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Anatomical Modifications in *Dalbergia sissoo* from the Faisalabad Region

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ABSTRACT

The Fabaceae family, known for its agricultural significance, includes *Dalbergia sissoo*, a multipurpose tree used in reforestation and timber production. This species plays a critical role in preventing soil erosion due to its root system's suckers and contributes to nitrogen fixation. To adapt to varying environmental conditions, plants exhibit anatomical changes across different habitats. This study aimed to investigate the comparative anatomy of *D. sissoo* specimens collected from 16 sites in the Faisalabad region of Pakistan, including Gutwala, Shahkot, Gatti, Gokhowal, and others. The plant samples were preserved using formalin acetic acid alcohol solution (FAA) for short-term preservation and acetic alcohol solution for long-term preservation. Stem and leaf sections were prepared using the free-hand sectioning technique, followed by staining using the double staining method for detailed anatomical analysis. Permanent slides were prepared with Canada balsam and observed under a stereo microscope. The study revealed significant anatomical variations in response to ecological factors such as salinity, pollution, and water stress. The adaxial epidermal and sclerenchyma thickness were highest in the saline Pakka Anna ecotype, while the cortical cell area of the leaf was maximum in the pollution-affected Shahkot ecotype. Notably, lamina, midrib, phloem thickness, and metaxylem cell area were minimum in the Sahianwala and Pakka Anna ecotypes. Stomatal area and density were minimum in the water-stressed regions. Statistical analysis using ANOVA (5% probability level) demonstrated significant differences, supporting the influence of environmental stressors on plant anatomy. This research enhances our understanding of how *D. sissoo* adapts to various ecological conditions, contributing valuable insights to its conservation and agricultural use.

INTRODUCTION

Dalbergia sissoo is an important plant of enormous commercial value. It is a deciduous tree of the family Fabaceae. It grows naturally in Sub Himalayan Tarai tract from Bangladesh to Afghanistan. It is widely planted throughout the subtropical parts of South Asia, particularly in Nepal, Pakistan and India. *D. sissoo* was brought to Pakistan in the mid-nineteenth century. It is frequently used as lumber, fuel, and shelterbelts in arid and semi arid regions (13, 24).

Climate change has a significant impact on plant health, distribution, and production. As a result, plants from various plant families have distinct genetic abilities to adapt to a wide variety of environmental situations. Differences in their resistance to environmental stressors might link to anatomical changes in the stem, leaf, and root as well as physiological adaptations in parts of plants (10).

Anatomical modifications in plant body are capable of minimizing detrimental effects of salt stress. Increased succulence, deposition of wax in the stem and root, many layered thick epidermis, widening of casparian band, greater density of salt glands and hairs, thick layer of cuticle, salt secretory trichomes, highly developed parenchyma cells in the cortical region which store water and increased development of endodermis in the root are anatomical modifications of plant species which are salt tolerant (9, 11).

In many terrestrial areas, drought is a constant stress that effect the productivity of wild and agricultural plants. To survive in water limited areas, plants develop various anatomical adaptations (2, 5). The development of abundant sclerenchyma, folding of leaf by specialized bulliform cells, minerals and sclereids deposition in the wall of the epidermal cells and stomata in the grooves on the lower surface of leaves are the xeromorphic adaptations of the members of the ecologically and commercially important family Poaceae (14).

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Roadside plants are negatively impacted by several toxic byproducts that are produced as a result of the pollution in soil and air. The development of cortical and pith cells significantly reduces in the stems of the plants which are near coal producing areas. (8, 22, 25). Decrease in the size of the stomata, increased thickness of leaf as well as deposition of phenolic compounds in the parenchyma cells of the spongy and palisade mesophyll tissues are the physiological and anatomical adaptations in the various species of the Fabaceae family in response to air pollution (7).

Plants respond to deficiency of oxygen during waterlogging by various morphological and anatomical adaptations like hypertrophy in the lenticels. The development of aerenchyma tissues in the stem, leaves and roots takes place during hypoxic conditions. Aerenchyma tissues due to their wide network of hollow spaces help the plant to survive in a low level of oxygen during hypoxic conditions and help the plant to store and transport gases within the plant (12, 15).

MATERIALS AND METHODS

Stem and leaf samples of different specimens of *Dalbergia sissoo* were collected from different sites of the Faisalabad region. *D. sissoo* samples of each leaf and stem were collected from Gutwala, Shahkot, Gatti, Gokhowal, Dijkot, Satiana, Abbaspur, Makkuana, Kohala Chak, Khurianwala, Chicha, Bawa Chak, Madina Abad, Samundri, Pakka Anna (waterlogged saline area) and Sahianwala (dry saline area) for comparing the anatomical modifications in different samples. Stem and leaf samples of *D. sissoo* were collected from all the above mentioned habitats and immediately placed and sealed in bags of polyethylene. Samples were conveyed to the Botany Laboratory at the University of Agriculture, Faisalabad. After that leaf and stem samples were cut into long pieces of about 5cm and preserved for anatomical studies. To study the leaf and stem anatomy of *D. sissoo* sections were preserved in formalin acetic acid alcohol solution (FAA). V/V 35% distilled water, 50% ethyl alcohol, 10% acetic acid and 5% formaldehyde solution were used to prepare the formalin acetic acid alcohol solution. After preservation of samples in formalin acetic acid alcohol solution for 48 hours, samples were then fixed in an acetic alcohol solution (V/V 75% ethyl alcohol and 25% acetic acid) to preserve for long time.

