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Leaf Anatomy of Herbal C_3, C_4, CAM, and Intermediate Plants from Arid and Semi-Arid Climate of Thar Desert

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ABSTRACT

Thar Desert is a home of a diverse array of flora utilizing various photosynthetic pathways—C_3, C_4, and intermediate species. How do local fluctuations in seasonal events such as temperature and rainfall affect the functional leaf anatomy will be a future question of study. Under ambient climate we present here leaf anatomical evidence for such plants as a repository for arid plant adaptations. We observed leaf anatomy of herbaceous plants like C_4-Dicot *Mollugo cerviana, Gisekia pharnaceoides, Tribulus* sp.*, Euphorbia* sp.*, Heliotropium* sp., and C_4-Monocot such as *Cenchrus* sp., *Brachiaria* sp.*, Lasiurus scindicus, Panicum* sp.*, Chloris* sp.*,* etc. for their exceptional strategies for coping with water scarcity during and after the brief rainy seasons. Their distinctive Kranz anatomy, well-developed bundle sheath cells, and unique chloroplast arrangements contribute to efficient CO_2 fixation, enabling them to thrive in arid climates. In contrast, C_3 plants such as Corbichonia decumbens*, H. curassavicum, Dipterygium glaucum* display distinctive leaf anatomies characterized by notable C_3 arrangements of palisade and spongy cells filled with chloroplasts. *M. nudicaulis, Parthenium hysterophorus, Farsetia hamiltonii* show intermediate C_3-C_4 and Portulaca grandiflora show C_4 and CAM leaf anatomies. This is the first anatomical record of Farsetia hamiltonii as C_3-C_4 intermediate based on leaf anatomy. The pictorial cataloguing of these plants' anatomical traits will shed light on evolutionary adaptations and aid in understanding how these plants respond to changing climatic conditions in the Thar Desert.

Key Words - : Kranz Anatomy; C_3-C_4 Intermediate; Leaf anatomy; Bundle Sheath; Desert Plants

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INTRODUCTION

Most plants rely on the ancient C_3 photosynthetic pathway, where atmospheric CO_2 is directly fixed by the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), initiating the Calvin–Benson–Bassham cycle (Alenazi *et al.,* 2023). Yet, Rubisco's ability to also bind O_2 initiates the energy-intensive photorespiratory pathway. To counter this, C_3 plants were gradually diversified into CAM and C_4 plants by elevating CO_2 concentration near Rubisco by following spatial (C_4 plants) and temporal (CAM) carbon concentration mechanism (CCM), enhancing photosynthetic efficiency. In C_4-CCM, the plants evolved to transport intermediate carbon compounds to bundle sheath cells whereas CAM-CCM plants fix the atmospheric carbon in night and stored in vacuole to avoid transpiration by keeping closed stomate in daytime (Mercado & Studer, 2022; Munekage & Taniguchi, 2022). This spatial isolation minimizes photorespiration Conversely, the intermediate C_2 pathway recycles photo-respired CO_2 via glycine shuttling to bundle sheath cells, while the more sophisticated C_4 pathway, featuring Kranz anatomy, mitigates photorespiration by initiating CO_2 fixation using phosphoenolpyruvate (PEP) carboxylase in mesophyll cells. This pathway produces a fourcarbon compound from PEP and CO_2, transferred to bundle sheath cells where all CO_2 fixation occurs in C_4 species (Mercado & Studer, 2022).

C_4 plants have a distinct leaf anatomy compared to C_3 plants, which significantly enhances their photosynthetic efficiency (Munekage & Taniguchi, 2022). A pivotal difference lies in the spatial separation of the CO_2 fixation and Calvin-Benson cycle processes within mesophyll and bundle sheath (BS) cells. In C_4 plants, BS cells take the lead in photosynthesis, while mesophyll cells focus solely on CO_2 fixation. This separation reduces carbon loss in mesophyll cells by notably reducing or eliminating RuBisCO expression there. Moreover, BS cells in C_4 plants are larger, house more chloroplasts, and maintain minimal to no RuBisCO, establishing a concentrated CO_2 environment that suppresses photorespiration. Their internal positioning within leaves further shields BS cells from atmospheric oxygen, sustaining the CO_2 to O_2 ratio and further curtailing photorespiration (Mercado & Studer, 2022; Munekage & Taniguchi, 2022). In earliest evidence, Haberlandt (Haberlandt 1882, 1914) identified the 'Kranz' arrangement of mesophylls in Cyperaceae and Gramineae families. The C_4 pathway's functionality requires metabolic cooperation between outer mesophyll (photosynthetic carbon assimilative) and inner bundle sheath (photosynthetic carbon reductive) tissues (Muhaidat *et al.,* 2007). Different patterns of mesophyll cell types in Chenopodiaceae have been identified, varying from non-Kranz-types to Kranz types (Jacobs 2001).

