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Carbon Isotope Discrimination in Accessions of *Urginea indica*, (*Kunth*) and *Urginea wightii* (*Lakxmin*), Hyacinthaceae

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ABSTRACT

The isotopic ratio of ^{13}C to ^{12}C in plant tissue is less than the isotopic ratio of ^{13}C to ^{12}C in the atmosphere, indicating that plants discriminate against ^{13}C during photosynthesis. Variation in discrimination against ^{13}C during photosynthesis is due to both stomatal limitations and enzymatic processes. Carbon isotope discrimination provides an integrated measure of water-use efficiency, samples are easily collected, and processed, and large numbers of samples may be collected in diverse environments.

Stomata play an important role in both the CO_2 assimilation and water relations of trees. Therefore, stomatal traits have been suggested as criteria for selection of clones or genotypes which are more productive and have larger water-use efficiency (WUE) than others.

Both CO_2 uptake and water losses are affected by stomatal dimensions and aperture. Consequently, stomatal traits such as stomatal density, length and responsiveness are considered key determinants of plant growth and water balance.

In this work we are trying to analyse the stomatal number and usage of ^{12}C and ^{13}C carbon from environment in 15 different accessions of Urginea indica and Urginea wightii. This work can help us to distinguish the Urginea indica and U.wightii accessions that are more mesophytic and tend to develop xerophytic characteristics.

In most of the dry land accessions or rock and hilly environment habituated accessions of U.indica showed low values of ^{13}C and their water usage is also less in them showing low stomatal numbets, indicating low utilisation of ^{13}C which is a tendency of dry land or xerophytic adaptation or C-4 pathway.

In those Urginea accessions that grow with moderate and more water (high rainfall) have more number of stomata and the values of ^{13}C is high. This is an indication of mesophytic habitat because it shows the tendency towards C -3 plants.

Key words: *Carbon discrimination, Water use efficiency (WUE), C-4 pathway, Urginea indica , Urginia wightii*

INTRODUCTION

There are two naturally occurring stable isotopes of carbon, $^{13}C/^{12}C$. Most of the carbon is ^{12}C (98.9%), with 1.1% being ^{13}C. The isotopes are unevenly distributed among and within different compounds, and this isotopic distribution can reveal information about the physical, chemical, and metabolic processes involved in carbon transformations. The overall abundance of ^{13}C relative to ^{12}C in plant tissue is commonly less than in the carbon of atmospheric carbon dioxide, indicating that carbon isotope discrimination occurs in the incorporation of CO_2 in to plant biomass. Because the isotopes are stable, the information inherent in the ratio of abundances of carbon isotopes, presented by convention as $^{13}C/^{12}C$, is invariant as long as carbon is not lost. Numerous contributions have been made to our understanding of carbon isotope discrimination in plants since this area was extensively reviewed by O'Leary (1997). Here we discuss the physical and enzymatic bases carbon isotope discrimination during photosynthesis, noting how knowledge of discrimination can be used to provide additional insight into photosynthetic metabolism and the environmental influences on that process.

Theoretical and empirical studies have demonstrated that carbon isotope discrimination is highly correlated with plant water use efficiency. Analysis of carbon isotope discrimination has conceptual and practical advantages over measuring water use efficiency by instantaneous measurements of gas exchange or whole-plant harvests.

The fact that all plants discriminate against ^{13}C in photosynthesis has been well documented. The range for 8013 relative to the Pee Dee belemnite PDB) standard of the Univ. of Chicago has been established as -20 to -30%0, an average of -25%, for wood and most terrestrial plant materials.

The isotope-ratio mass spectrometer (IRMS) allows the precise measurement of mixtures of naturally occurring isotopes. Most instruments used for precise determination of isotope ratios are of the magnetic sector type .

An isotope ratio mass spectrometer (IRMS) is an instrument that measures the ratios of different isotopes of particular elements (Fig - 1). All elements have isotopes that differ from one another only in the number of neutrons in the nucleus, giving them different atomic weights.