Sectioning of stem and leaf samples was done by the free hand sectioning technique. The sections were dehydrated with a series of ethanol (30%, 50%, 70%, 90% and 100%), stained with safranin (1%) and fast green (1%), and mounted on slides using Canada balsam as described by Ruzin (1999). Samples were observed under stereo microscope (Nikon 104, Japan) containing a digital camera. Measurements of parameters were taken with the help of photographs taken by using stereo microscope.

Statistical analysis

The data of different samples of *Dalbergia sissoo* collected from different sites of the Faisalabad region were statistically analyzed by using Analysis of Variance (ANOVA) to investigate differences in the anatomy of the different parts of the plants including leaf and stem. Statistix 8.1 software was also used.

RESULTS

Stem anatomy

Khurianwala ecotype showed the maximum epidermal thickness. Madina Abad, Shahkot and Gokhowal ecotypes showed moderate values, while the minimum epidermal thickness was recorded in the Sahianwala ecotype.

Madina Abad ecotype showed the maximum vascular bundle area. The minimum vascular bundle area was recorded in the Sahianwala ecotype, while the Samundri, Gutwala and Chicha ecotypes showed moderate values.

Bawa Chack ecotype showed the maximum cortical thickness, while the minimum cortical thickness was recorded in the Kohala Chak ecotype. Gatti and Samundri ecotypes showed moderate values.

The maximum phloem thickness was recorded in the Gutwala ecotype, while the minimum phloem thickness was present in the Sahianwala ecotype. Abbaspur and Shahkot ecotypes showed moderate values of phloem thickness.

Metaxylem cell area was the maximum in the samples of *D. sissoo* collected from Abbaspur, while metaxylem cell area was the minimum in the Pakka Anna ecotype. Gatti, Dijkot and Abbaspur ecotypes showed moderate values.

The number of vascular bundles were the maximum in the Khurianwala ecotype. The minimum number of vascular bundles were recorded in the Pakka Anna ecotype, while the number of vascular bundles from Abbaspur, Madina Abad and Shahkot ecotypes showed moderate values.

Shahkot ecotype showed the maximum sclerenchyma thickness, while the minimum sclerenchyma thickness was recorded in the Pakka Anna ecotype. Gatti and Gokhowal ecotypes showed moderate values.

Gokhowal ecotype showed the maximum stem radius. The minimum stem radius was recorded in the Pakka Anna ecotype, while Khurianwala and Bawa Chak ecotypes showed moderate values of stem radius.

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Figure 1
Stem anatomy of *Dalbergia sissoo* collected from the Faisalabad region