Examining how plants functionally adapt to changing environments often involves studying leaf anatomy. Leaves typically showcase arrangements of epidermal cells, mesophyll cells, bundle sheaths, and vascular bundles, which provide insights into the plant's physiology regarding photosynthesis (Pathare *et al*., 2020). Dicot leaves notably differ from monocot leaves in their arrangement of photosynthetic cells.

The study of leaf anatomy in plant populations within a specific area serves as a valuable tool for monitoring ecological shifts such as aridization, climate change, and the diminishing availability of water resources (Ivanova *et al.*, 2019). An increase in the prevalence of plant species exhibiting Kranz anatomy in their leaves is regarded as an indicator of heightened adaptation to dry, xerophilous habitats (Ivanova *et al.,* 2019; Heilmeier, 2019)

Here we are presenting leaf anatomical pictorial evidence of all four C_3, C_4, CAM and intermediate types of plants growing under ambient climate of Thar arid regions.

MATERIALS & METHODS

Twenty-seven (27) plant species encompassing C_3, C_4, CAM and intermediate photosynthetic pathways were collected from the local arid and semi-arid regions of Jodhpur and Jaisalmer during 2016 to 2019 (Table 1). Species selection was based on availability within their natural habitat at the time of collection. At least three mature leaves were collected from each individual plant.

Leaf sectioning and Staining

The leaves were first washed with clean water and submerged in the water till use. Each leaf was cut for thin transverse sections by using a sharp razor such as blade. Hand-sections of the leaf with a uniform thickness of 0.02-0.03 mm were selected for staining. The sections were stained with either a 0.01% dilution of safranin (HiMedia©) or Toluene blue (HiMedia©).

Microscope Analysis

The stained sections were mounted in 50% Glycerin and observed under compound Microscope (Olympus) with 100X magnification. Images of leaf anatomy were captured using a digital camera (Canon) attached to the microscope.

RESULTS & DISCUSSION

The western Thar Desert represents one of India's hottest regions characterized by low rainfall, intense temperatures, significant day-night temperature fluctuations with minimal humidity for more than half months of the year. Over the last 50 years, anthropogenic activities and global climate change have significantly impacted the desert's natural ecosystem, leading to the proliferation of weedy species during the rainy season. During peak floral diversity in the monsoonal season, these ephemeral plants complete their life cycle rapidly. The flora in this area faces various stresses, and these stresses are likely reflected in leaf functional anatomy. This highlights the resilience of desert flora, which has adapted to these challenging conditions through various mechanisms, including C_4 photosynthetic pathways (Tian *et al.,* 2016; Guo *et al.,* 2017).

Dominance of C_4 Anatomy and Variations

Our study found a predominance of C_4 leaf anatomy among the investigated plants, which align with evolutionary adaptation of desert plants to arid environments. These plants often employ carbon concentration mechanisms (CCM) for efficient photosynthesis and water use efficiency.