The simplest case to analyse is that of diffusive entry of CO_2 followed by C3 metabolism.C3 metabolism means that 295% of the inorganic C is fixed by the CO_2-using RUBISCO (ribulose bisphosphate carboxy- lase-oxygenase) while the remaining 15% of the C found in the accumulated organic C in the plant is fixed by anaplerotic carboxylases. The main anaplerotic carboxylase contributing to harvested organic C is one which catalyses the production of a C4 dicarboxylic acid by a C3+C1 reaction. The enzymes involved are PEPC (phosphoenolypyruvate carboxylase) in the Chlorophyta and higher plants as well as the Rhodophyta, cyanobacteria and some Dinophyta and Haptophyta[4].

Species that occupy large geographic ranges or a variety of habitats within a limited area deal with contrasting environmental conditions by genotypic and phenotypic variation[6].

Stomata play an important role in both the CO_2 assimilation and water relations of trees. Therefore, stomatal traits have been suggested as criteria for selection of clones or genotypes which are more productive and have larger water-use efficiency (WUE) than others. However, the relationships between plant growth, WUE and stomatal traits are still unclear depending on plant material (genus, species, families, genotypes) and, more precisely, on the strength of the relationships between the plants[5].

In the present study, the correlations between these stomatal traits were compared and related with carbon isotope discrimination among different accessions of *Urginea indica*.

Stomata play an important role in both the CO_2 assimilation and water relations of plants.

Therefore, stomatal traits have been suggested as criteria for selection of clones or genotypes which are more productive and have larger water-use efficiency (WUE) than others. However, the relationships between plant growth, WUE and stomatal traits are still unclear depending on plant material (genus, species, families, genotypes) and, more precisely, on the strength of the relationships between the plants as previously conducted by Sophie *et.al.*,[1]. Both CO_2 uptake and water losses are affected by stomatal dimensions and aperture. Consequently, stomatal traits such as stomatal density, length and responsiveness are considered key determinants of plant growth and water balance. Evidently, environmental factors (light, temperature, vapour pressure deficit, etc.) also play a role (Dillen *et al.,* 2008 — Genetic Variation of Leaf Traits in Poplar)

Carbon isotope discrimination provides an integrated measure of water-use efficiency, samples are easily collected, and processed, and large numbers of samples may be collected in diverse environments. Moreover, in woody plants, carbon isotope discrimination can be determined on annual ring samples, providing a historical analysis of plant response to environmental conditions.

In this work we are trying to analyse the stomatal number and usage of ^{12}C and ^{13}C carbon from environment in 15 different accessions of *Urginea indica* and *Urginea wightii.* This work can help us to distinguish the *Urginea indica* and *U.wightii* accessions that are more mesophytic and tend to develop xerophytic characteristics. It also gives information on evolutionary trends in *Urginea indica* and *U. wightii* that might be getting to C-4 plants although showed no Krantz anatomy.

This is altogether a new approach to study a wild plant species for its physiological behaviour, speciation and evolutionary trends among different accessions of single species of *Urginea indica* and *Urginea wightii.*

MATERIALS AND METHOD

Stomatal measurements

Adaxial and abaxial imprints of epidermis at maximum leaf width near the central vein of the first fully expanded leaf were made using clear nail varnish and adhesive cello-phane tape (Ceulemans *et al.*, 1995). All imprints were fixed onto glass microscope slides and examined at 100 magnification using a digital camera (Olympus MJU1010 Digital still Camera, I-Chrome , Tokyo, Japan.) attached to microscope LAB. EN Stereo zoom lense, . micro-scope (Japan). At least ten microscopic fields from each imprint of the adaxial and abaxial surfaces were randomly sampled and photographed. The average number of stomata was calculated for each imprint and then for each replicate. Stomatal density was defined as the number of stomata per unit of leaf area (mm^2) and was determined as below.

1. A thin layer of tissue from the underside of a leaf is peeled carefully.
2. The leaf tissue was laid on the microscope slide. Few drops of water was placed over the tissue, then cover slip on it was glided on it.
3. The slide was mounted on microscope on the stage of light microscope and observed the leaf tissue under a magnification of 400 times. Stomata were visible among the epidermal cells. (As *Urginea* show Isobilateral leaf only on lower epidermal leaf peel was collected as similar number of stomata were available on both epidermis)
4. Counted the number of stomata within 10 grid squares of cover slip. For example, when we found 40 stomata in 10 grid squares.
5. Calculated stomatal density by dividing the number of stomata we counted by 10 times the area of 1 grid square.

