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Seasonal and Salinity-Induced Variations in Leaf Midrib Anatomy of  
*Prosopis cineraria* (L.) DruceShivani Tiwari\*<sup>1</sup>, Anupama Goyal<sup>2</sup><sup>1</sup>Research Scholar, Department of Science and Technology, Jayoti Vidyapeeth Women's University (JVWU), Jaipur, Rajasthan, India.<sup>2</sup>Professor, Department of Science and Technology, Faculty of Education and Methodology, Jayoti Vidyapeeth Women's University (JVWU), Jaipur, Rajasthan, India.\*Corresponding Author Email: [shivaniabhitiwari1902@gmail.com](mailto:shivaniabhitiwari1902@gmail.com)

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## ABSTRACT

*Prosopis cineraria* (L.) Druce, a keystone plant of arid and semi-arid environments, has notable anatomical features that facilitate it to survive in challenging environmental conditions, such as salinity and seasonal changes. The present work explores the seasonal and salinity-induced differences in the midrib anatomy of *P. cineraria* plants sampled from saline and non-saline areas during three different seasons—summer, monsoon, and winter. Comparative anatomical studies disclosed uniform alterations in midrib thickness, vascular bundle organization, mechanical tissues, cuticle formation, and parenchyma arrangement. Under salinity stress, the midrib was thicker, with increased lignification of the mechanical tissues, narrower xylem vessels, and a continuously thicker cuticle throughout all seasons, indicating structural adaptations against osmotic and ionic stress. Seasonal effects were observed in saline as well as normal conditions, with summer exhibiting maximum xeromorphic traits, monsoon revealing lenient anatomical patterns, and winter exhibiting intermediate characteristics. The research points to the active anatomical plasticity of *P. cineraria* as key to its ecologic resilience and offers great potential as a species for reforestation as well as soil rehabilitation in saline-affected habitats.

## INTRODUCTION:

*Prosopis cineraria* (L.) Druce, a dominant tree in Indian arid and semi-arid zones, shows impressive plasticity in its anatomical features against seasonal and environmental stress factors like salinity. One such key anatomical feature is the leaf midrib, which is crucial for vascular conduction, mechanical support, and adaptability (Zahedi *et al.*, 2025). Seasonal fluctuations, especially between summer, monsoon, and winter, play an important role in the development, thickness, and cellular arrangement of the midrib (Dolezal *et al.*, 2019). In summer, with high evaporative pressure, the midrib tends to display increased development of collenchymatous tissue and thick-walled xylem vessels, perhaps to support water conduction and structural strength (Pallardy, 2010).

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During monsoon, with increased availability of moisture, the midrib shows greater development of mesophyll and broader vascular bundles, favouring greater translocation of nutrients and photosynthates. The vessels in the xylem at this time can be seen to be relatively narrower and more in number, indicating lower water stress. On the other hand, during winter, low temperatures and shorter photoperiods cause vascular activity to slow down, as evidenced by narrower vascular bundles and fewer phloem elements. This midrib plasticity by season is reflective of the resilience of the species and its adaptive modulation of the physiological processes with respect to environmental fluctuation (Madhavan *et al.*, 2025).

Salinity is yet another important stressor affecting midrib anatomy in *P. cineraria*. Salinity, particularly in areas such as Sambhar Lake with high salt deposition, affects the leaf midrib by evident anatomical changes. These are in the form of higher lignification, better developed sclerenchyma, and fewer intercellular spaces that, together, lower water loss and enhance support. The xylem vessels, or vascular bundles, also get smaller with thicker walls, which is a characteristic of improved salt-induced cavitation and ion toxicity resistance. These anatomical features assist the tree in being halotolerant and its ecological dominance in salt environments (Kaleem *et al.*, 2024).