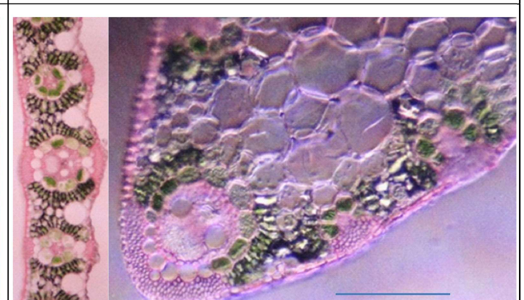
This finding aligns with established knowledge – C_4 plants dominant in grass species, with their characteristic Kranz anatomy (bundle sheath encircling vascular bundles), are particularly suited for xeric climates due to their enhanced water use efficiency and CO_2 fixation rates (Guo *et al.,* 2017). While grasses comprise roughly 60% of all C_4 plants, i.e. around 7500 species across various families (Sage, 2004). C_4 anatomy extends beyond this group. We also recorded C_4 anatomy in species of Cyperaceae, a family within the Poales order and in numerous eudicot families such as Asteraceae, Brassicaceae, Euphorbiaceae, and families within the extend of Caryophyllales order (Huang *et al*., 2022). It is observed that these C_4 anatomies differ in the structure and arrangement of the dual layered chlorenchyma adjacent to vascular bundles. These variations involve features like water storage tissue, hypodermal cells, sclerenchyma, and continuous or interrupted Kranz tissue (Fig 1 and 2). Such anatomical disparities within C_4 plants reflect adaptations to diverse environmental conditions and resource availability. For instance, grass species like L. scindicus and P. turgidum showcase enlarged bundle sheath cells, while perennial grasses often possess a higher count of bulliform cells (Fig 1 and 2). Apart from differences in mesophyll and bundle sheath tissues, three biochemical subtypes exist in C_4 photosynthesis based on the principal decarboxylation enzyme used in the Bundle Sheath: NADP-malic enzyme (NADP-ME), NAD-malic enzyme (NAD-ME), and Phosphoenolpyruvate carboxykinase (PCK). PEPC, the initial enzyme common to all three subtypes, resides in mesophyll cells (Hatch, 1987; Kanai & Edwards, 1999). The distribution of these subtypes is significantly correlated with aridity and human domestication. NAD-ME species tend to dominate in arid C_4 vegetation, while NADP-ME species prevail in moister environments. Most of our domesticated majority of C_4 row crops, pasture plants, and weeds are NADP-ME type (Wang *et al*., 2014). Furthermore, dicot plants with C4 anatomy typically exhibit centripetally arranged chloroplasts in bundle sheath cells (Fig 2), while C_4 grasses showcase differing chloroplast arrangements based on their biochemical subtype (Fig 1).

Additionally, leaf cross-sections contain mesophyll cells, primary sites of photosynthesis, comprising palisade (upper side) and spongy (lower side) cell types. C4-CAM intermediate *P. grandiflora* a small herbaceous annual plant, shows the C_4 photosynthetic pathway with characteristic of Pilosoid-type Kranz leaf anatomy (Fig 2). *Parthenium hysterophorus*, *M. nudicaulis*, and *Farsetia hamiltonii* exhibited C_3-C_4 intermediate anatomical features, indicating a transitional state between C_3 and C_4 pathways (Fig 2). Notably, *P. grandiflora* displayed C_4-CAM intermediate anatomical characteristics. The key finding of the observations is identification of Farsetia hamiltonii as a new member of C_2 type plants (C_3-C_4 intermediate) based on preliminary anatomical features. As per the literature survey, the plant is not recorded for its photosynthetic type. The leaf anatomy of F. hamiltonii shows the presence of bundle sheath around vascular bundles filled with chloroplasts arranged in both centripetal and centrifugal patterns, alongside typical C_3 leaf anatomy characteristics such as differentiation of mesophyll cells into palisade and spongy cells (Fig 3). However, other evidence such as photosynthesis rate for the CO_2 compensation point, C^{13} radio labelling for carbon trace in Bundle sheath, and TEM for chloroplast arrangement in bundle sheath will be further required confirm C_2 physiology in this plant.

Table1- Plant species selected for Leaf anatomy repository

Species	Family	Local Name	Type
Monocot			
Pennisetum glaucum (L.) R. Br	Poaceae	Bajra	C_4
Chloris virgata Sw.	Poaceae	jaranio	C_4
Brachairia reptans (L.) C.A. Gardner & C.E. Hubb.	Poaceae	Murat	C_4
Brachairia remosa (L.) Stapf	Poaceae	Murat	C_4
Lasiurus scindicus Henrard	Poaceae	Sewan	C_4
Panicum turgidum Forssk	Poaceae	Switch grass	C_4
Dichanthium annulatum (Forssk.) Stapf	Poaceae	Marvel grass	C_4
Cenchrus setigerus Vahl	Poaceae	Bhurut	C_4
Oryza sativa L.	Poaceae	Rice; Chawal	C_3
Triticum aestivum L.	Poaceae	Wheat, Genhu	C_3
Cyperus rotendus L.	Cyperaceae	Java grass	C_4
Dicots			
Mollugo cerviana (L.) Ser.	Molluginaceae	Chiria-ro-khet	C_4
M. nudicaulis Lam.	Molluginaceae	Naked Stem Carpetweed; Paprastaka	C_3-C_4
Parthenium hysterophorus L	Asteraceae	Santa-Maria, Gajar Ghans	C_3-C_4
Farsetia hamiltonii Royle	Brassicaceae	Hiran Chabo	C_3-C_4
Gisekia pharnaceoides Linn.	Gisekiaceae		C_4
Corbichonia decumbens (Forrssk.) Exell	Lophiocarpaceae	Carpet-Weed	C_3
Tribulus terrestris L.	Zygophyllaceae	Chhota Gokhru	C_4
T. rajasthensis Bhandari & V.S.Sharma	Zygophyllaceae	Chhota Gokhru, Kanti	C_4
Dipetrygium glaucum Decne.	Cleomaceae	Safrawi	C_3
Phyllanthus amarus Schumach. & Thonn	Phyllantheceae	Stone breaker, Gugario	C_3
Portulaca oleracea L.	Portulacaceae	Purskane, chicken weed	CAM
P. umbraticola L.	Portulacaceae	Purskane, chicken weed	CAM
P. grandiflora Hook.	Portulacaceae	Moss rose purslane	C_4-CAM
Heliotropium zeylanicum (Burm.f.) Lam.	Boraginaceae	Kali Bui	C_4
H. bacciferum Forssk.	Boraginaceae	Kali Bui	C_4
H. marifolium Koen. Ex Retz.	Boraginaceae	Chhoti- Santari	C_4
H. curassavicum L.	Boraginaceae	Salt heliotrope	C_3

