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Seasonal and salinity-induced variations in stem anatomy of *Prosopis cineraria* (L.) Druce

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Abstract
The present study investigates the seasonal and salinity-induced anatomical changes in the stem of *Prosopis cineraria* (L.) Druce, a drought- and salt-tolerant tree species native to arid and semi-arid regions of India. Comparative anatomical analysis was conducted on stem samples collected from two ecologically distinct regions of Rajasthan—saline soils near Sambhar Lake and non-saline soils around Jaipur—across three seasons: summer, monsoon, and winter. Transverse sections of stems were examined for structural variations in the epidermis, cortex, sclerenchyma, vascular system, and pith. The results revealed significant anatomical modifications in response to salinity stress and seasonal variation. In saline environments, traits such as thickened epidermis and cuticle, compact cortex, continuous and lignified sclerenchyma bands, narrower xylem vessels, and frequent tyloses were observed, indicating adaptive strategies for water conservation and salt tolerance. In contrast, plants from non-saline areas exhibited anatomical features favouring active growth and efficient water transport, including wider xylem vessels and loosely arranged cortical and pith tissues. These findings underscore the ecological plasticity and structural resilience of *P. cineraria*, highlighting its potential role in afforestation and saline land reclamation efforts in arid regions.

Keywords: Prosopis cineraria, stem anatomy, seasonal variation, salinity stress, arid and semi-arid regions, Rajasthan

Introduction

Plants inhabiting arid and semi-arid environments are constantly challenged by abiotic stressors such as extreme temperatures, low water availability, and high soil salinity (Hussain *et al.,* 2019) [1]. These stresses can lead to significant modifications in plant structure and function, particularly in anatomical traits that directly influence physiological performance. Among these, stem anatomy plays a vital role in plant adaptation as it governs the transport of water and nutrients, structural support, and storage of essential compounds. Anatomical plasticity in response to seasonal fluctuations and environmental stress, such as salinity, reflects a species' ecological versatility and adaptive strategies (Dildar *et al.,* 2025) [2].

Prosopis cineraria (L.) Druce, commonly known as khejri, is a keystone leguminous tree of the Indian Thar Desert and adjoining dry regions. It is renowned for its ecological resilience, nitrogen-fixing capability, and socioeconomic importance in traditional agroforestry systems. As a drought-tolerant and salt-tolerant species, *P. cineraria* provides an ideal model for studying adaptive anatomical responses under environmental stress. Although previous studies have recognized its physiological and ecological importance, detailed investigations into its stem anatomical responses to seasonal and salinity gradients remain scarce (Madhavan *et al.,* 2025).

The present study aims to explore the seasonal and salinity-induced anatomical changes in the stem of *P. cineraria*, comparing individuals from saline habitats near Sambhar Lake, Rajasthan—characterized by hypersaline soil conditions—with those growing in non-saline conditions around Jaipur. By analyzing structural changes in tissues such as the epidermis, cortex, vascular system, and medullary region across different seasons (summer, monsoon, and winter), this work seeks to provide insights into the species' adaptability and functional morphology.

The findings are expected to enhance our understanding of how *P. cineraria* survives and thrives across contrasting environmental regimes, thereby contributing to conservation, afforestation, and saline land reclamation strategies.

Methodology

Study Site and Plant Material

The study was conducted at two contrasting ecological sites in Rajasthan, India

1. **Sambhar Lake Region:** Representing saline conditions, this site is located in the Sambhar Salt Lake area, which is characterized by high soil salinity, seasonal waterlogging, and an arid microclimate. Soil conductivity levels here are significantly higher due to salt accumulation.

2. **Jaipur Region:** Representing non-saline conditions, this site is situated in the semi-arid zone of Jaipur, which has relatively neutral soil salinity and serves as the control location.

Both sites experience extreme temperatures and seasonal climatic variation, making them suitable for studying both salinity and seasonal anatomical responses.

Seasonal Sampling

Stem samples were collected during three distinct seasons
Summer (March–June)
Winter (November–February)
Monsoon (July–October)

Anatomical Analysis

Mature and healthy individuals of *Prosopis cineraria* were selected from both sites. Stem samples (young to semi-mature branches) were collected from a uniform height (approximately 1.5 meters above ground level) to maintain consistency.

Fixation and Sectioning

Immediately after collection, stem samples were trimmed and fixed in FAA solution (formalin: acetic acid: alcohol in a ratio of 5:5:90) for 48–72 hours. The fixed samples were then washed with 70% ethanol and stored until further processing.

Free-hand transverse sections of the stems were prepared using a sharp razor blade. Thin sections were stained using safranin and fast green to differentiate lignified and non-lignified tissues, respectively. The sections were mounted in glycerin on clean glass slides and covered with cover slips.