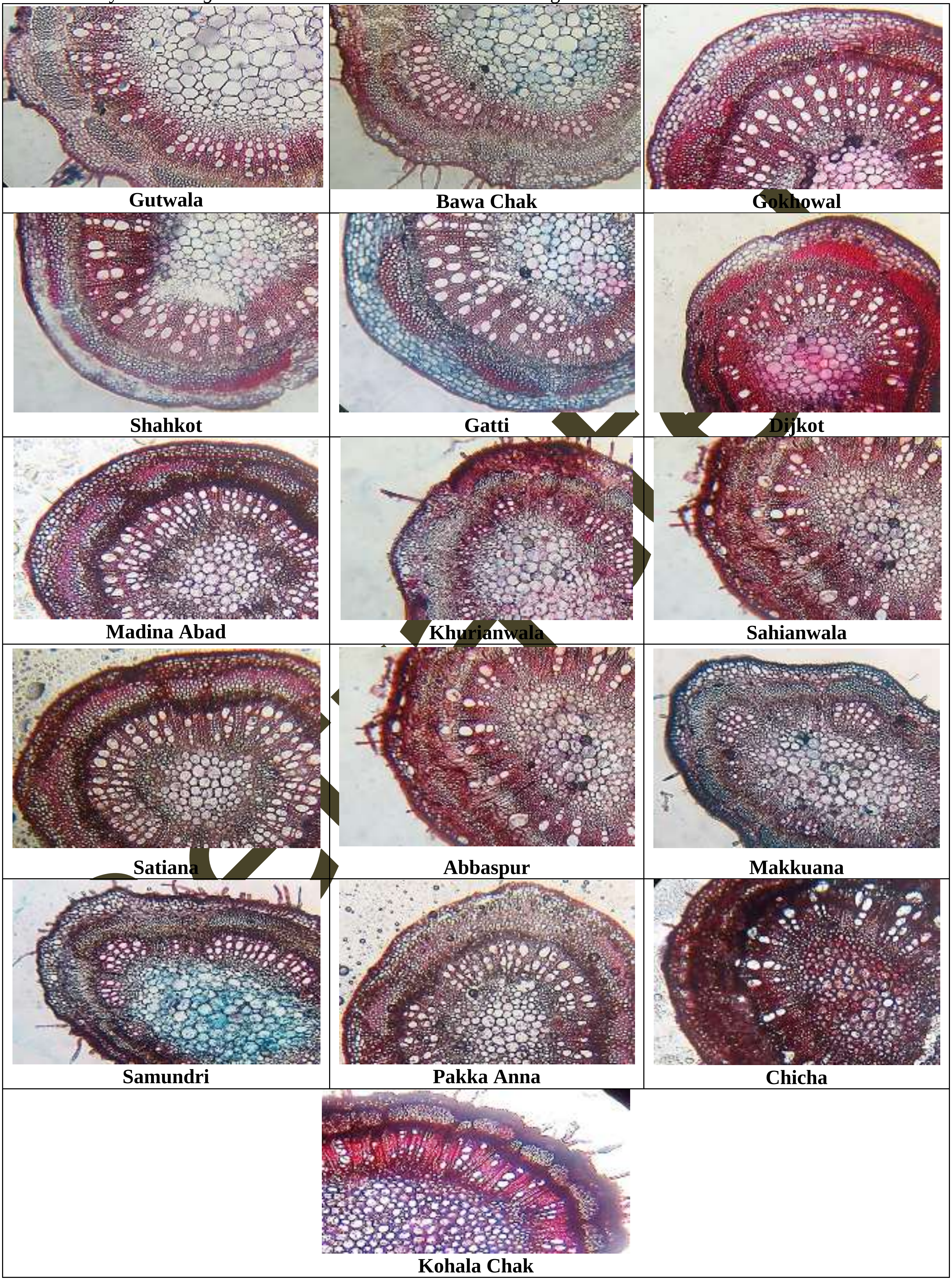

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Figure 2
Stem anatomical characteristics (epidermal thickness, vascular bundle area and cortical thickness) of *Dalbergia sissoo* collected from the Faisalabad region

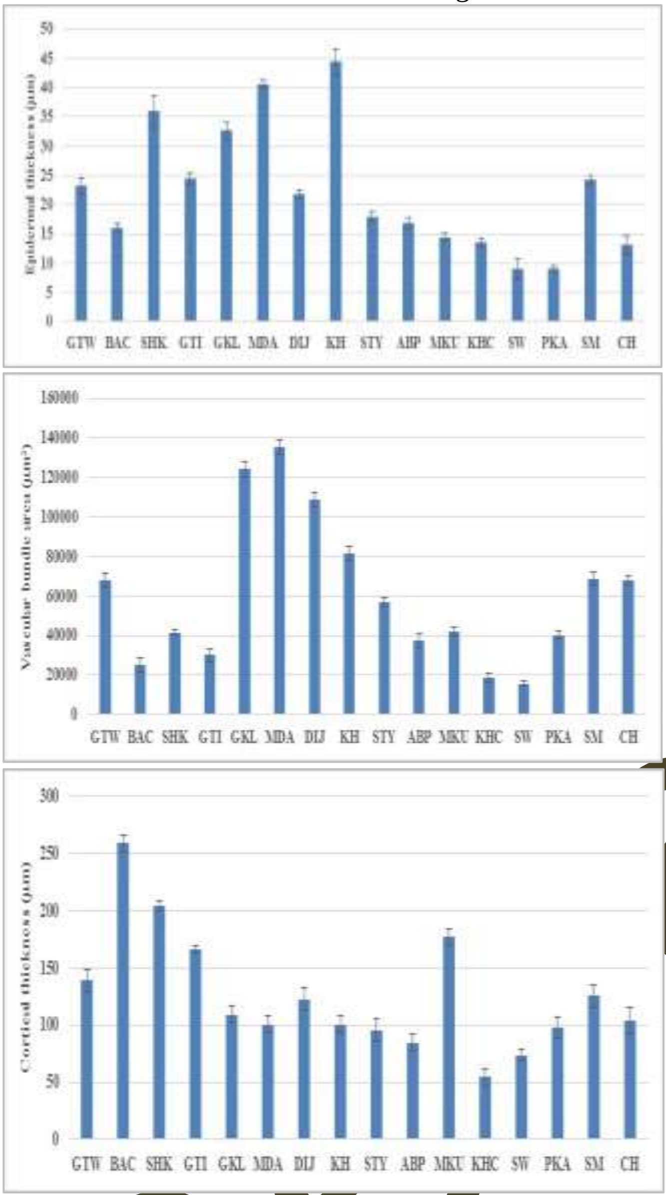
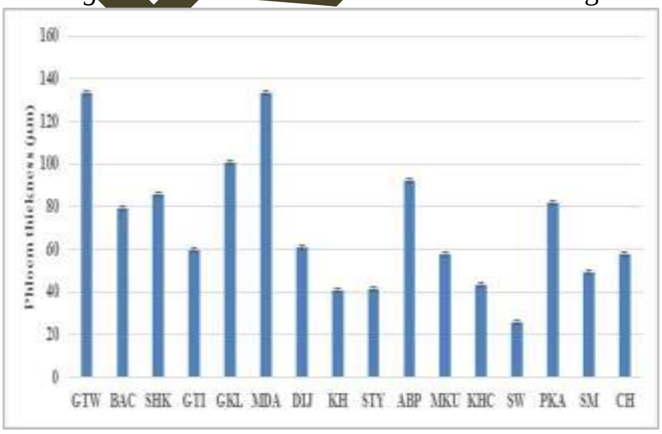


