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Stem Morphometric of Selected *Indigofera* Species under Climate Variability and Land Use Change in Nigeria

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ABSTRACT

Understanding the response of plant's stem anatomy to environmental heterogeneity is crucial for predicting the resilience of plants to climate variability and land-use change. Despite the ecological relevance of the *genus Indigofera*, information on how climate variability and land-use disturbance jointly influence stem anatomical attributes across contrasting tropical ecosystems remains scanty, especially in Nigeria. This study aimed to fill this gap by comparing stem anatomy across seven *Indigofera* species (*I. arrecta*, *I. macrophylla*, *I. pulchra*, *I. spicata*, *I. hirsuta*, *I. tinctoria*, and *I. subulata*) sampled from disturbed and undisturbed rainforest and savanna habitats in Nigeria. Transverse sections of the stems were prepared using standard paraffin embedding techniques, stained, and then examined with light microscopy for detailed anatomical characterization. From the result, there existed interspecific variation and habitat-related variations in cuticle thickness, epidermis configuration, cortical organization, vessel diameter, sclereid occurrence, crystal distribution, intercellular spaces, and pith development. Those species inhabiting more humid environments, like *I. arrecta* and *I. macrophylla*, tended to possess larger vessel diameters, thicker cuticles, and wider pith areas, which are characteristics typical of enhanced hydraulic efficiency. In contrast, species confined to xeric or disturbed habitats, such as *I. tinctoria, I. hirsuta,* and *I. subulata,* showed narrow vessels, densely lignified fibres, and thickened periderm, all structural features indicative of adaptations to water stress and environmental disturbance. These results demonstrate that anatomical traits in the stems of *Indigofera* express functional plasticity and adaptive differentiation and are thus particularly valuable as plant indicators of climate variability and land-use change across diverse tropical landscapes.

1. INTRODUCTION

The ability of the plant to adapt and survive across diverse ecosystems depends mainly on some structural and functional mechanisms that deal with dynamism in environmental factors. These arise from the interactive effects of various climatic variables, edaphic conditions, and their inherent anatomical features, which depend on the particular species, its ecological performance, distribution, and evolutionary history (Preston, 2022). Changes in temperature, rainfall, and light availability determine growth patterns and regulation of resource allocation, influencing morpho-anatomical differentiation and thus the success or decline of species under changing environment (Geange *et al.,* 2017). Growing climate variability, along with human-induced land use change through deforestation, intensive agriculture, and urban expansion, has increasingly subjected plant structure and function to selective pressures that are enforcing morphological and anatomical changes as survival strategies **(**Guo *et al.,* 2017)**.** Following De Baerdemaeker *et al.* (2020); González-Castro *et al.* (2023), many plants have developed morpho-anatomical plasticity in the shoot system, like stems and leaves, where changing structural characteristics maintain physiological functions under fluctuating environmental factors.

Pawade & More. (2018). revealed that at different climatic conditions, plant growth and survival are significantly influenced by stem-related traits, particularly xylem vessel diameter and wood density. In more arid and disturbed habitats, xylem becomes denser with smaller vessel to reduce the risk of cavitation (reduce water flow), however, species from humid forests have larger vessels, which enable efficient water transport. Cochard & Delzon, 2021; De Baerdemaeker *et al*. (2020) corroborate this finding. These are some of the adaptive variation in stem anatomy that are important to maintaining plant performance at different environmental zones. The *genus Indigofera* provides a viable model for studying morpho-anatomical adaptation of the stem in the described environmental setting. The genus is ecologically and morphologically diverse with over 700 species distributed worldwide across many tropical and subtropical environments (Zhou *et al.,* 2023). Members of this genus contribute to the fixing of nitrogen, amelioration of soil, control of erosion, and production of secondary metabolites, they are economically economically important because of their values as forage, dye, and medicinal products (Sharma &Prakash, 2019). Despite their important ecological and economic roles, few studies have examined how *Indigofera* species adapt structurally, through stem anatomy, to the combined pressures of climate variability and land use change. Stem as the major organ of transport of water and solutes, mechanical support, and storage, the stem is central to the plant's adaptation, with its internal anatomy reflecting the integrated responses of plants to environmental stressors (Li *et al.,* 2024). Main traits linked to cuticle, outer and inner bark xylem vessel diameter, cortical thickness, and pith structure have consequences for hydraulic efficiency, mechanical stability, and drought tolerance. Mainly in legumes, including *Indigofera L,* most environmental fluctuations caused thickening of the periderm and reduction in the vessel lumens; these adaptations maintain hydraulic safety under limiting water conditions **(**Zimmerman *et al.,* 2021). This study intend to examine the stem morphometric response of seven selected *Indigofera* species to climate variability and land use change.

Information on the morphology and anatomy of plants, and how such features change over time, is limited for the *Indigofera* species in Nigeria. However this has implications for the assessment of the species' resilience and land management across different vegetation zones. The relationship between various anatomical features that exit in disturbed and undisturbed habitats has important implications for ecological adaptation. The prediction of ecosystem stability under various pressures from climate and land-use demands is essential for effective conservation strategies. The members of the *Indigofera genus* are of ecological and economic importance for example, soil stabilization, production of forage, and medicinal uses which support both conservation and livelihoods (Sharma & Prakash, 2019).This study explores adaptive stem morpho-anatomical plasticity in selected Indigofera species in Nigeria, considering morphological and anatomical features in the context of background climatic variability and land use. The anatomical structures of stems from disturbed and undisturbed conditions will be compared to determine the ecological importance of the stem attributes for plant survival and growth under environmental stress (Zhang & Liu, 2024).Knowledge derived will be used to understand plant functional traits and resilience strategies, thereby informing predictions of vegetation dynamics in the tropics. The findings from the study therefore address conservation efforts and land use management practices by providing baseline information useful for agriculture, soil restoration, ecosystem services, and biodiversity conservation for human well-being.

2.0 Materials and Methods

2.1 Study Area

The tropical rainforest and savanna are the two major vegetation zones of Nigeria, which were differentiated into disturbed and undisturbed in each vegetation zones (Disturbed Tropical Rainforest-DTR, Undisturbed Tropical Rainforest-UTR, Disturbed Savannah-DSS, and Undisturbed Savannah-USS).