Comparing saline and normal habitats seasonally, however, a complex interaction occurs. For example, in summer in saline habitats, the anatomy of the midrib is strongly xeromorphic with decreased vascular area, denser tissues, and smaller stomatal cavities, whereas in monsoon, partial restoration of vascular structure with enhanced phloem development occurs. It points to an active anatomical response in which *P. cineraria* oscillates between maintaining physiological function and reducing stress damage. These adaptations are important for maintaining photosynthesis and water economy under combined stress conditions of salinity and seasonal extremes.

Knowing these seasonal and salinity-caused changes in leaf midrib anatomy not only offers information on the adaptive mechanisms of *P. cineraria* but also has implications for ecological restoration and agroforestry intervention in salt-affected and arid lands. These observations indicate that anatomical characteristics may be used as effective bioindicators of environmental stress and can assist in choosing stress-tolerant genotypes for afforestation schemes.

## **MATERIALS AND METHODS:**

### **Study Site and Plant Material:**

The Sambhar Lake area, in the northwestern part of Rajasthan, India, is a distinctive and ecologically valuable landscape. Geographically, the lake occurs between 26°52' to 27°02' N latitude and 74°54' to 75°14' E longitude, stretching across the districts of Jaipur, Nagaur, and Ajmer. It is India's largest freshwater saline lake, measuring about 190 to 230 square kilometers in area, though the real water-spread area varies seasonally due to fluctuations in rainfall. It is elliptical in shape and is supplied by several seasonal rivers like Mendha, Rupangarh, Kharian, and Samas.

The area falls within the ecosystem of the Thar Desert and within the semi-arid climatic region. It has very hot summers, gentle winters, and low annual rainfall (averaging about 500 mm or less), the majority falling during the monsoon season. Soils tend to be alkaline to saline, and the general topography is dry scrublands, sand dunes, and poor vegetation. The lake itself is seasonally inundated and is of high salinity, particularly during the dry season, as it is a natural repository of salt.

Owing to its saline character and variable hydrology, the area around Sambhar Lake is home to a specialized plant and animal life comprising a number of halophytic plant species and an immense avifaunal population comprising migratory birds such as Greater and Lesser Flamingos. Its geographical characteristics render it a very suitable location for ecological and environmental research, especially those concerning salt tolerance, water stress adaptation, and xerophytic plant behaviour. Vegetation around it, including *Prosopis species*, forms a useful framework for analysis of plant reactions to harsh edaphic and climatic factors.

Along with sambhar lake, samples were also collected from Jaipur, Rajasthan which has normal salinity conditions.

### **Seasonal Sampling:**

Leaf samples were collected during three distinct seasons:

Summer (March–June)

Winter (November–February)

Monsoon (July–October)

### **Anatomical Analysis:**

Transverse sections of leaf midrib were prepared using a microtome and stained with safranin and fast green. Sections were observed under a compound microscope. Morphometric data on midrib thickness, xylem area, cuticle thickness, and cell arrangement were recorded and compared across seasons and conditions.

**RESULTS AND DISCUSSION:****Summer season:**

*Prosopis cineraria* showed remarkable anatomical variations in the structure of the midrib between the plants from saline and ordinary climatic conditions during the summer season. In the case of plants from the ordinary climate, the midrib was found to be thickened and made up of dense tissues - a response to minimize transpirational water loss under desiccating conditions. Yet in the saline environment, this thickening was exaggerated, with the additional well-marked increase in mechanical tissues like sclerenchyma. This increased development most likely offers greater mechanical strength and helps to preserve turgor under saline-induced osmotic stress (Singh *et al.*, 2024).

The architecture of the vascular bundle was also distinctly altered. In control conditions, the midrib had well-differentiated xylem with small vessels, a characteristic xerophytic trait that enabled effective water conduction while reducing the threat of embolism. In saline conditions, xylem elements were more compacted and the vessel lumen was profoundly narrowed. This vessel reduction is probably a defensive strategy against high salt levels in the soil, which can enhance cavitation risk and reduce hydraulic conductivity (Stiller, 2009). By decreasing vessel size, the plant reduces its susceptibility to air bubble formation and hence ensures functional water transport under stress (Quaderiet *al.*, 2009).