Figure 3
Stem anatomical characteristics (phloem thickness, metaxylem cell area and number of vascular bundles) of *Dalbergia sissoo* collected from the Faisalabad region



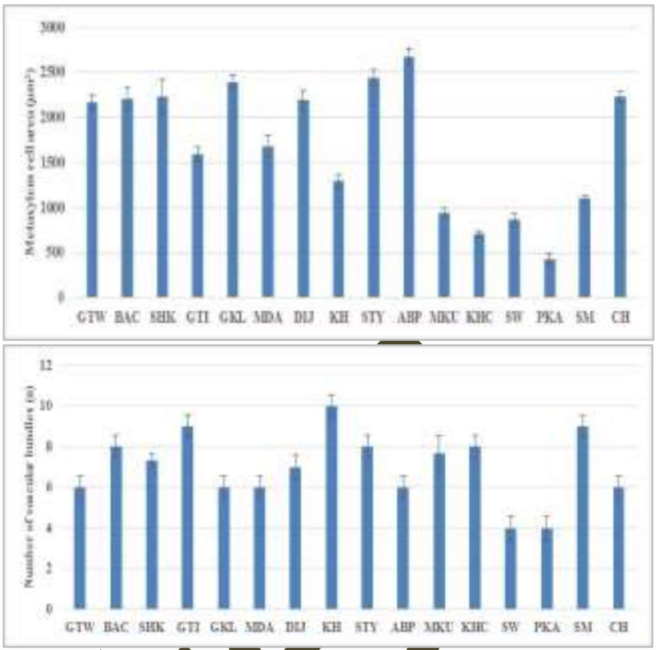
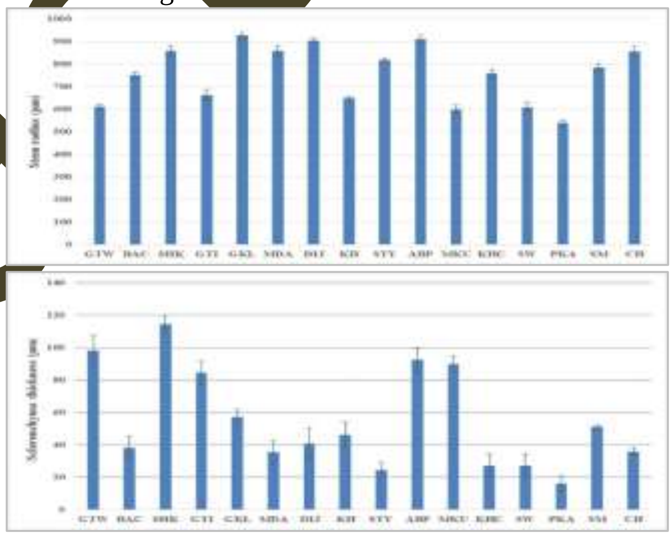


Figure 4
Stem anatomical characteristics (sclerenchyma thickness and stem radius) of *Dalbergia sissoo* collected from the Faisalabad region



Leaf Anatomy

Satiana ecotype showed the maximum lamina thickness, while the minimum lamina thickness was recorded in the Sahianwala ecotype. Abbaspur and Makuana ecotypes showed moderate values.

Pakka Anna ecotype showed the maximum adaxial epidermal thickness, while the minimum adaxial epidermal thickness was recorded in the Shahkot ecotype. Gatti and Gokhowal ecotypes showed moderate values.

Dijkot ecotype showed the maximum midrib thickness, while Makuana and Bawa Chak ecotypes showed moderate value. The minimum midrib thickness was present in the Kohala Chak ecotype.