For example:

Stomatal Density = Number of Stomata / (10 x area of 1 grid square)

Stomatal Density = 40 / (10 x 0.36 square millimeters (mm^2)) = 11.1 stomata per $mm^{2.}$

Carbon isotope discrimination

Oven-dried foliar samples (leaves following the first fully expanded leaf, 150 leaves for each accession of *Urginea indica* and *U. wightii*) were ground to fine powder for analysis of Carbon isotope composition $\delta\ ^{13}C$ carbon isotope composition using a continuous flow isotope ratio mass spectrometer (Delta - V Advantage – Thermo Scscher Scientifics, Bremen- Germany) interphased with a continuous flow device (Con flow-III) for high throughput measurements.

A sample of oven-dried, powdered foliar sample was crimpled in a silver cup and used for the analysis. This Internal standard was pre-calibrated using the international Pee Dee Belemnite standard (Farquhar et al., 1989): and ANU (Austrelian National University) Sucrose.

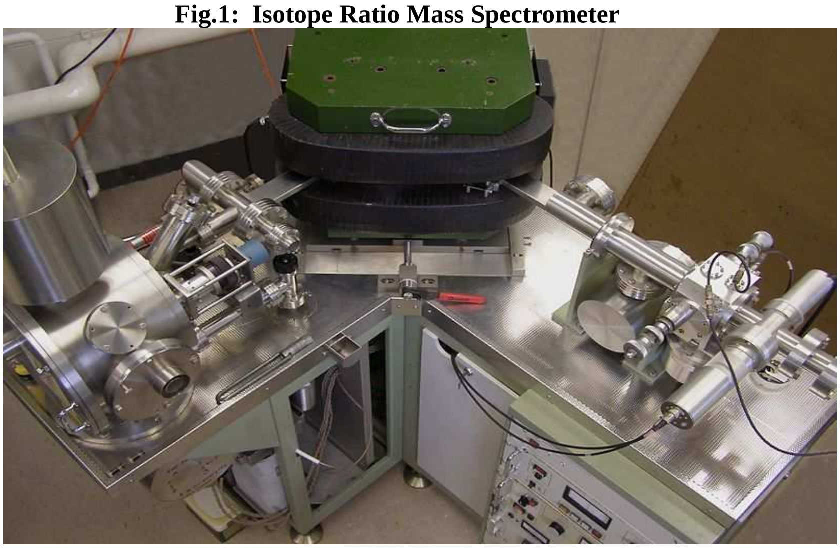
Carbon isotope composition (d) was calculated relative to the international Pee Dee Belemnite standard (Farquhar et al., 1989): dplant¼(Rsa–Rsd)/Rsd 1000 [‰] where Rsa and Rsd are the ^{13}C - ^{12}C ratios of the sample and the standard, respectively (Craig, 1957), where R is the ratio of ^{13}C - $^{12}C^2$.

RESULTS

Among 14 accessions selected from various parts of India $\delta\ ^{13}C$ % values varied as given in the table – 1, Graph – 1 and the amount of annual rainfall with the number of stomata in leaves is given in Table – 2 and Graph – 2 .

- Accessions of *U.indica* that are collected from Bellary, Bettahalli, Kerala, Banganawadi, Gopalaswamy betta, Gorur are showing low utilisation of ^{13}C and accessions of *U. wightii* collected from Gulbarga also showing low utillization of ^{13}C .
- Where as the accessions of *U.indica* collected from Ramanagara, Seethampundi, Udupi and Bellur along with u.wightii accession from Shimoga are showing higher values of ^{13}C indicating more utilisation of ^{13}C.
- In second group of experiments (table – 2, Graph – 2) the ^{13}C values were compaired to the average rainfall and the number of stomata in given area of leaf in all accessions.
- Accessions of *U.indica* showing more number of stomata are collected from Ramanagara, Seethampundi, Kerala and Udupi also from *U wightii* Shimoga.
- Accessions showing less number of stomata in *U.indica* are from Ranganathittu, Bellary, Bettahalli, Banganawadi, Gopalaswamy betta and Nagamangala.
- The annual rainfall recieved by the accessions with low stomatal number is less where as those accessions that grow at high rainfed areas show more number of stomata.

