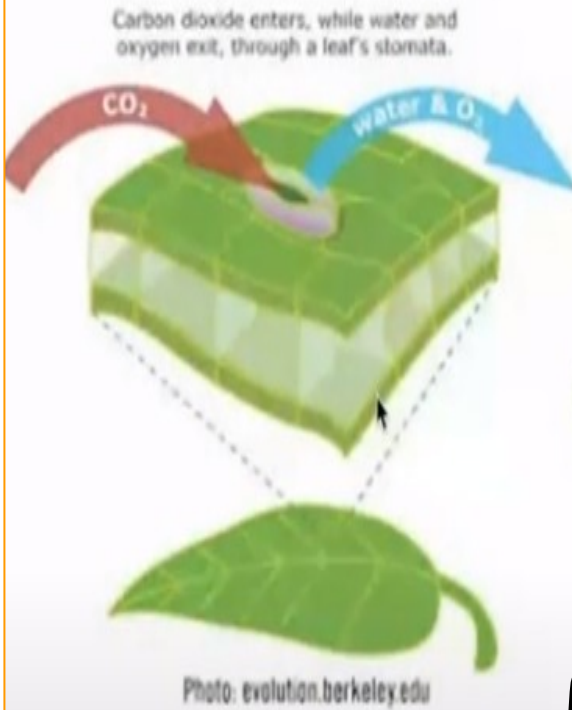
Figure 1. Results of the statistical tests for WUE following dry and rainy seasons.

VI. Conclusion

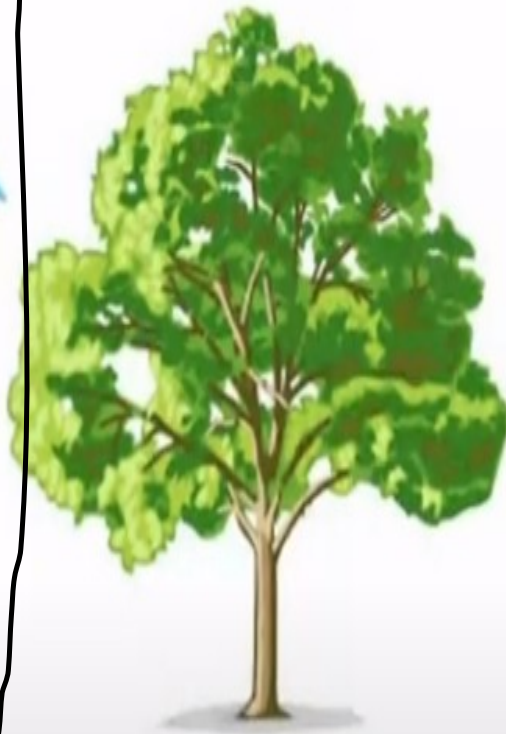
- The water use efficiency index, which was changed by the care period, tree growth and harvesting of the tree was determined. In the dry season, the WUE value was ~ 0.153 and increased to about ~ 2 in the rainy season.
- This study only investigated the method to determine WUE based on $\delta^{13}\text{C}$ of leaves. Surveying and evaluating the water use efficiency of coffee trees in Lam Dong in particular, and the Central Highlands, in general needs to be conducted with different varieties, ages, climate and weather conditions.
- Further study may provide a better understanding and an overall picture of the most effective irrigation regime and calculation of the most optimal water use efficiency of the coffee garden types.

WUE?

Level 1



Spatial scale: Leaf



Whole plant



Ecosystem or field

**Thank you for your
attention!**