Gatti ecotype showed the maximum abaxial epidermal thickness, while the minimum abaxial

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epidermal thickness was recorded in the Pakka Anna ecotype. Kohala Chak and Gokhowal ecotypes showed moderate values.

Cortical cell area was the maximum in the Shahkot ecotype. Samundri, Gatti and Kohala Chak ecotypes showed moderate values. Cortical cell area was the minimum in the Pakka Anna ecotype.

Pakka Anna ecotype showed the maximum sclerenchyma thickness, while the Gatti ecotype showed the minimum sclerenchyma thickness. Kohala Chak, Sahianwala and Khurianwala ecotypes showed moderate value.

Metaxylem cell area was the maximum in the Samundri ecotype, while the minimum metaxylem cell area was recorded in the Sahianwala ecotype. Gokhowal and Shahkot ecotypes showed moderate value.

The maximum phloem thickness was present in the Kohala Chak ecotype, while the minimum phloem thickness was recorded in the Sahianwala ecotype. Gatti and Shahkot ecotypes showed moderate value.

The number of vascular bundles were the maximum in the Bawa Chak ecotype, while the minimum number of vascular bundles were recorded in the Pakka Anna ecotype. Abbaspur, Satiana and Gokhowal ecotypes showed moderate values.

Cortical thickness was the maximum in the Gatti ecotype, while Abbaspur and Makkuana ecotypes showed moderate values. The minimum cortical thickness was recorded in the Sahianwala ecotype.

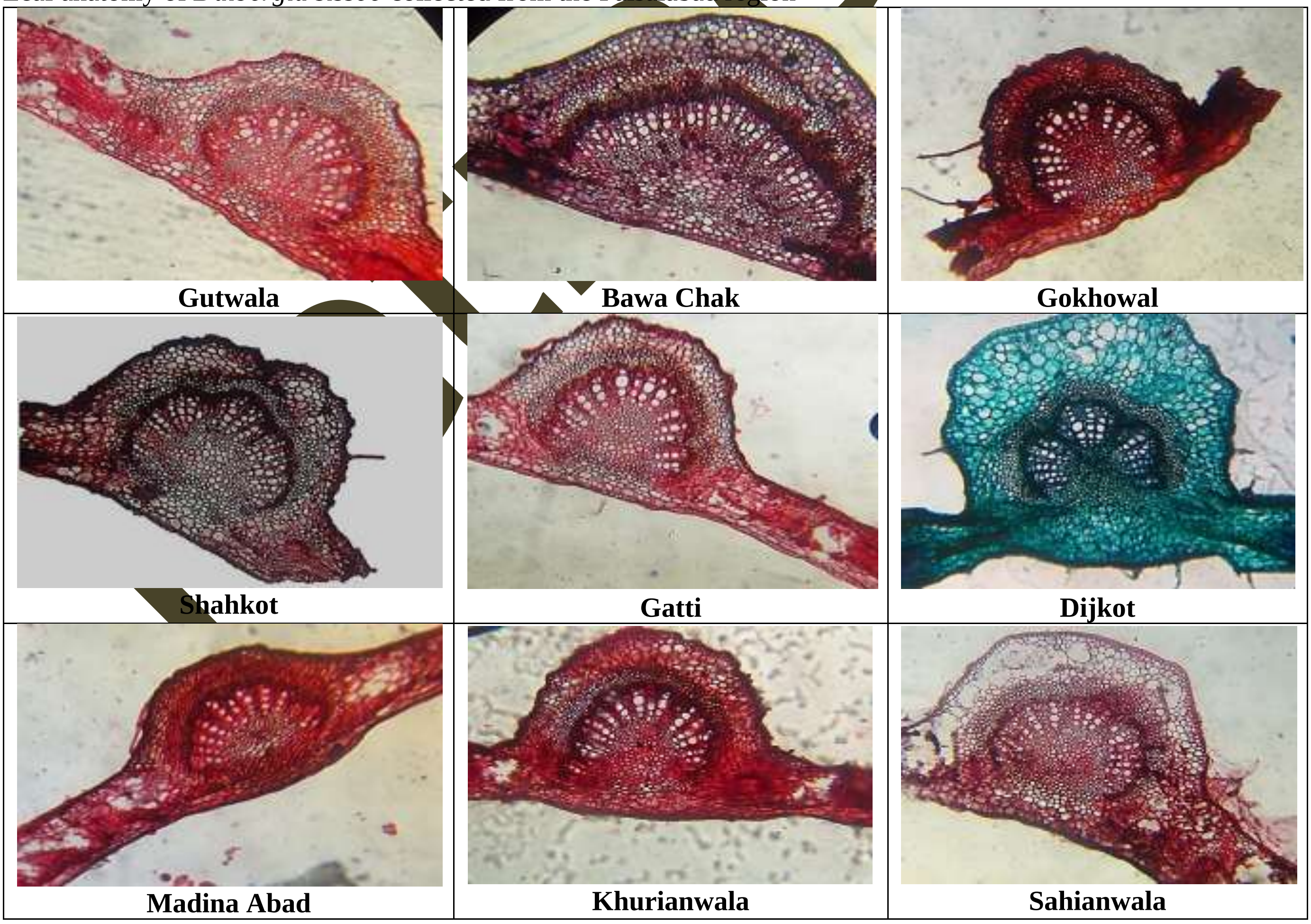
Gutwala ecotype showed the maximum abaxial stomatal numbers, while Gatti and Khurianwala ecotypes showed moderate values. The minimum abaxial stomatal numbers were recorded in the Pakka Anna ecotype.