Microscopic Observation and Imaging

Prepared slides were observed under a compound light microscope (Magnus MLX model). Photomicrographs were taken using a digital camera attachment. Anatomical features were studied and recorded, including:

- Thickness of epidermis and cuticle
- Number and arrangement of cortical layers
- Presence of sclerenchyma bands
- Vessel diameter and density
- Xylem/phloem proportion
- Presence of tyloses or crystals
- Medullary cell characteristics

Results

The stem anatomy of *Prosopis cineraria* exhibits notable structural modifications across seasons in response to saline stress at Sambhar Lake compared to the relatively non-saline conditions at Jaipur. These changes reflect the plant's adaptive mechanisms to cope with seasonal variations in temperature, moisture availability, and soil salinity.

During the summer season, anatomical features of plants growing under normal conditions in Jaipur showed a typical xerophytic pattern with a thin epidermis, a moderately thick cuticle, and a broad cortex composed of loosely arranged parenchyma. The sclerenchyma bands were present but not highly lignified, indicating minimal mechanical stress. Vascular bundles were widely spaced and xylem vessels were broad and well-developed, supporting efficient water conduction during arid summer conditions. The pith region appeared large and spongy, indicating active storage of metabolic reserves. In contrast, plants growing under saline conditions near Sambhar Lake displayed significant alterations. The epidermis was slightly thickened, with a noticeably more developed cuticle, serving as a barrier against water loss and salt intrusion. The cortex appeared narrower and more compact, while sclerenchyma tissue formed thick, continuous bands likely providing enhanced structural support in the harsh saline environment. Xylem vessels were narrower and more lignified, possibly as an adaptation to regulate water flow under saline stress. The pith was smaller and denser, and tyloses were frequently observed, suggesting xylem occlusion as a stress response.

Table 1: Stem Anatomical Characteristics in Summer Season

Feature	Normal Condition (Jaipur)	Saline Condition (Sambhar Lake)
Epidermis	Thin, single-layered with compact cells	Slightly thickened, more protective cuticle layer
Cuticle	Thin, less prominent	Well-developed, thick cuticle as protective adaptation
Cortex	Broad cortex with loosely arranged parenchyma	Narrow cortex with compact cells
Sclerenchyma	Moderately developed discontinuous bands	Highly thickened and continuous bands for mechanical support
Xylem	Wide vessels and active secondary growth	Reduced vessel size; more lignified secondary xylem
Vascular Bundles	Prominent, widely spaced	More compact and closer together
Pith	Broad with large, loosely arranged cells	Compressed pith with dense parenchyma
Tyloses	Rare or absent	Frequently observed indicating stress response

In the monsoon season, both saline and non-saline conditions led to changes in stem anatomy, although the differences were more nuanced. Under normal conditions, the epidermis remained intact and smooth, with a thin and uniform cuticle. The cortex was well-developed, parenchymatous, and characterized by large intercellular spaces, indicating active physiological processes. Sclerenchyma tissues formed discontinuous patches around the vascular bundles, and the xylem vessels were wide and well-organized in a regular radial pattern. The pith region appeared large and spongy, typical of active vegetative growth. Under saline conditions, however, the epidermis showed signs of thickening, and periderm development was occasionally observed, hinting at the initiation of protective secondary growth. The cortex was less expansive and tightly packed, and sclerenchyma bands were more continuous and denser compared to the non-saline site. Vascular bundles were slightly distorted and appeared closer together, possibly reflecting stress-induced cambial adjustments. The xylem vessels were narrower, and the pith was moderately reduced and compact. Occasional tyloses were observed, reinforcing the idea of internal stress even during the relatively favourable monsoon period.

Table 2: Stem Anatomical Characteristics in Monsoon Season

Feature	Normal Condition (Jaipur)	Saline Condition (Sambhar Lake)
Epidermis	Thin and smooth, intact epidermal cells	Slight thickening, occasional periderm formation
Cuticle	Thin and uniform	Moderate thickening observed
Cortex	Loose, well-developed, with intercellular spaces	Less developed, compactly arranged
Sclerenchyma	Intermittent patches around vascular bundles	Dense bands surrounding bundles
Vascular Bundles	Organized and radial in pattern	Slightly distorted arrangement due to salt stress
Pith	Large and spongy	Dense and moderately reduced
Tyloses	Absent	Occasionally seen in some xylem vessels

During the winter season, anatomical traits again highlighted the influence of environmental conditions. In Jaipur's normal environment, the epidermis remained thin and intact, with a moderately thick cuticle. The cortex was parenchymatous and well-differentiated, and sclerenchyma bands were present in moderate thickness around vascular bundles. Xylem development was active but less extensive compared to summer, with moderately wide vessels and organized vascular arrangements. The pith retained its spongy structure with large parenchymatous cells. On the other hand, plants exposed to saline stress at Sambhar Lake exhibited further thickening of the epidermis, often accompanied by periderm formation. The cuticle was notably thickened, indicating enhanced protective measures against salt and cold-induced dehydration. The cortex was reduced and showed tightly arranged cells with fewer intercellular spaces. Sclerenchyma was highly lignified and formed continuous dense bands, while the xylem showed increased lignification and compact vessels. Vascular bundles appeared irregular and closer, indicating anatomical modifications for efficient internal regulation. The pith was significantly compressed, with reduced cell size, and tyloses were commonly observed in the xylem vessels, again marking stress-induced physiological responses.