Supportive tissues like collenchyma and sclerenchyma also differed significantly. A

moderate growth of collenchymatous layers promoting leaf rigidity and wilting resistance in the normal climate was observed. Plants from the saline region, however, exhibited a remarkable increase in both the sclerenchymatous and collenchymatous layers. The hardened layers offer not just mechanical support but also serve a crucial function of withstanding the combined impact of salt toxicity and severe heat stress common in saline deserts (Hussain *et al.*, 2019).

The outer epidermal layer had clear xeromorphic characters in both conditions but especially in the saline environment. Whereas the normal climate plants had a thick, cutinized epidermis, such a layer was much thicker in saline-grown plants. A well-developed cuticle is a protective mechanism against excessive loss of water and also against the entrance of salt ion, hence increasing the resistance of the plant to stressful conditions (Devi *et al.*, 2017). This is in agreement with adaptation in other desert or salt-tolerant plants.

Finally, the parenchyma tissue in the midrib area was dense and compact in both conditions, although it was denser in saline. The greater density could imply the deposition of osmoprotective metabolites like proline or other compatible solutes, which assist in preserving cell turgor and enzyme activity in saline stress. These changes in structure together emphasize the extreme plasticity of *P. cineraria*, enabling it to survive and flourish under conditions of extreme temperature as well as high salinity.

**Table 1: Anatomical features of mid rib of *Prosopis cineraria* collected from saline and normal climates during Summer Season.**

Feature	Normal Climate (Non Saline Region: Jaipur)	Saline Climate (Saline Region: Sambhar Lake)
Midrib Thickness	Thickened midrib with compact tissues to minimize water loss.	Further thickened midrib; increased mechanical tissues as an adaptive strategy to osmotic stress.
Vascular Bundles	Well-developed xylem with narrow vessels for efficient water conduction under drought.	More densely packed xylem with reduced lumen diameter to prevent cavitation under salinity-induced water stress.
Collenchyma/Sclerenchyma	Prominent for structural support against wilting.	Highly developed sclerenchyma and collenchyma layers to combat both salt and heat stress.
Cuticle	Thick, cutinized epidermis.	Thicker cuticle than normal; a xeromorphic feature enhancing salt resistance.
Parenchyma Tissue	Dense and compact.	More compact; possible accumulation of osmolytes.

**During Winter Season:**

In winter months, the anatomical makeup of the midrib in *Prosopis cineraria* showed minute but remarkable variations between plants from saline and normal environments. In normal environments, the midrib possessed a moderately thickness with equal tissue arrangement, showing less stressful conditions and relatively stable physiological processes. Yet, in the saline environment, there was a slight thickening of the midrib, even though this was less marked compared to summer. This indicates a residual but persistent anatomical adaptation to salinity even in months when temperatures are lower and both rates of evapotranspiration and metabolic processes are lower (Shabala and Muns, 2017).

The vascular system, especially the xylem and phloem, was functionally active in both climatic conditions in winter. Under normal climate, xylem vessels and phloem tissues were healthy and well distributed, reflecting unobstructed translocation processes. In saline conditions, while the xylem still possessed functional ability,

there was some amount of lignification, perhaps as a defence mechanism against ionic stress over time. Saline-induced lignification can act to strengthen vascular tissues against salt-induced mechanical and oxidative stress, increasing structural stability and avoiding vascular collapse (Mwamba *et al.*, 2019).

Mechanical tissues also indicated the effect of environmental stress. Whereas normally grown plants had moderately developed sclerenchyma layers, salt-grown ones had more lignified sclerenchymatous tissue. Such an enhancement of lignification brings structural support that is apparently needed to maintain leaf function under prolonged salinity stress even during winter. It also means that anatomical responses to stress in *P. cineraria* are not seasonally specific but continue throughout seasons in saline conditions (Riaz *et al.*, 2023).