Abbaspur ecotype showed the maximum abaxial stomatal area. Gatti and Satiana ecotypes showed moderate values. The minimum abaxial stomatal area was recorded in the Pakka Anna ecotype.

Abbaspur ecotype showed the maximum adaxial stomatal area. Gatti and Shahkot ecotypes showed moderate values, while the minimum adaxial stomatal area was recorded in the Pakka Anna ecotype.

Madina Abad ecotype showed the maximum adaxial stomatal numbers, while Dijkot and Gutwala ecotypes showed moderate values. The minimum adaxial stomatal numbers were seen in the Sahianwala ecotype.

Figure 5
Leaf anatomy of *Dalbergia sissoo* collected from the Faisalabad region



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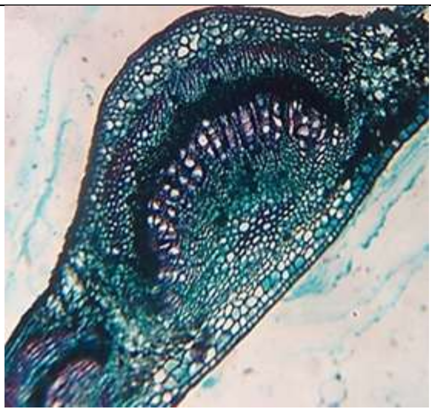
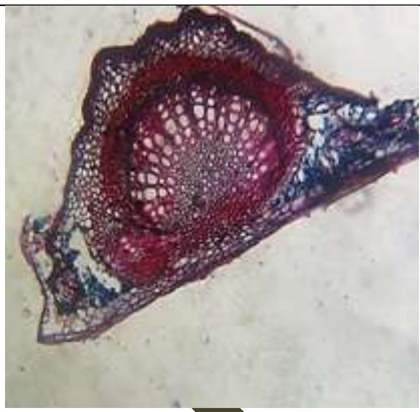
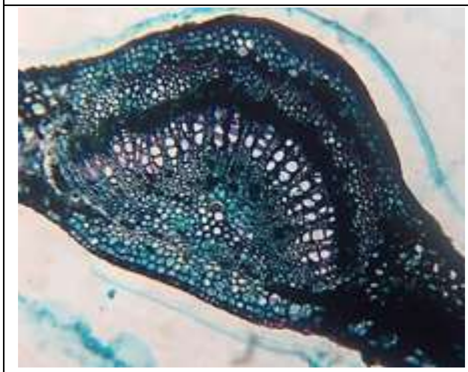
Satiana


Abbaspur


Makkuana

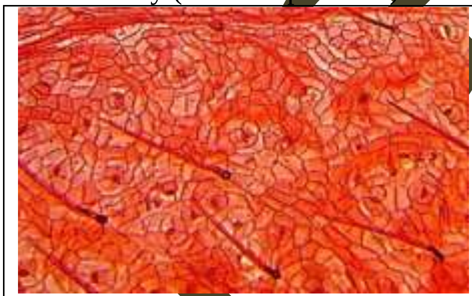

Samundri


Pakka Anna


Chicha


Kohala Chak

Figure 6
Leaf anatomy (abaxial epidermis) of *Dalbergia sissoo* collected from the Faisalabad region


Gutwala


Bawa Chak


Gokhowal


Shahkot


Gatti


Dijkot

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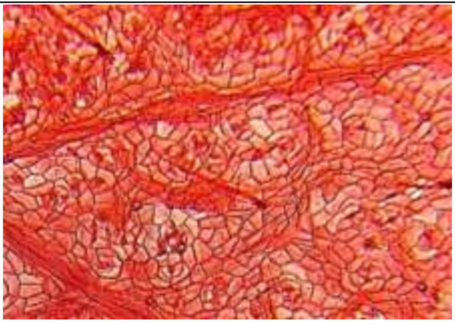
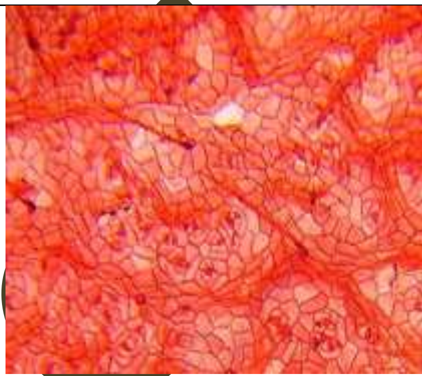