Fig 1: - Monocot species with leaf anatomy. Crop plants *Triticum aestivum* and *Oryza sativa* are C_3 crops plants taken as comparison with desert grown natural grasses. The anatomy evidenced here the cell thickness, bundle sheath area and bulliform cells. C_4 grasses clearly show the presence of Kranz pattern of mesophyll cells. Scale bar= 1 mm

Species	Morphology	Leaf Anatomy
Brachiaria ramosa (L.) Stapf		
Brachiaria reptans (Linn.) Gardner & Hubbard		
Dichanthium annulatum (Forssk.) Stapf		
Chloris virgata Sw.		
Lasiurus scindicus Henrard Sewan grass		

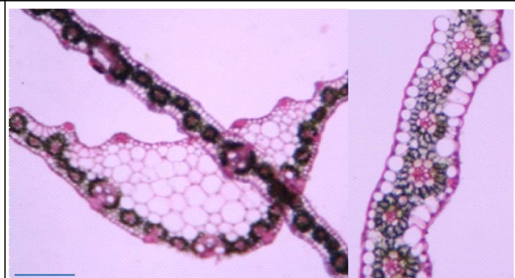
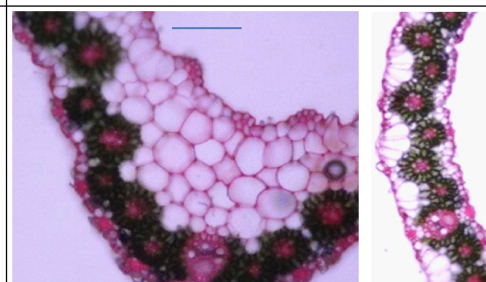