Cuticle thickness also differed with climate. Under regular conditions, the winter epidermal cuticle was comparatively thinner because low temperatures naturally diminish transpiration requirements. In

the saline climate, however, a moderate cuticle thickening persisted. This characteristic, probably a residual xeromorphic adaptation from past high-stress environments, still offers protective coverage against intrusion of salt and dryness, showcasing the long-term anatomical acclimatization of the plant (Madhavan *et al.*, 2025).

Parenchymatous tissues in the midrib also displayed contrasting features. Under the normal climate, these tissues were loosely packed, as would be expected with lower levels of stress and less osmotic regulation requirement. Under saline conditions, the parenchyma was more compact, perhaps with higher levels of vacuolation. This would indicate the presence of an active ion sequestration mechanism in vacuoles to prevent cytotoxicity caused by excess salt accumulation. These anatomical features highlight *P. cineraria*'s enduring capacity to adjust internal tissue structure to counter ionic imbalance and environmental change, leaving it well adapted to peripheral and saline environments even in winter (Wu and Li, 2019).

**Table 2: Anatomical features of mid rib of *Prosopis cineraria* collected from saline and normal climates during Winter Season.**

Feature	Normal Climate (Non Saline Region: Jaipur)	Saline Climate (Saline Region: Sambhar Lake)
<b>Midrib Thickness</b>	Moderately thick with balanced tissue arrangement.	Slight thickening compared to normal, but less than summer.
<b>Vascular Bundles</b>	Functionally active xylem and phloem.	Xylem remains functional but may exhibit signs of salt-induced lignification.
<b>Mechanical Tissue</b>	Moderately developed.	Enhanced lignification of sclerenchyma to support structure under ionic stress.
<b>Cuticle</b>	Relatively thinner.	Moderate thickening persists as a residual response to salinity.
<b>Parenchyma</b>	Loosely arranged.	Relatively compact, possibly showing increased vacuolation to sequester ions.

**Monsoon Season:**

*Prosopis cineraria* relaxed, relatively anatomical adaptations both in saline and normal climates during the monsoon season due to enhanced water supply and lower environmental stress. Under the normal climate, the midrib remained relatively thinner and consisted of softer tissues, indicating lower mechanical constraints resulting from high soil moisture supply and lower evapotranspiration. Yet, the plants growing in the saline areas still had a moderately thickened midrib. Although osmotic stress pressure was lower during monsoon, the recurrence of salt in the rhizosphere caused the maintenance of some adaptive characteristics as a carry-over effect from earlier dry periods (Richards *et al.*, 2007).

The vascular bundles reacted differently to the seasonal change. In ordinary conditions, there were larger vessels of xylem, probably optimized for greater water conduction and nutrient supply during the active growing season. Such anatomical adaptations facilitate high metabolic activity and

photosynthesis under favorable water conditions. Xylem vessels in plants from salt areas, however, were slightly smaller in diameter. This indicates a residual constraint on hydraulic conductivity imposed by long-term monsoonal salinity, which suppresses complete anatomical relaxation even during the comparatively optimal monsoon season. This characteristic is a conservative adaptation for preventing vessel cavitation under unreliable moisture conditions (Shabala and Munns, 2017).

Mechanical tissues, such as sclerenchyma, were less prominent in normal climate plants during the monsoon. This investment reduction in structural tissue might be explained by lower mechanical stress and greater plasticity of leaf tissues in a water-rich environment. Yet, in the saline climate, a moderate degree of sclerenchymatous development was maintained. This anatomical consistency represents a type of structural memory or preconditioning that is preserved and guarantees midrib rigidity despite temporary relief from environmental stress.

The cuticle layer, an important barrier against extrinsic stressors, also underwent seasonal moderation. Under normal climatic conditions, the cuticle was thin, as expected under reduced requirements for transpiration control and salt exclusion. By contrast, the cuticle in saline climate specimens retained a moderate thickness. Even after seasonal rainfall diluted surface salts, the maintenance of a thicker cuticle indicates that the plant still protects itself against passive salt entry through diffusion through the leaf surface—a residual characteristic of xeromorphic adaptation (Schonherr *et al.*, 2006).