Madina Abad


Khurianwala

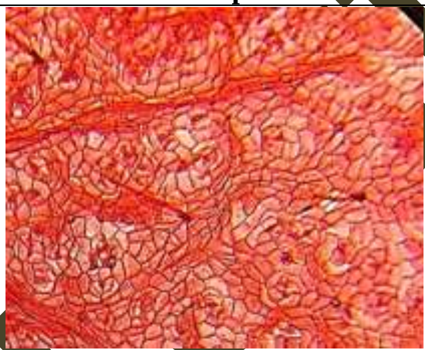

Sahianwala


Satiana


Abbaspur


Makkuana


Samundri


Pakka Anna


Chicha


Kohala Chak

Figure 7
Leaf anatomy (adaxial epidermis) of *Dalbergia sissoo* collected from the Faisalabad region


Gutwala


Bawa Chak


Gokhowal

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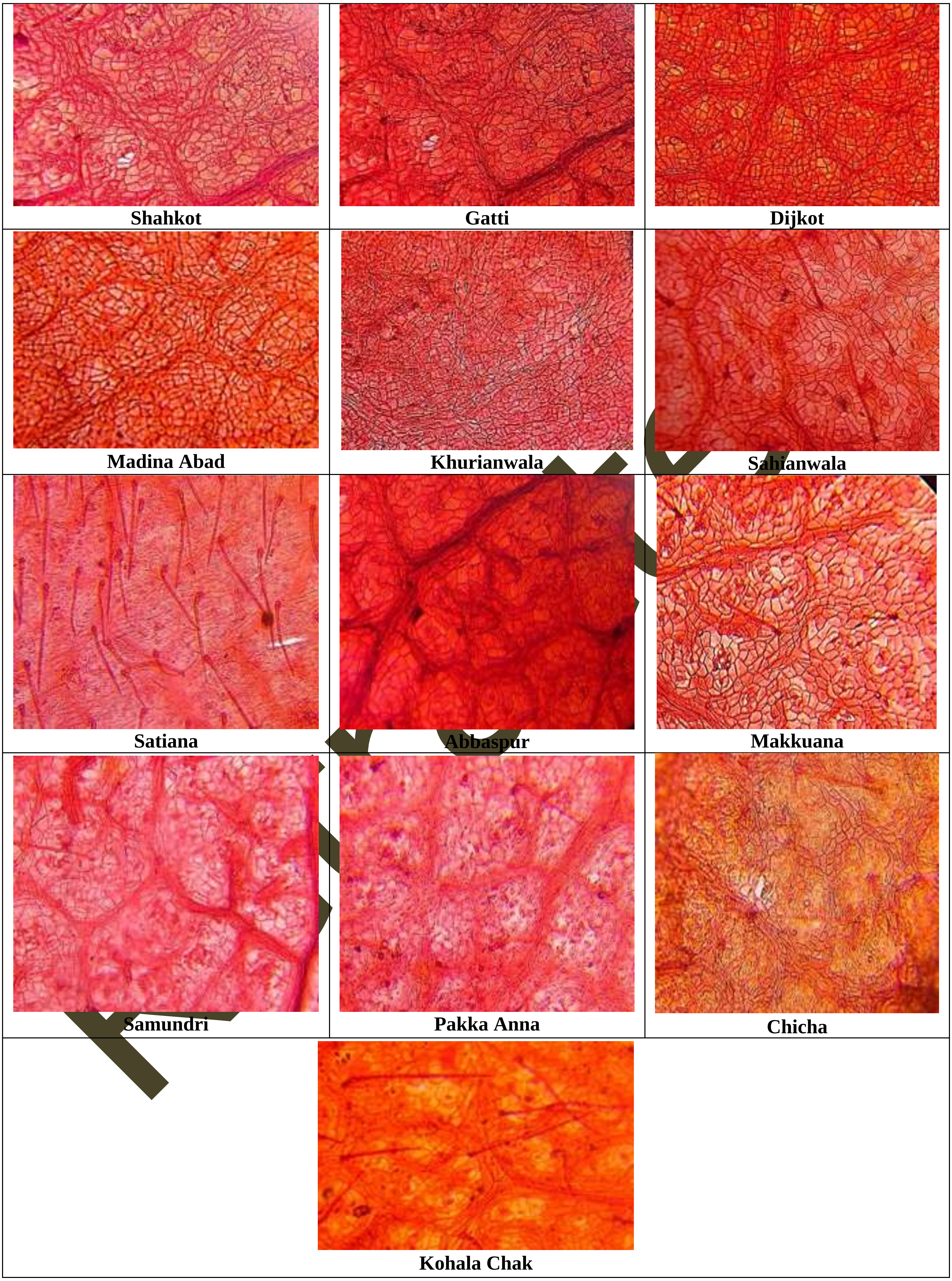