The rainforest sites (Oyo, Ogun and Ekiti state) were located in the southwestern zone between approximately 6°30′ to 7°30′N, 3°30′ and 4°30′E, with mean annual rainfall of 1800 to 2500 mm, relative humidity over 75%, and a mean temperature of 26 to 28°C. On the other side, savanna sites comprises of (Kaduna, Katsina and Sokoto lay between 8°00′–9°30′N and 3°50′–5°00′E, with the precipitation range between 900-1500 mm and a mean annual temperature within the range of 28 to 32°C (Odekunle & Adejuwon, 2020; Adeyemi, 2022)

2.2 Species Selection and Sampling Strategy

From the study area, the following *Indigofera* species, namely *Indigofera arrecta* Hochst. ex A. Rich. *Indigofera macrophylla* Schumach. & Thonn, *Indigofera pulchra* Willd, *Indigofera spicata* Forssk, *Indigofera subulata* Vahl ex Poir, and *Indigofera hirsuta* L. *Indigofera tinctoria* L.were collected from the four sites.

Table 1: Species identification was based on Flora of West Tropical Africa (Hutchinson & Dalziel, 1958) and authenticated at the Forestry Herbarium, Ibadan (FHI). In each habitat, five healthy mature individuals per species were randomly sampled, giving a total of 140 stem samples (7 species × 4 habitats × 5 replicates). Stem segments were collected from the

mid-portion (chronological 3and 5) of the youngest fully developed secondary stem during the active months of June to September when both anatomy and physiology were at their peak. Each sample measured 1.0–1.5 cm in diameter and 2–3 cm in length. All the specimens were labelled by species and site and were immediately fixed in FAA according to standard anatomical procedures (Johansen, 1940; Ruzin, 1999).

Table 1: Inventory of Collected Samples and Collection Records

		Inventory of Collected Samples and Collection Records				
S/N	**Species**	**Site of Collection**	**Location**	**Latitude/Longitude**	**Collector**	**Voucher No.**
1	*I. arrecta* Hochst. ex A. Rich.	DSS	Among grassses,Kuwdu, Zaria, Kaduna	LAT. N11^{o} 53^{i} 79^{ii} LOG E7^{o} 29^{i} 35^{ii}	Ogundele	FHI. 114243
2	*I. macrophylla Schumach. & Thonn.*	DSS	Farmland, Jamaa village, Zaria, Kaduna	LAT. N11^{o} 85^{i} 31^{ii} LOG E7^{o} 40^{i} 86^{ii}	Ogundele	FHI. 114239
3	*I. pulchra Willd.*	DSS	Farmland Wusasa, Zaria, Kaduna	LAT. N11^{o} 53^{i} 80^{ii} LOG E7^{o} 29^{i} 49^{ii}	Ogundele	FHI. 114245
4	*I. spicata Forssk.*	DSS	Kafuna, Edge of the road, Zaria, Kaduna	LAT. N11^{o} 83^{i} 10^{ii} LOG E7^{o} 40^{i} 21^{ii}	Ogundele	FHI. 114235
5	*I. hirsuta Vahl ex Poir*	DSS	Farmland, Kahiyayi village, Zaria, Kaduna	LAT. N11^{o} 84^{i} 90^{ii} LOG E7^{o} 40^{i} 16^{ii}	Ogundele	FHI. 114232
6	*I. subulata L.*	DSS	Road side Kufena, Zaria, Kaduna	LAT. N11^{o} 05^{i} 05^{ii} LOG E7^{o} 43^{i} 80^{ii}	Ogundele	FHI. 114241
7	*I. tinctoria L.*	USS	Farmland kunran Jamaa, Kaduna	LAT. N11^{o} 93^{i} 83^{ii} LOG E7^{o} 29^{i} 49^{ii}	Ogundele	FHI. 114234
8	*I. arrecta*	USS	Woodland Zango, Zaria, Kaduna	LAT. N11^{o} 83^{i} 71^{ii} LOG E7^{o} 43^{i} 23^{ii}	Ogundele	FHI. 114242
9	*I. macrophylla*	USS	Woodland Tundun-junlan, Kaduna	LAT. N11^{o} 83^{i} 71^{ii} LOG E7^{o} 43^{i} 23^{ii}	Ogundele	FHI. 114238
10	*I. pulchra*	USS	Woodland Karau-karau,	LAT.N11^{o} 41^{i} 70^{ii} LOG E7^{o}47^{i}54^{ii}	Ogundele	FHI. 114244
11	*I. spicata*	USS	Woodland Dandumi, Katsina State.	LAT. N11^{o} 57^{i} 14^{ii} LOG E7^{o} 30^{i} 93^{ii}	Ogundele	FHI. 114237
12	*I. hirsuta*	USS	Dandumi villilage, Funtua, Katsina State.	LAT. N11^{o} 83^{i} 80^{ii} LOG E7^{o} 40^{i} 23^{ii}	Ogundele	FHI. 114231
13	*I. subulata*	USS	Kafune, Edge of the road, Zaria, Kaduna	LAT. N11^{o} 12^{i} 24^{ii} LOG E7^{o} 72^{i} 54^{ii}	Ogundele	FHI. 114241
14	*I.tinctoria*	USS	Woodland Jamaa Village, Katsina	LAT. N11^{o} 82^{i} 55^{ii} LOG E7^{o} 40^{i} 52^{ii}	Ogundele	FHI. 114233
15	*I. arrecta*	DTR	Road side Temidire-Esunmo, Ekiti State	LAT. N7^{o} 32^{i} 33^{ii} LOG E3^{o} 13^{i} 36^{ii}	Ogundele	FHI. 114420
16	*I. macrophylla*	DTR	FRIN Premises Ibadan, Oyo State	LAT. N7^{o} 28^{i} 35^{ii} LOG E3^{o} 44^{i} 52^{ii}	Ogundele	FHI. 114417
17	*I. pulchra*	DTR	Premises EKSU, Ekiti State	LAT. N7^{o} 31^{i} 33^{ii} LOG E3^{o} 13^{i} 36^{ii}	Ogundele	FHI. 114413
18	*I. spicata*	DTR	Road side, Olokemeji, Ogun State	LAT. N7^{o} 23^{i} 33^{ii} LOG E3^{o}38^{i} 52^{ii}	Ogundele	FHI. 114419
19	*I. hirsuta*	DTR	FRIN Premises Ibadan, Oyo State	LAT. N7^{o} 23^{i} 14^{ii} LOG E3^{o} 39^{i} 33^{ii}	Ogundele	FHI.114422
20	*I. subulata*	DTR	Road side Ajibode, Ibadan, Oyo State	LAT. N7^{o} 31^{i} 33^{ii} LOG E3^{o} 13^{i} 37^{ii}	Ogundele	FHI. 114414
21	*I. tinctoria*	DTR	Ojo barracks, Ibadan, Oyo State	LAT. N7^{o} 40^{i} 18^{ii} LOG E3^{o} 14^{i} 39^{ii}	Ogundele	FHI. 114410
22	*I. arrecta*	UTR	Woodland Temidire-Esunmo, Ekiti State	LAT. N7^{o} 32^{i} 59^{ii} LOG E3^{o} 39^{i} 54^{ii}	Ogundele	FHI. 114421
23	*I. macrophylla*	UTR	Woodland Ido- Eruwa, Oyo State	LAT. N7^{o} 30^{i} 32^{ii} LOG E3^{o} 13^{i} 35^{ii}	Ogundele	FHI. 114416
24	*I. pulchra*	UTR	woodfarm Ago-adulogu, Ado-Ekiti, Ekiti s	LAT. N7^{o} 23^{i} 89^{ii} LOG E3^{o}39^{i} 54^{ii}	Ogundele	FHI. 114412
25	*I. spicata*	UTR	Woodland Olokemeji, Ogun State	LAT. N7^{o} 23^{i} 04^{ii} LOG E3^{o}38^{i} 57^{ii}	Ogundele	FHI. 114418
26	*I. hirsuta*	UTR	Olokemeji road, Ogun State	LAT. N7^{o} 23^{i} 54^{ii} LOG E3^{o} 45^{i} 46^{ii}	Ogundele	FHI.114236
27	*I. subulata*	UTR	Woodland Laniba, Ibadan, Oyo State	LAT. N7^{o} 23^{i} 41^{ii} LOG E3^{o} 38^{i} 57^{ii}	Ogundele	FHI. 114415
28	*I. tinctoria*	UTR	Igbo-Elerin, Ibadan, Oyo State	LAT. N7^{o} 25^{i} 28^{ii} LOG E3^{o} 31^{i} 38^{ii}	Ogundele	FHI. 114411