Parenchymatous midrib tissue also showed

differential organization. In the regular climate, such tissues were loosely organized and photosynthetically active, indicating favorable physiological conditions for growth. Under saline conditions, the parenchyma was still compact to some extent, possibly showing continued sequestration of ions in vacuoles. Such continuous compartmentalization would most likely serve as a detoxification mechanism to preserve cytoplasmic homeostasis. In general, the monsoon morphology of *P. cineraria* reflects both climatic seasonality and residual impact of saline stress, indicating the species' propensity to accommodate growth and survival strategies across different climatic regimes (Dagar *et al.*, 2016).

**Table 3: Anatomical features of mid rib of *Prosopis cineraria* collected from saline and normal climates during Monsoon Season.**

Feature	Normal Climate (Non Saline Region: Jaipur)	Saline Climate (Saline Region: Sambhar Lake)
<b>Midrib Thickness</b>	Relatively thinner midrib; softer tissues due to abundant water.	Moderately thick; shows reduced adaptive pressure but retains some salt-resistance structures.
<b>Vascular Bundles</b>	Larger xylem vessels for maximum water conduction.	Xylem vessels slightly reduced in diameter due to residual salt effects.
<b>Mechanical Tissue</b>	Less prominent sclerenchyma.	Moderate sclerenchyma persists to maintain rigidity.
<b>Cuticle</b>	Thin cuticle.	Still moderately thick to prevent salt entry via surface diffusion.
<b>Parenchyma</b>	Loosely packed, more active photosynthetic tissue.	Less loose than in normal conditions; increased ionic sequestration in vacuoles.

#### Key adaptation in mid rib in saline conditions during all seasons:

In all seasons, *P. cineraria* shows a uniformly thicker midrib in saline conditions than in normal climates. It is thickest in the summer, moderately maintained during the monsoon, and weakly expressed in winter. The thicker condition is linked with increased mechanical support and conservation of water during osmotic stress (Salinas *et al.*, 2019).

Xylem vessels in salt-rich environments are usually narrower and closely packed, particularly in summer, so as to avoid cavitation due to salt-stress water tension. Even during monsoon, when water is relatively plentiful, the xylem has smaller diameters, reflecting a persisting salt-adaptive feature. Lignification in vascular tissues is stronger during winter, providing structural support.

There is a significant concentration of sclerenchymatous and collenchymatous tissues in the midrib under salinity, especially in summer and winter. Such tissues provide structural support and resistance to ionic toxicity that maintains integrity of the midrib against salt loading and desiccation stress (Wasim and Naz, 2020).

The cuticle layer is always moderately to highly thickened in all seasons within saline environments. These xeromorphic characteristic

acts to avoid intrusion of salt by surface diffusion and minimizes transpiration for water economy and ion regulation.

Parenchyma cells in saline conditions are denser and could exhibit enhanced vacuolation, particularly during winter and monsoon periods. Such a structure allows for the sequestration of excess salts into the vacuoles, thus preserving cytoplasmic enzymatic activity and osmotic balance.

#### Common seasonal changes in mid rib in both saline and normal conditions:

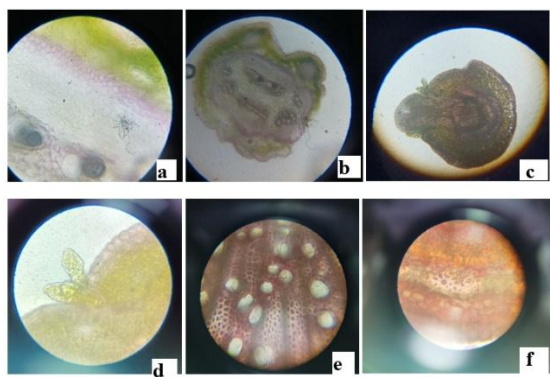
In both normal and saline environments, *Prosopis cineraria* has clear and persistent anatomical modifications of the leaf midrib in accordance with seasonal changes. In summer, during maximum thickening of the midrib in both types of habitats, it is an adaptive feature to withstand high temperature, strong solar radiation, and low water supply. This thickening is coupled with the growth of dense tissues and well-differentiated mechanical support layers, like sclerenchyma and collenchyma, that assist in wilting reduction and structural maintenance. The xylem vessels during this time are typically smaller, allowing effective water conduction with reduced danger of embolism with high transpiration pressure. There is also a thick cuticle, which provides protection against drying out. Parenchymatous cells become denser, maybe