Fig.1: Isotope Ratio Mass Spectrometer



Graph – 1: Graph showing different accessions of *Urginea indica* and their utilisation of carbon ^{13}C

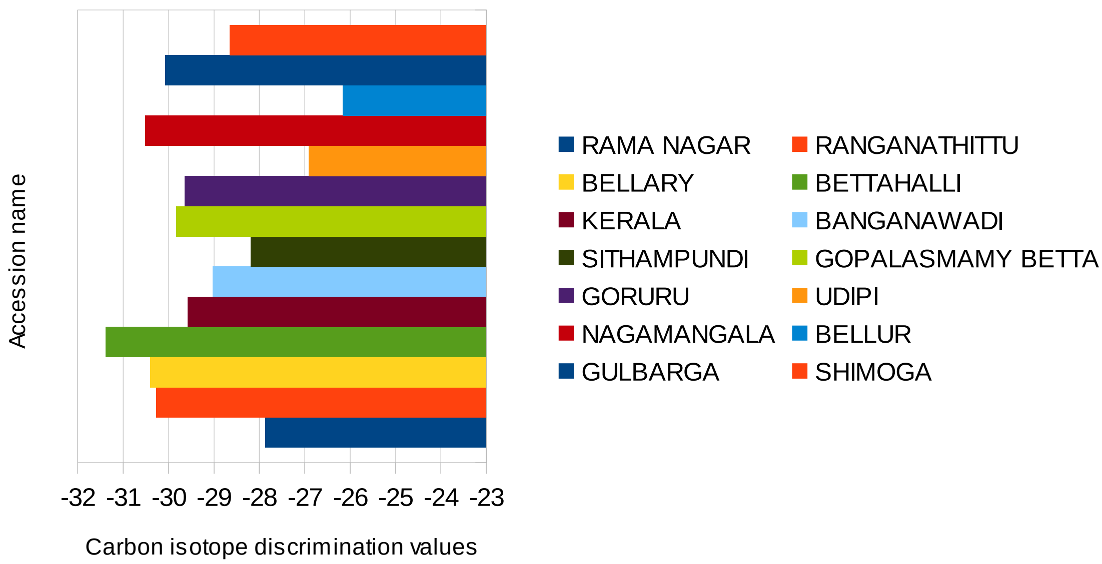


Table – 1: Table showing carbon isotope discrimination in accesions of *Urginea indica* and *U. wightii*

No.	Accession No.	Name of Accessions	δ ^{13}C %0
1	835	RAMA NAGAR	- 27.865
2	804	RANGANATHITTU	- 30.269
3	851	BELLARY	- 30.398
4	843	BETTAHALLI	- 31.381
5	842	KERALA	- 29.573
6	815	BANGANAWADI	- 29.024
7	846	SITHAMPUNDI	- 28.1828
8	807	GOPALASMAMY BETTA	- 29.831
9	814	GORURU	- 29.637
10	840	UDIPI	- 26.91
11	831	NAGAMANGALA	- 30.517
12	828	BELLUR	- 26.163
13	825	GULBARGA (*U. wightii*)	- 30.071
14	802	SHIMOGA (*U.wightii*)	- 28.647

Graph – 2: Graph showing relationship between carbon isotope discrimination and number of stomata in leaf