Disturbed Tropical Rainforest-DTR, Undisturbed Tropical Rainforest-UTR, Disturbed Savannah-DSS, and Undisturbed Savannah-USS

2.3 Anatomical Preparation of Stems

Anatomical studies followed a modified paraffin-embedding technique suitable for tropical herbaceous stems. Fixed samples were washed in 50% ethanol, dehydrated through a graded ethanol series (50%, 70%, 90%, 95%, and absolute), and infiltrated with paraffin wax at 58°C. Transverse sections (12–15 µm) were prepared using a Leica RM2125 rotary microtome, stained with safranin O and fast green to distinguish lignified and non-lignified tissues (Ruzin, 1999), and mounted in DPX. Microscopic examination was carried out with an Olympus CX43 light microscope fitted with an HD digital camera and calibrated micrometer. The anatomical features observed during the course of study are the cuticle, epidermis, bark tissues, crystals, sclereids, xylem, vessel pores, and pith.

Result

Comparative Anatomical Assessment of Stem Transverse Sections in Seven *Indigofera* Species from both Disturbed and Undisturbed Tropical Rainforests and Savannah

Cuticle

Compared across the seven *Indigofera* species, the cuticle in the disturbed rainforest sites remained generally thin, undulating, and finely striated, with *I. macrophylla* being an exception, which developed a remarkably thicker and more prominently wavy cuticle layer. In the undisturbed rainforest habitat, most species retained a thin, tightly waved cuticle. Meanwhile, *I. macrophylla* consistently showed a thick and strongly striated surface (Plate. 1).

Epidermis

The study indicates clear epidermal diversity among the *Indigofera* species in the disturbed rainforest habitats, as most species develop a thin uniseriate epidermis comprising cells of different shapes. *I. macrophylla* exhibited a thicker multiseriate epidermis, which was indicative of higher structural development. In undisturbed rainforest habitats, epidermal layers are remaining uniseriate but more complex, except in *I. macrophylla*, which keeps a multiseriate pattern as revealed (Plate.1).

Outer bark (Phloem)

The cortex of *Indigofera* species in disturbed rainforests exhibited explicit variation. *Indigofera arrecta, I. subulata, I. hirsuta*, and *I. spicata* grow 7-15 layers of living parenchyma, while those of *I. macrophylla, I. tinctoria*, and *I. pulchra* form 4-8 layers of cells with partial cell death, indicating early tissue differentiation. In the undisturbed rainforests, *I. macrophylla* and *I. pulchra* expressed 12-25 layers of partially dead parenchyma. Lastly, *I. arrecta, I. hirsuta*, and *I. subulata* retain 5-10 living layers, while *I. spicata* and *I. tinctoria* show 4-8 phloem-oriented layers (Plate 1.).

Inner

In the transverse stem anatomy of *I. arrecta, I. spicata, I. subulata, I. pulchra, I. macrophylla,* and *I. hirsuta*, there is an inner cortex with 9 to 18 layers of living parenchyma with round, oval, cylindrical, or polygonal cells, together with large brachysclereids near the phloem and smaller ones towards the xylem. Undisturbed rainforest samples of all seven species develop a thicker inner cortex comprising 12 to 28 living layers with the same diverse cell forms as those shown in (Plate.1).