playing a role in osmotic regulation or water storage (Skinner *et al.*, 2021).

Conversely, at the monsoon period, when water supply is in excess and environmental stress is low, midrib anatomy relaxes. Saline as well as non-saline plants have relatively thinner midribs, softer tissues, and larger xylem vessels to ensure maximum water conduction and facilitate active photosynthesis and growth. Mechanical tissues like sclerenchyma are less developed, indicating the lower requirement of rigidity in water-saturated conditions. The cuticle thins down, permitting enhanced gas exchange, and parenchyma tissues are loosely packed with wider intercellular spaces, which improve efficiency in photosynthesis (Wang *et al.*, 2012).

The winter season, *P. cineraria* exhibit intermediate anatomical traits. The midrib is moderately thick with a balanced structure for the cold conditions and reduced metabolic processes. Vascular tissue, particularly xylem, is operational under both conditions, albeit with some minimal lignification as a result of reduced temperatures and continuous ionic stress in salt regions. Mechanical tissue such as sclerenchyma exhibits moderate development, offering adequate structural support without expensive resource use. The cuticle is quite thin under normal circumstances but is still moderately thick in saline areas as a residual adaptation from previous seasons. Parenchyma tissues are loosely packed under normal circumstances, whereas in saline circumstances, they are slightly more dense, perhaps indicative of ongoing ionic sequestration and vacuolation.

Generally, the seasonal anatomical variation in the midrib of *Prosopis cineraria* under either salinity or notis evidence for its dynamic structural plasticity. It shows the fine-tuning of its internal anatomy under external seasonal signals to guarantee survival and functionality under contrasting environmental conditions.



**Figure 1:** Microscopic images of mid rib sections in different seasons and from different places.

- a- Non saline (Winter) showing well hydrated tissues with loosely arranged parenchyma, thinner cuticle, and large open xylem elements visible;
- b- Saline (Winter) showing the combination of thick midrib, dense vascular tissues, prominent sclerenchyma, and compact parenchyma;
- c- Non saline (Monsoon) showing thinner midrib, less sclerenchyma, loosely packed parenchyma, and broader vascular bundles;
- d- Saline (Monsoon) showing cuticle thickening, compact mesophyll, reinforced tissues, and pronounced vascularization justify that the leaf section was developed under saline monsoon conditions;
- e- Non saline (Summer) showing well-developed xylem vessels suited for efficient water transport. The arrangement and size of xylem vessels, along with the dense surrounding parenchyma;
- f- Saline (Summer) thicker protective layers, reinforced vascular bundles, dense ground tissue, and probable salt-sequestering zones

#### CONCLUSION:

The research establishes the fact that *Prosopis cineraria* has pronounced seasonal and salinity-triggered anatomical plasticity in its leaf midrib. These anatomical modifications are paramount for ensuring water balance, photosynthetic capacity, and mechanical rigidity under stress conditions. Such characteristics render *P. cineraria* an extremely resilient species to be used in reforestation and agroforestry initiatives in saline and desert habitats. Subsequent research must incorporate physiological measurements (e.g., osmolyte concentration, photosynthetic rate) to better define the functional importance of such anatomical plasticity. *Prosopis cineraria* is a xerophytic tree species from arid and semi-arid regions, and it possesses extensive morphoanatomical plasticity in its leaf midrib in order to respond to both seasonal and saline stress.

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