Table 3: Stem Anatomical Characteristics in Winter Season

Feature	Normal Condition (Jaipur)	Saline Condition (Sambhar Lake)
Epidermis	Intact, protective layer	More prominent with periderm development
Cuticle	Slightly thick, stable	Noticeably thicker as an adaptation to dry-salty air
Cortex	Parenchymatous, well differentiated	Slightly collapsed, reduced intercellular spaces
Sclerenchyma	Present in bands, moderately developed	Highly lignified and continuous
Xylem	Moderate secondary xylem development	Extensive lignification and compact xylem vessels
Vascular Bundles	Regular pattern	Slight irregularity, denser packing
Pith	Prominent with healthy parenchyma	Shrunken with reduced cell size
Tyloses	Not observed	Frequently formed in xylem vessels

Overall, the anatomical responses of *P. cineraria* under saline conditions across all seasons indicate clear morphological and internal tissue-level adaptations. The formation of a thicker cuticle, denser sclerenchyma, reduced cortical and pith regions, and narrower xylem vessels all suggest strategies to conserve water, resist mechanical stress, and survive in saline environments. Tylosis formation, frequent under saline conditions, further underscores xylem stress and potential loss of hydraulic conductivity as the plant attempts to compartmentalize injury or salt accumulation. In contrast, plants growing under non-saline conditions show broader, less lignified structures, optimized for active growth, efficient conduction, and metabolic storage. These anatomical variations not only reflect the ecological resilience of *Prosopis cineraria* but also underline its adaptive plasticity in surviving Rajasthan's harsh and fluctuating environmental conditions.

Discussion

The anatomical plasticity exhibited by *Prosopis cineraria* across seasons and under saline conditions highlights the species' robust adaptation mechanisms. The significant differences observed between the Sambhar Lake (saline) and Jaipur (non-saline) populations underline how environmental stress shapes internal plant structure to optimize survival. A consistently thickened epidermis and cuticle in saline-exposed plants serve as protective barriers against excessive water loss and salt entry—key survival traits under osmotic stress (Sledge *et al.,* 2025). Furthermore, the narrower, highly lignified xylem vessels and dense sclerenchyma bands suggest an anatomical trade-off: reducing hydraulic conductivity to prevent cavitation and mechanical collapse, even at the cost of reduced water flow (Plavcova *et al.,* 2019).

Another noteworthy adaptation is the frequent development of tyloses in the xylem vessels under saline stress, particularly in winter and summer. Tyloses are indicative of vascular stress and serve to block dysfunctional vessels, preventing the spread of salt or embolism (Micco *et al.,* 2016). This supports the idea that *P. cineraria* actively compartmentalizes stress to maintain physiological integrity. The compressed and compact pith observed in saline conditions likely aids in reducing internal water loss and reflects limited metabolic activity during harsh periods (Deng *et al.,* 2018) [6].

Seasonal differences further emphasize the dynamic nature of stem anatomy in response to changing environmental cues. During monsoon, despite increased moisture availability, plants under saline stress retained features indicative of ongoing physiological strain. In contrast, the non-saline population showed traits favorable for active growth and efficient nutrient transport, such as a broad cortex and spongy pith (Ahmed *et al.,* 2013; Steppe *et al.,* 2015) [7, 8].

Overall, the interplay between anatomical structure, salinity, and seasonal variation in *P. cineraria* exemplifies its xerohalophytic nature. The species not only tolerates but functionally adjusts to fluctuating environmental pressures, making it an ideal candidate for ecological restoration programs in arid and salt-affected landscapes.

Conclusion

This study demonstrates that *Prosopis cineraria* displays significant anatomical modifications in its stem tissue in response to both salinity and seasonal variation. The adaptive traits—such as thicker epidermis and cuticle, compact cortical tissue, lignified sclerenchyma, reduced xylem vessel diameter, and frequent tylosis formation—enable the plant to maintain structural stability and physiological function under environmental stress. These features are particularly prominent in populations growing in the saline conditions of Sambhar Lake, reflecting the species' adaptive resilience to high salinity and water scarcity.

In contrast, individuals growing in non-saline conditions exhibit anatomical structures that support active physiological processes, suggesting that *P. cineraria* optimizes its internal morphology depending on habitat conditions. The study not only enhances our understanding of anatomical adaptations in xerophytic and halophytic tree species but also reinforces the potential of *P. cineraria* in afforestation, agroforestry, and the rehabilitation of degraded saline soils. Future work could focus on the correlation between these anatomical traits and physiological parameters such as transpiration rate, hydraulic conductivity, and ion accumulation to further elucidate stress adaptation mechanisms.

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