Crystal

In the disturbed rainforests, most *Indigofera* species, including *I. macrophylla, I. spicata, I. arrecta, I. tinctoria, and I. pulchra*, have abundant druse crystals, whereas. *I subulata* has a few, while *I. hirsuta* has even fewer. However, undisturbed rainforest species of *I. macrophylla, I. hirsuta, I. subulata,* and *I. spicata* largely lacked druses, but *I. arrecta, I. tinctoria*, and *I. pulchra* contained only rare druse sand, just as was observed (Plate.1)

Sclereids

It was observed that *I. pulchra, I. hirsuta,* and *I. arrecta* form only small, irregular brachysclereids close to the phloem (Plate.1) in disturbed rainforest sites, whereas *I. macrophylla, I. subulata, I. tinctoria,* and *I. spicata* show no sclereids. In undisturbed rainforest conditions, sclereid development is well expressed, with large brachysclereids near the phloem (Plate.1) and smaller ones extending toward the xylem in *I. macrophylla, I. tinctoria,* and *I. subulata*. In the same undisturbed forests, *I. pulchra, I. spicata, I. arrecta,* and *I. hirsuta* retain only small-sized sclereids.

The seven *Indigofera* species, *I. arrecta, I. pulchra, I. spicata, I. macrophylla, I. subulata, I. tinctoria*, and *I. hirsuta* present xylem variations that are quite distinct between disturbed and undisturbed tropical rainforest.

Pores

Cortical pore adaptation in disturbed and undisturbed tropical rainforests in the seven *Indigofera* species. *I. spicata, I. hirsuta* and *I. pulchra* have been identified. *I. pulchra*, found in disturbed sites, developed small, irregular, and poorly distributed pores (Plate.2), and *I. arrecta, I. macrophylla,* and *I.subulata* and *I. tinctoria* were found to have fewer well-defined pores. *I. tinctoria* possessed fewer well-defined pores.

Pith

The seven *Indigofera* species show clear pith plasticity across disturbed and undisturbed tropical rainforests (Plate.2). The disturbed sites have resulted in narrow piths with small, irregular parenchyma cells, especially in *I. arrecta, I. spicata*, and *I. hirsuta*, while the rest, like *I. subulata, I. tinctoria, I. macrophylla*, and *I. pulchra*, showed slightly more developed pith tissue. In contrast, undisturbed forests support wider, better-organised piths with thicker parenchyma layers, particularly in *I. arrecta, I. macrophylla, I. tinctoria*, and *I. subulata*.

Cuticle (Savannah)

The cuticle variations of the seven species of *Indigofera I. arrecta, I. pulchra, I. spicata, I. hirsuta, I. tinctoria, I. subulata*, and *I. macrophylla* demonstrate clear patterns of cuticle adaptation across the disturbed and undisturbed savannahs (Plate.3). Stems from disturbed savannahs have softly thickened and wavy, striated cuticles, reflecting responses to fluctuating temperature and reduced moisture.

Epidermis

From the observation of seven *Indigofera* species, *I. macrophylla, I. pulchra, I. spicata, I. hirsuta, I. tinctoria, I. arrecta*, and I. subulata, all display sharp epidermal contrasts between disturbed and undisturbed savannahs. The disturbed site develops thin, uniseriate epidermal layers that are irregularly shaped in their parenchyma cells, whereas the sites that are not disturbed develop more uniform, structurally organized epidermis. *I. macrophylla* stands out for maintaining multiseriate epidermis of mixed cell shapes as revealed (Plate 3).

Outer bark (Phloem)

The anatomy of the outer bark of seven *Indigofera* species, *I. arrecta, I. spicata, I. subulata, I. hirsuta, I. macrophylla, I. tinctoria,* and *I. pulchra,* differs considerably between DSS and USS (Plate 3). In DSS, *I. arrecta, I. spicata, I. subulata*, and *I. hirsuta* showed loosely packed, living parenchyma (7–15 radial layers), but in *I. macrophylla, I. tinctoria,* and *I. pulchra*, the epidermal cells were dead. In USS, the bark of all species is denser and darker, having more compact and partly lignified parenchyma (5–10 layers).

Inner bark

Ontogenetic divergence in the inner bark of seven *Indigofera* species thus expresses the adaptive responses to environmental stress from climate and land use change. In disturbed savannahs, fluctuating moisture, temperature, and soil disturbance all impede cellular growth and the development of sclereids, leading to very sparse parenchyma and low sclereid frequency.

Crystal

The stems of *I. arrecta, I. spicata, I. pulchra, I. subulata, I. tinctoria,* and *I. macrophylla*, with all producing abundant druse and styloid crystals in disturbed savannahs (DSS), unlike *I. hirsuta,* which lacked crystals. In undisturbed savannahs (USS), all species showed a few crystals, while in *I. macrophylla*, crystals were absent as observed (Plate 3).

Sclereids

In the DSS, abundant druse and styloid crystals were present in *I. arrecta, I. spicata, I. pulchra, I. subulata, I. tinctoria,* and *I. macrophylla* but absent in I. hirsute as shown (Plate 3). On the other hand, few or no crystals were formed by all species in the USS. This obviously reflects the lower environmental stress in the USS. Crystals are implicated in herbivore deterrence, ion regulation, and calcium storage.

Xylem

In DSS, xylem vessels are narrower, have a smaller diameter, with less secondary tissue and fewer rows of radial parenchyma, a reflection of adaptation to water stress and high evapotranspiration that decreases the risk of embolism (Plate 3).

Pore space

In DSS species, the frequency of pores is low; they are irregularly distributed and tend not to form chains, except in *I. arrecta* and *I. hirsuta*, with numerous solitary pores (Plate 3). USS samples from all species feature ordered chain pores and some clustered arrangements, showing higher anatomical plasticity.

Pith

The pith of all seven *Indigofera* species, *I. arrecta, I. spicata, I. pulchra, I. subulata, I. tinctoria, I. hirsuta* and *I. macrophylla*, growing on disturbed savannahs consists of loosely arranged, circular to cylindrical parenchyma with narrow intercellular spaces. By contrast, piths from undisturbed savannahs are large and well-developed, with oval to polygonal parenchyma layers as revealed (Plate.4).