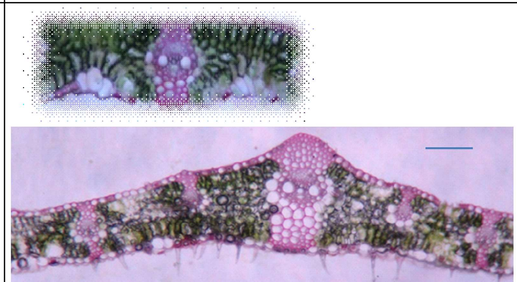
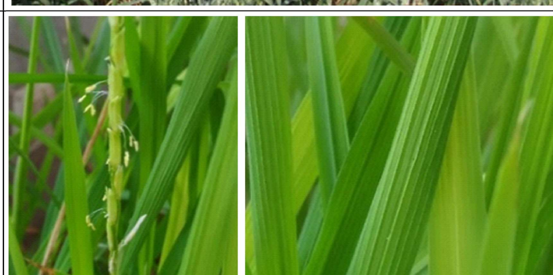
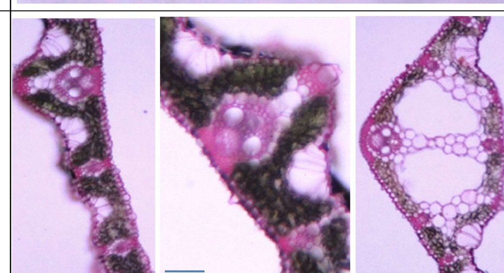
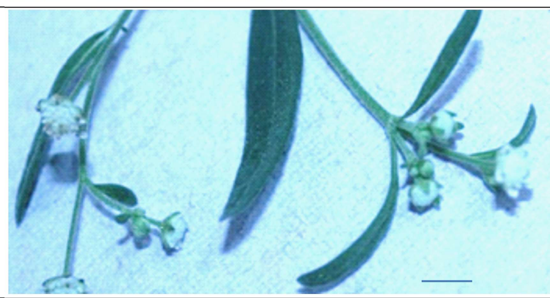
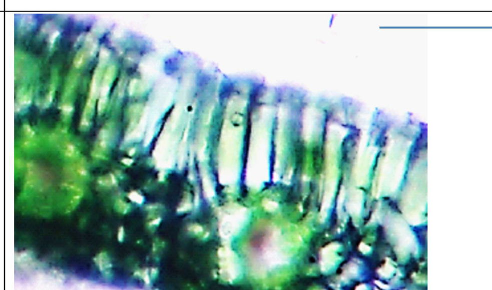
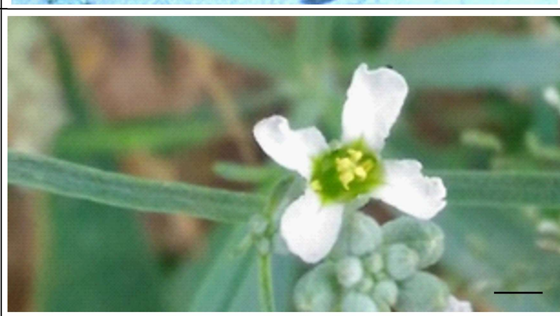
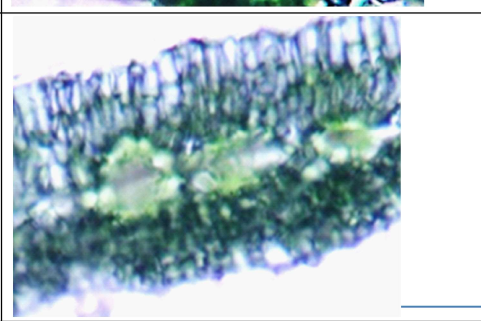
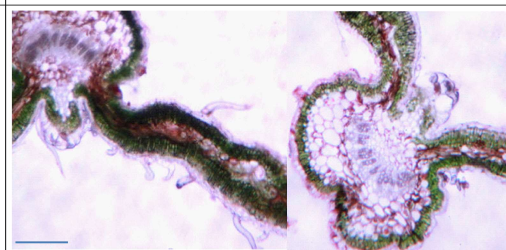
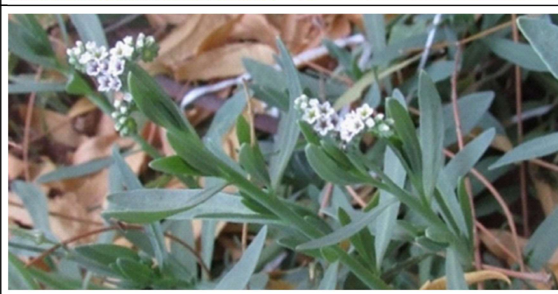
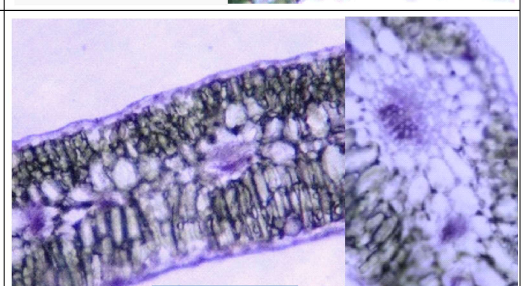
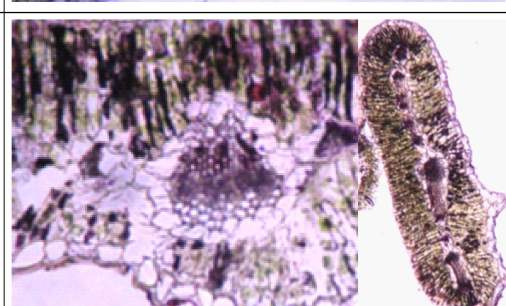
Panicum turgidum Forssk.		
Pannisetum glaucum (Linn.) R. Br.		
Cenchrus setigerus Vahl		
Triticum aestivum L.		
***Oryza sativa Linn*.**		
Cyperus rotendus Linn.		