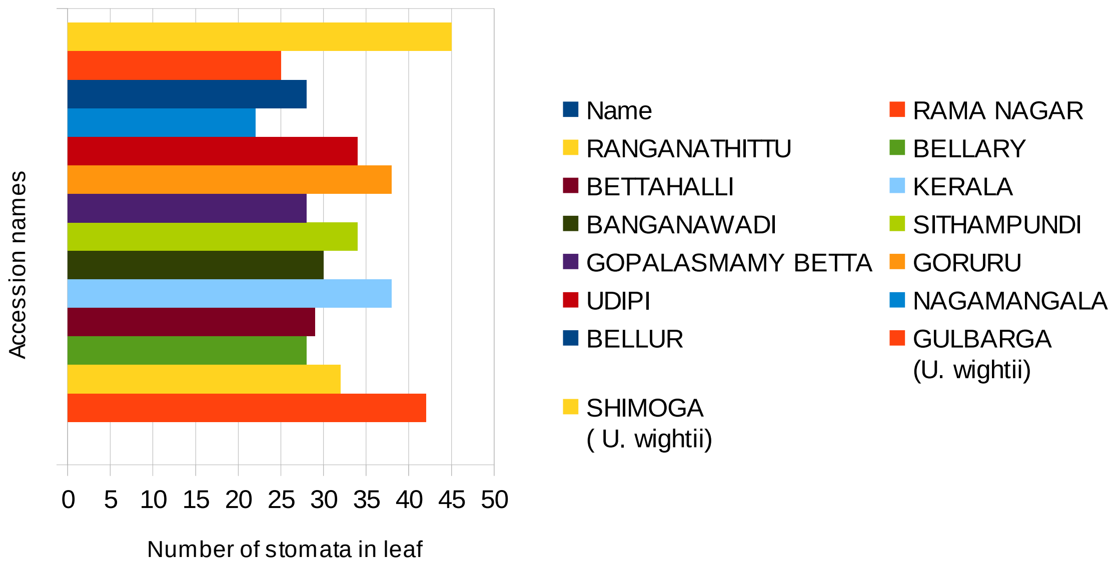


Table – 2: Table showing numer of stomata different accessions of *U. indica* and *U. wightii* compaired with Annual rainfall and Carbon isotope discrimination

No.	Accession No.	Name	Number of stomata	Average rainfall	δ ^{13}C %0
1	835	RAMA NAGAR	42	Moderate	- 27.865
2	804	RANGANATHITTU	32	Heavy	- 30.269
3	851	BELLARY	28	Low	- 30.398
4	843	BETTAHALLI	29	Low	- 31.381
5	842	KERALA	38	High	- 29.573
6	815	BANGANAWADI	30	High	- 29.024
7	846	SITHAMPUNDI	34	Moderate	- 28.182
8	807	GOPALASMAMY BETTA	28	High	- 29.831
9	814	GORURU	38	High	- 29.637
10	840	UDIPI	34	High	- 26.91
11	831	NAGAMANGALA	22	Low	- 30.517
12	828	BELLUR	28	Low	- 26.163
13	825	GULBARGA *(U. wightii)*	25	Low	- 30.071
14	802	SHIMOGA *(U. wightii)*	45	High	- 28.647

CONCLUSIONS

Carbon isotope discrimination have been discussed in two species of Urginea namely *U.indica* and *U.wightii.*

- The accessions collected in moderate and with high rainfed areas show more utilisation of carbon isotope ^{13}C, where as those that grow in low rainfall show less utilization of ^{13}C more discrimination to ^{13}C.
- The accessions of *U.indica* that use more ^{13}C are , Ramanagara, Seethampundi and Bellur and the accessions from *U. wighti* like Shimoga. These values were correlated to the number of stomata in a given part of leaf. Their stomatal number is higher that also indicate their geographic adaptation for mesophytic habitat.
- But accession Udupi shows less stomatal number but recieve higher annual rainfall. Its usge of ^{13}C is also high. It shows mesophytic habitat but less number of stomata might be indicating other physiological characters that may be responsible for this .
- Accessions collected from areas of lower annual rainfall like Bellary, Bettahalli, Gopalaswamy betta and Nagamangala of *U.indica* and that of Belluru from *U. wightii* show low usage of ^{13}C and less number of stomata that can be directly correlated to their habitat. These accessions are indicating their transition to xeric conditions and may lead to C-4 pathway.
- In *U.indica* accessions there is variability with respect to their geographic locations, that matches the

annual rainfall and their number of stomata. At the same time there is a variability among the accessions in terms of carbon isotope discrimination.