Discussion

The cuticle from the seven selected species of Indigofera reflect an adaptive response to climatic and land-use stress, whereby the thin cuticle corresponds to low moisture stability, changeable temperature, and limited nutrient conditions in disturbed areas (Ni *et al.,* 2022; Yavas *et al.,* 2023). On the other hand, the persistent thick cuticle of I. macrophylla enhances protection against evapotranspiration and UV exposure (Heredia, 2024; Fernández *et al.,* 2017), and also thin cuticles in rainforest plants enable efficient permeability under reduced evaporative stress (Slot *et al.,* 2021). The consistent thickness in *I. macrophylla* may be due to intrinsic, phylogenetically constrained structural stability. The epidermis layers of the *genus Indigofera* show anatomical responses to climatic and land-use pressure: the multiseriate tissue provides the plant with mechanical support, water storage, and protection against stress (Zhang *et al.,* 2021). Thin uniseriate layers in other species correspond to the stable and humid microclimate of the forests with a closed canopy. Persistence of multiseriate epidermis in *I. macrophylla* reflects plasticity at the species level and points toward its value as an adaptive and taxonomic trait (Slot *et al.,* 2021). Structural differences were observed therefore showing that climatic and land use change have an impact on the survivability of cortical cells and the development of the cortex. Disturbed sites may have limited resource availability and might be erosion-prone, thereby promoting partial cell death or reducing the growth of parenchyma (Ni *et al.,* 2022; Pandey *et al.,* 2018).). At the same time, stable undisturbed environments promote thick cortices and greater secondary differentiation that enhance the plant's mechanical and physiological resilience (Giraldo-Kalil *et al.,* 2022; He *et al.,* 2018). The regular brachysclereid gradient from phloem to xylem reflects a reinforcement zone that provides strength and protection. Change in climate and human activities on land are the factors responsible to the developmental strategies adopted by plant to survival in their habitat. Changes in temperature and rainfall affect the proliferation and storage functions of parenchyma, as reported by Ni *et al.* (2022). On the other hand, disturbance-driven soil and mechanical stresses influence the distribution pattern of sclereids to maintain the rigidity of the stem under abiotic pressure, as reported by Giraldo-Kalil *et al.* (2022) and (Pandey *et al.,* 2018).

The observed trends in crystals reflect adaptive responses to microenvironmental shifts regulated by climate and land use change. In disturbed habitats, high abiotic and biotic stress increases in the production of druses for protection and detoxification. The presence of crystals and their allies is an indication of stress observed by the plant, while those plants located in an undisturbed habitat have few or no crystals of any form except those species-specific (Ni *et al.*, 2022; Khan *et al.*, 2023). In the same undisturbed forests, *I. pulchra, I. spicata, I. arrecta,* and *I. hirsuta* retain only small-sized sclereids. These differences indicate adaptive responses to climatic and land-use pressures that influence structural reinforcement needs. According to Giraldo-Kalil *et al*. (2022) and He *et al*. (2018), nutrient-rich undisturbed rainforests promote the development of rigid sclerenchymatous tissue that enhances mechanical strength and resilience, whereas nutrient-poor habitats show limited sclereid development (Ni *et al.,* 2022; Pandey *et al*., 2018).

Disturbed sites resulted in fewer, irregular layers of parenchyma, (5-10), reflecting stress-driven anatomical reductions. Undisturbed forests have more regular, radially arranged parenchyma (8-25 layers) that enhance hydraulic efficiency and structural stability as observed in fig.1. Such variations are indicative of response mechanisms to climatic and land use pressures in line with ecological findings (He *et al.,* 2018, Giraldo-Kalil *et al.,* 2022). The undisturbed forests have larger, fairly uniformly distributed pores with interconnected clusters, particularly in *I. macrophylla, I. tinctoria,* and *I. subulata*. These adaptive responses are consistent with other studies carried out by Nwachukwu et al. (2022). These anatomical differences reflect adaptive responses to climatic stress and land-use disturbance, consistent with Pawade & More (2018), Nwachukwu *et al.* (2022), Ni *et al.* (2022), and Giraldo-Kalil *et al*. (2022).

5.0 Conclusion

5.1 Conclusion

This study, analyzed the stem anatomy of seven *Indigofera* species across Nigeria, including *I. arrecta*, *I. macrophylla*, *I. pulchra*, I. *spicata, I. subulata, I. hirsuta, and I. tinctoria,* indicates that the studied species exhibit considerable stem-anatomical plasticity along climatic and contrasting land-use gradients. Specific variations in the studied anatomical structure types, i.e., vessel diameter, cortex width, vascular bundle arrangement, fibre density, periderm tissue formation, and arrangement of the pith tissue, clearly distinguish the species under study. Species typical of humid, less disturbed habitats, such as *I. arrecta* and *I. macrophylla*, showed wider vessels, thicker cortical areas, less compact vascular elements, and larger pith areas. On the contrary, species that are typical in drier habitats, disturbed habitats, or habitats with less water, such as *I. tinctoria, I. hirsuta,* and *I. subulata*, showed vessels that are narrower, less compact vascular elements, fibre elements with greater density, areas that are more lignified, and greater periderm thickness.

More importantly, disturbed habitats showed features of steadily increasing tissue compactness, shrinking intercellular spaces, and stronger supporting tissues, indicating a correspondence between anatomical changes and increased anthropogenic influence and climatic fluctuations. In undisturbed situations, however, there is more relaxed tissue patterns, which denote efficient transport and growth. Overall, there was evidence of anatomical gradients that reflect tuning of hydraulic safety and efficiency, as well as mechanical strength and growth potential, in response to environmental pressures, confirming the importance of stem anatomical traits as being indicative of ecological adaptation and success, and emphasising the significance of studying how Indigofera spp. might structurally respond to climatic and anthropogenic changes in heterogeneous landscapes of the tropics.