Fig 2: Leaf anatomical features of Dicot species of C3, CAM, C4 and intermediate plants. Scale bar = 1 mm

Species	Morphology	Leaf Anatomy
Tribulus terrestris Linn.		
T. rajasthanensis Bhandari *et* Sharma		
Parthenium hysterophorus L.		
Farsetia hamiltonii Royle		
Mollugo nudicaulis Lam.		

Heliotropium paniculatum R. Br		
Heliotropium bacciferum *Forssk.*		
Heliotropium marifolium Koen. ex Retz		
Heliotropium zeylanicum (Burm.f.) Lam.		
Heliotropium curassavicum *Linn.*		
Dipterygium glaucum Decne.		

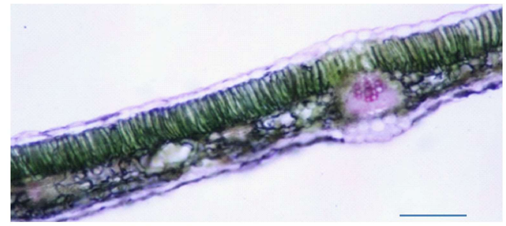
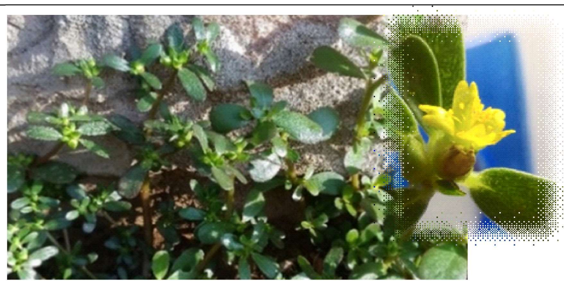
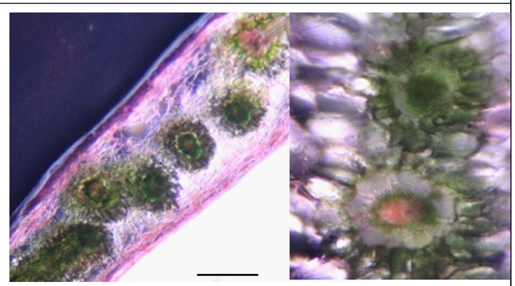
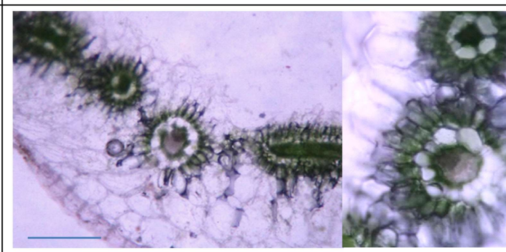
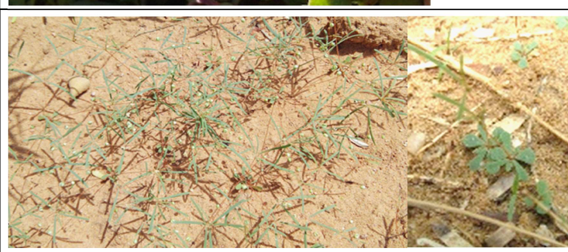
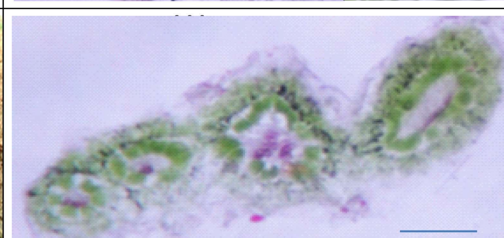
Phyllanthus amarus Schum. And Thonn.		
Gisekia pharnaceoides Linn.		
Portulaca oleracea L.		
P. umbraticola Kunth		
Mollugo cerviana (Linn.) Ser.		

Fig. 3: Leaf anatomy of *Farsetia hamiltonii*: Typical anatomical characteristics, indicating C_3 features, include both types of mesophyll cells (PMC, Palisade Mesophyll Cells, and SMC, Spongy Mesophyll Cells) containing chloroplasts. Furthermore, the leaf sections exhibit two types of chloroplasts within the bundle sheath-centripetal and centrifugal-reminiscent of a C_3-C_4 intermediate plant. Scale bar = 5 mm. All images are copyrighted to authors.

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