- Similarly the results of *U.wightii* accessions aslo show the variability in their stomatal number and carbon isotope discrimination.
- To summerise the germplasm of *Urginea* shows clear variation with respect to carbon discrimination which plays important role in its physiology of photosynthesis. But it also is related to amount of rainfall recieved and stomatal number in leaves.
- In most of the dry land accessions or rock and hilly environment habituated plants showed low values of ^{13}C indicating low utilisation of ^{13}C which is a tendency of dry land or xerophytic adaptation or C-4 pathway.
- In the plant accessions that grow with moderate and more water (high rainfall) the values of ^{13}C is high. This is an indication of mesophytic habitat because it shows the tendency towards C -3 plants.
- Those that show less carbon isotope discrimination are Mesophytic accessions and adopted to C-3 pathway.
- Those accessions that show High carbon isotope discrimination are showing tendency for Xerophytic habitat and tendency to adopt C-4 pathway.

This is an inadequate experimental support to draw clear conclusions about physiological adaptibility of plants during evolution. A more systematic investigation needs to be conducted to exploit the advantage of stable isotope of carbon in identifying the habitat or geographical adaptibility in plants.
Further the work needs more intense action to work on the real habitat adaptation by growing various accessions of *Urginea indica* in dry and wet conditions to assess their physiological behaviour.

REFERENCES

1. Sophie, Y., Dillen, *et.al.*, Genetic Variation of Stomatal Traits and Carbon Isotope Discrimination in Two Hybrid Poplar Families, *Annals of Botany*, **102:** 399-407 (2008).
2. Farquhar, G.D., *et.al*., Carbon isotope discrimination and photosynthesis, *Annu. Rev. Plant Physiol. Plant Mol. Bio*., **40:** 503-37 (1989).
3. Bender, M.M., Mass spectrometric studies of carbon- 1 3 variations in com and other grasses, Radiocarbon, **10:** 468-72 (1968).
4. Bender, M.M., Variation in the l3C/l2C ratios of plants in relation to the pathway of photosynthetic carbon dioxide fixation, Phytochemistry, **10:** 1339-44 (1971).
5. Cowan, I .R., Stomatal physiology and gas exchange in the field. In Flow and Transport in the Natural Environment, ed. O. T. Denmead. New York: Springer-Verlag. In press (1988).
6. Abrams, M.D., Genotypic and phenotypic variation as stress adaptations in temperate tree species: a review of several case studies, Tree Physiol., **14(79):** 833-842 (1994).
7. Chliyeh, M., Touhami, A.O., Filali-Maltouf, A., Modafar, C.El., Moukhli, A., Oukabli, A., Benkirane, R. and Douira, A., Effect of a composite endomycorrhizal inoculum on the growth of olive trees under nurseries conditions in Morocco, *Int. J. Pure App. Biosci.* **2(2):** 1-14 (2014).
8. Sundar Raj, A.A., Samuel, J., Joshva, M., Ranganathan, T.V. and Tiroutchelvamae, D.,A Review – Recent Advances in Nutrition of Health Benefits and their Significance of Indian Spices in Foods, *Int. J. Pure App. Biosci.* **2(2):** 15-23 (2014).
9. Huh, M.K., Fluvial Landscape Ecology and Water Quality at the Jupo River, Korea *Int. J. Pure App. Biosci.* **3 (3):** 1-9 (2015).
10. Ouabbou, A., Saifeddine, El. K., Touhami, A.O., Benkirane R. and Douira, A., Study of some new Lepioteae for the Morocco's fungal flora, *Int. J. Pure App. Biosci.* **3 (3):** 28-34 (2015).
11. Zuraida, A.R., Erny Sabrina, M.N., Mohd Shukri, M.A., Razali, M., Norma, H., Wan Zaliha, W.S. and Ayu Nazreena, O., *In vitro* Micropropagation of a Valuable Medicinal Plant, Piper crocatum. *Int. J. Pure App. Biosci.* **3 (3):** 10-16 (2015).