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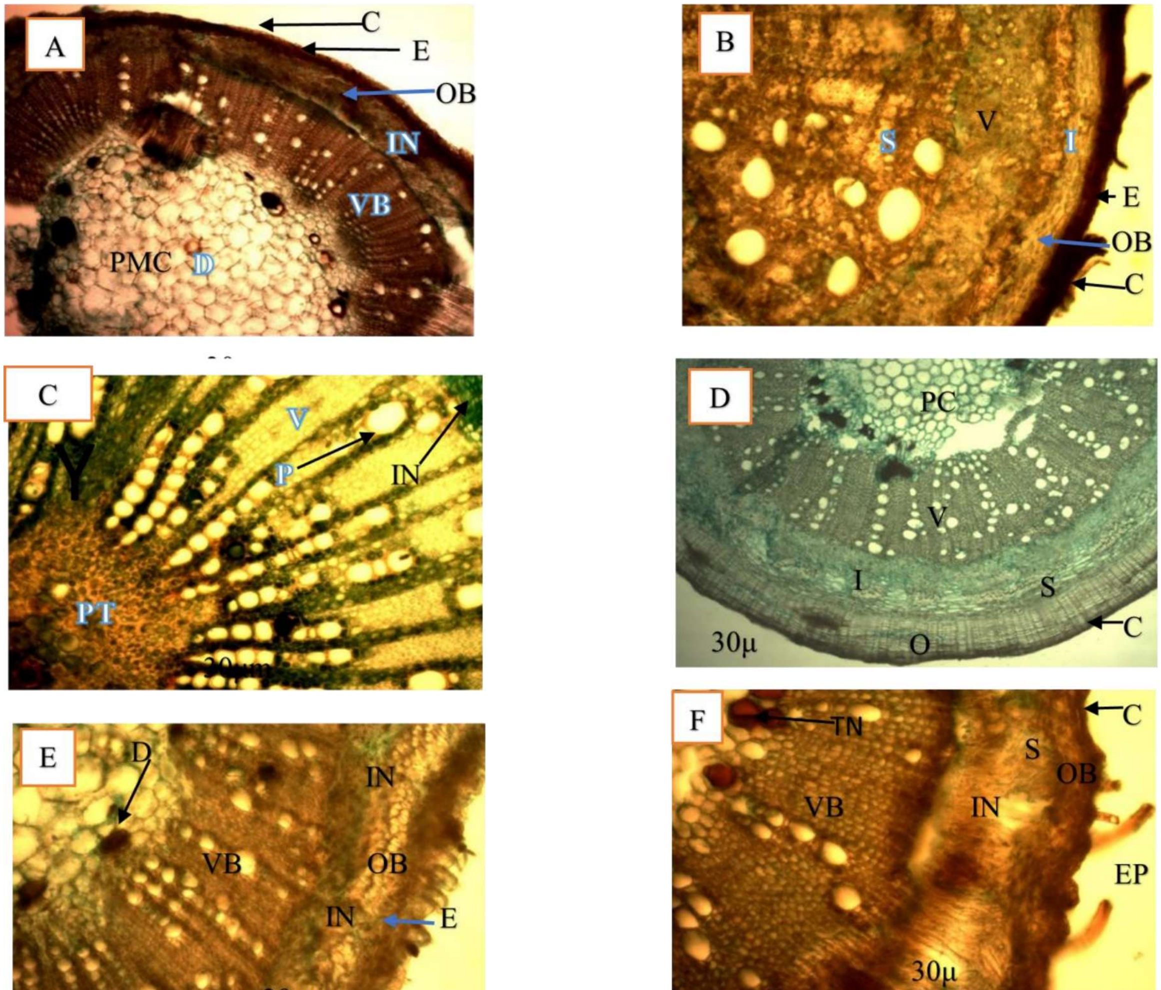


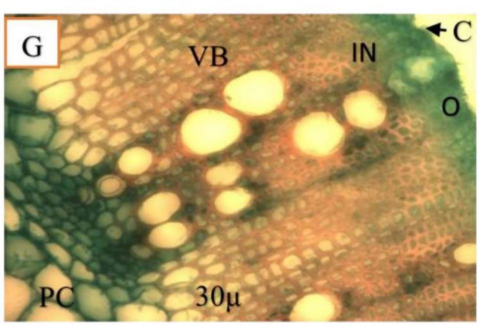


Plate 1: A- I. arrecta, B- I. macrophylla, C-I. tinctoria, D-I. pulchra, E-I.spicata, F-I. hirsuta, G-I. subulata C—cuticle, EP- epidermis, OB- outer bark, IN-inner bark, VB- vascular bundle,SC-sclereids PCM- parenchymal cell (cortex). The placement of the features described

Plate 1: Photomicrographs showing the transverse sections (T.S) of stem tissues -cuticle, epidermis, outer bark, inner bark, vascular bundle, and cortex of seven selected *Indigofera* species collected from disturbed and undisturbed tropical rainforest

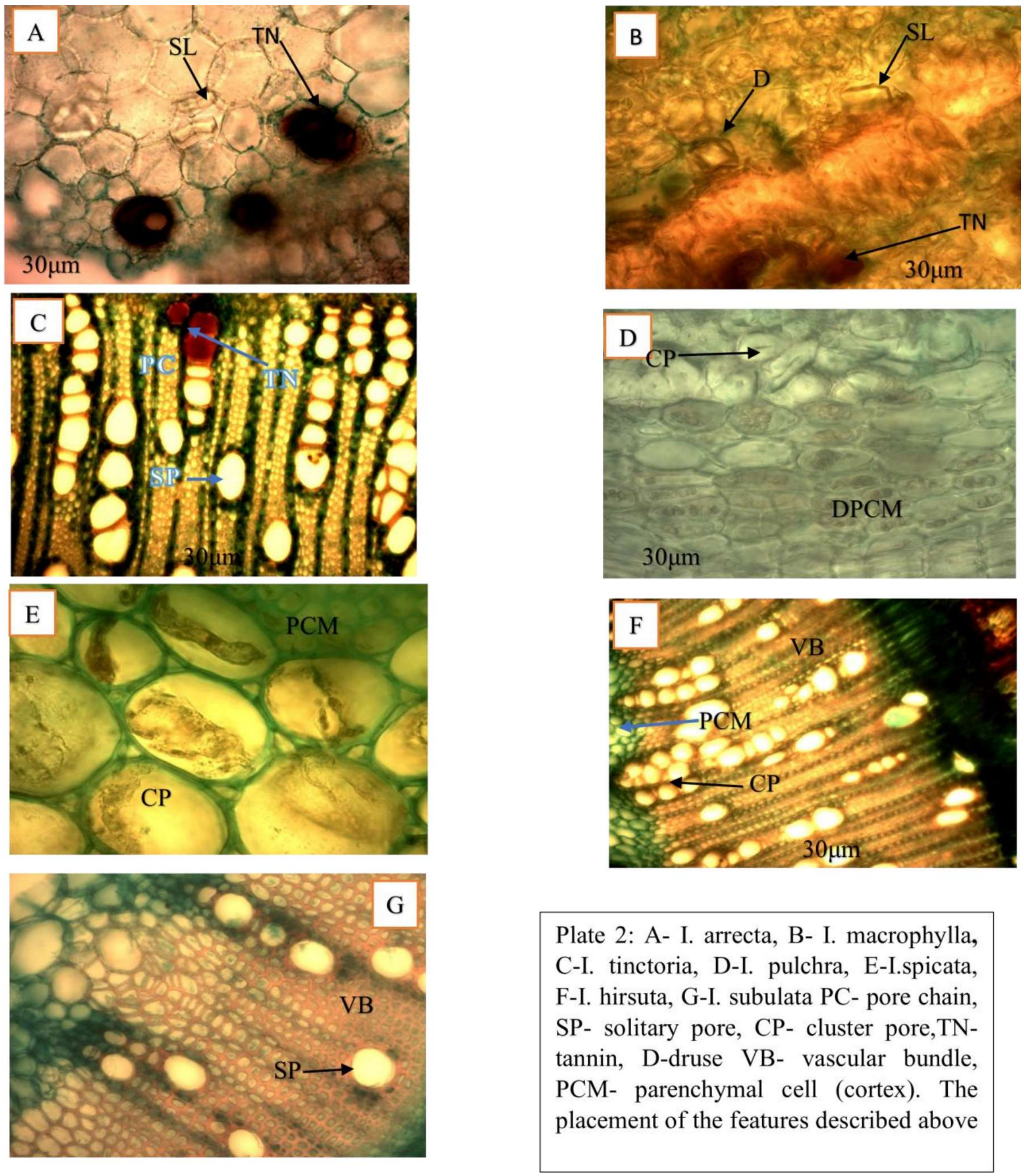
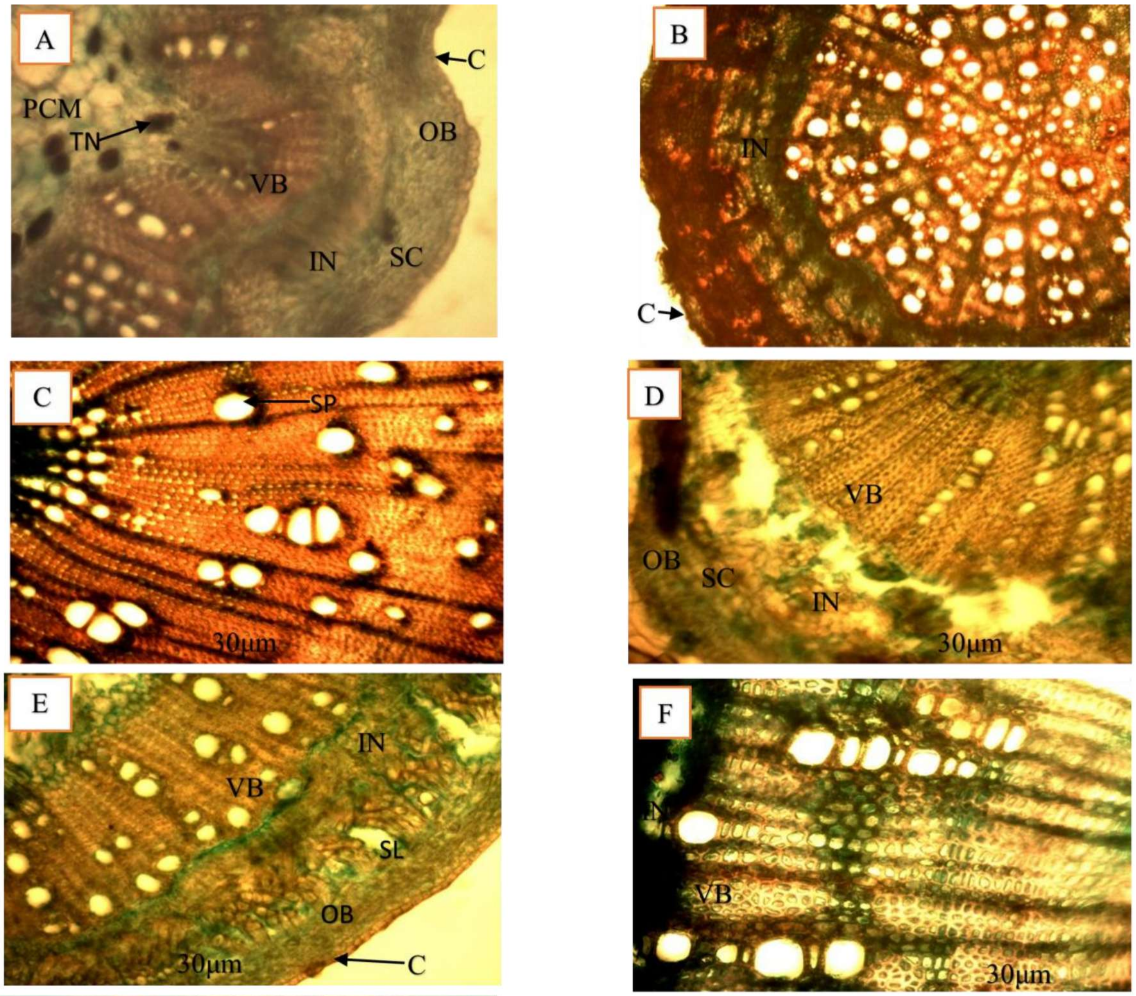


Plate 2: A- I. arrecta, B- I. macrophylla, C-I. tinctoria, D-I. pulchra, E-I.spicata, F-I. hirsuta, G-I. subulata PC- pore chain, SP- solitary pore, CP- cluster pore,TN- tannin, D-druse VB- vascular bundle, PCM- parenchymal cell (cortex). The placement of the features described above

Plate 2: Photomicrographs illustrating the stem crystal and pore from the seven selected *Indigofera* species collected from undisturbed tropical rainforest



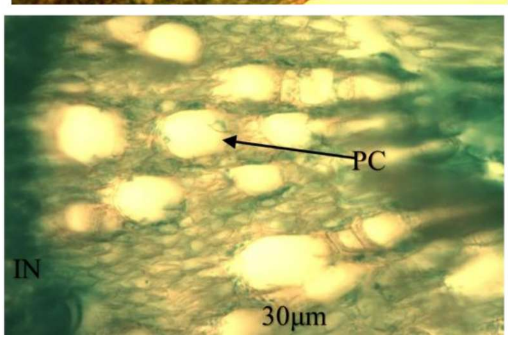
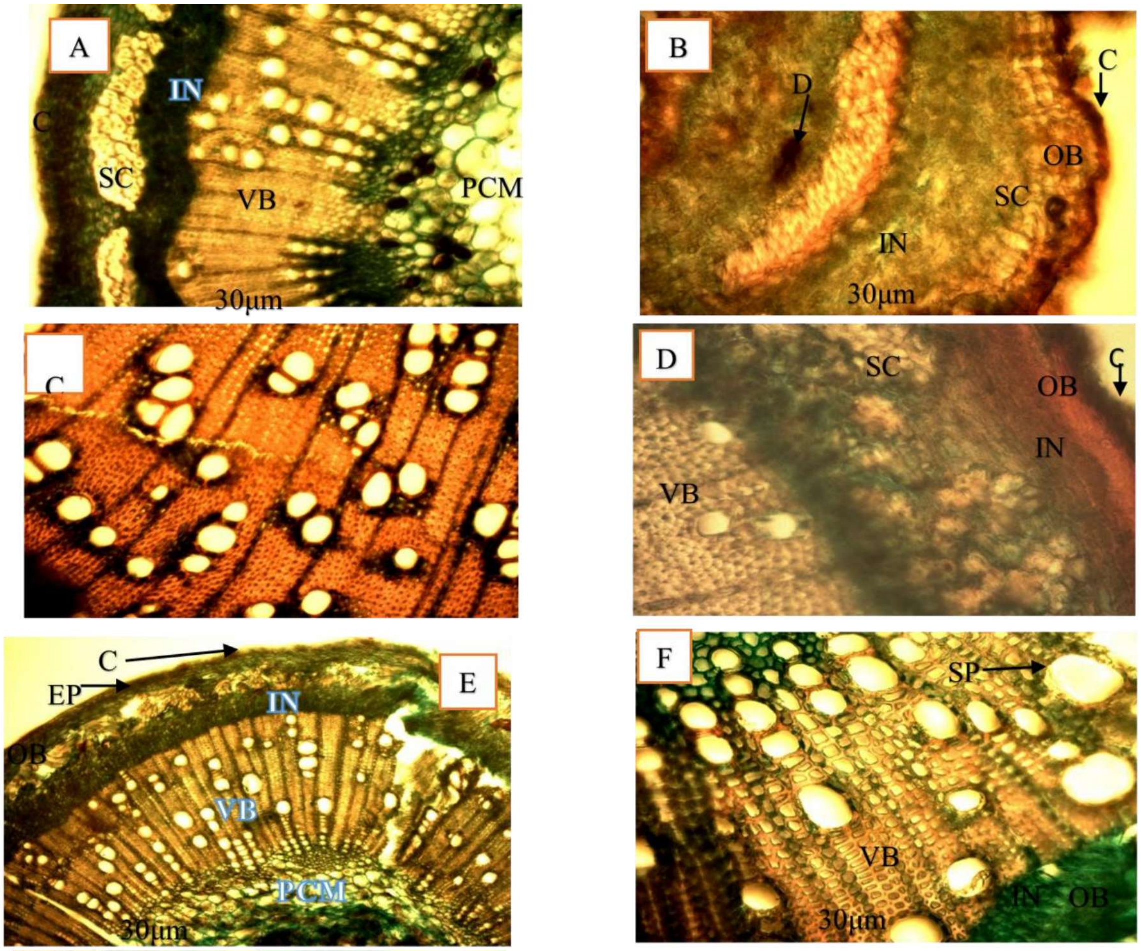


Plate 3: A- I. arrecta, B- I. macrophylla, C-I. tinctoria, D-I. pulchra, E-I.spicata, F-I. hirsuta, G-I. subulata C-cuticle, EP-epidermis, OB- outer bark, IN-inner bark, VB-vascularbundle,SC-sclereidsPCM-parenchymal cell (cortex). The placement of the features described above for each sample species.

Plate 3: Photomicrographs showing the transverse sections (T.S) of stem tissues: cuticle, epidermis, outer bark, inner bark, vascular bundle, and cortex of seven selected *Indigofera* species collected from disturbed and undisturbed savannah



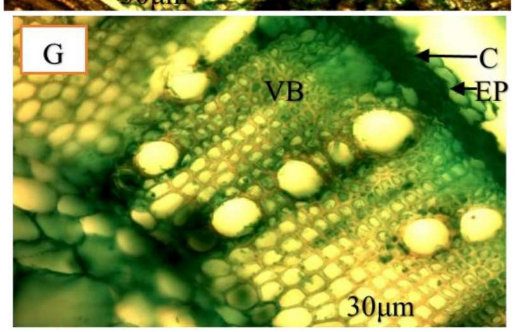


Plate 4: A- I. arrecta, B- I. macrophylla, C-I. tinctoria, D-I. pulchra, E-I.spicata, F-I. hirsuta, G-I. subulata C-cuticle, EP-epidermis, OB- outer bark, IN-inner bark,VBvascularbundle,PCM parenchymal cell (cortex). The placement of the features described

Plate 4: Photomicrographs illustrating the stem crystal and pore from the seven selected *Indigofera* species collected from disturbed and undisturbed savannah