Figure 8

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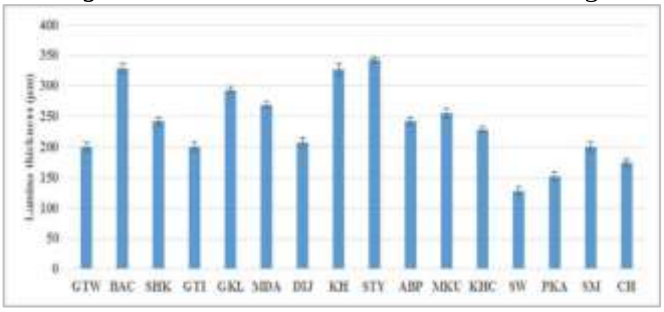
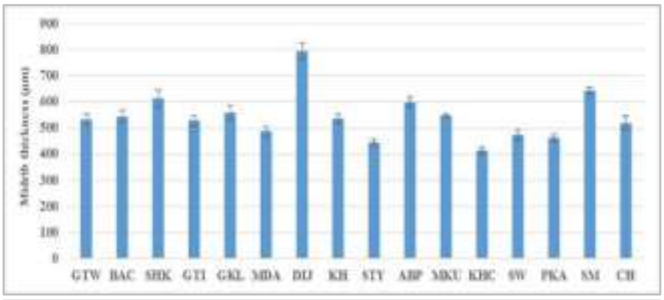
Leaf anatomical characteristics (lamina thickness, midrib thickness and adaxial epidermal thickness) of *Dalbergia sissoo* collected from the Faisalabad region





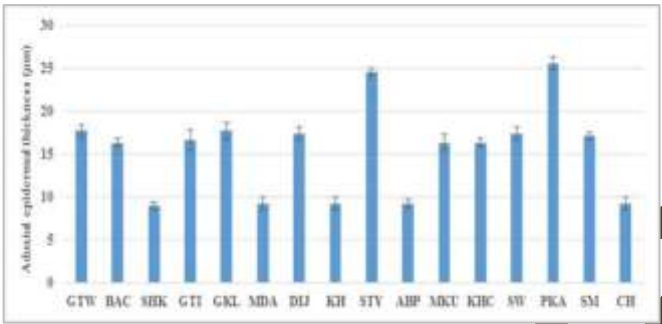
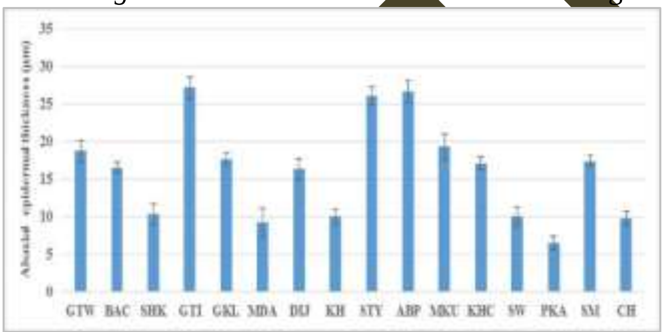
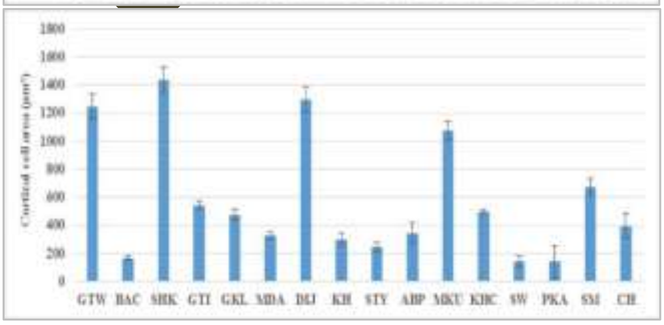


Figure 9

Leaf anatomical characteristics (abaxial epidermal thickness, cortical cell area and sclerenchyma thickness) of *Dalbergia sissoo* collected from the Faisalabad region





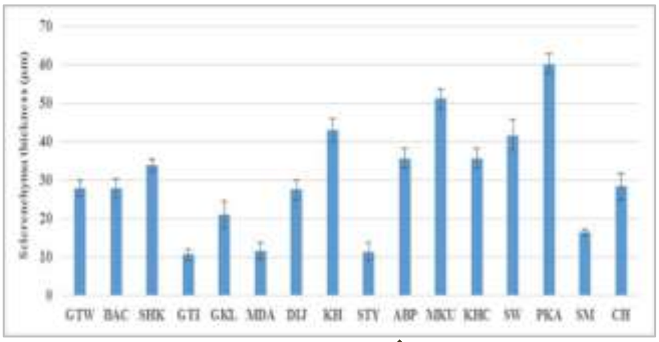
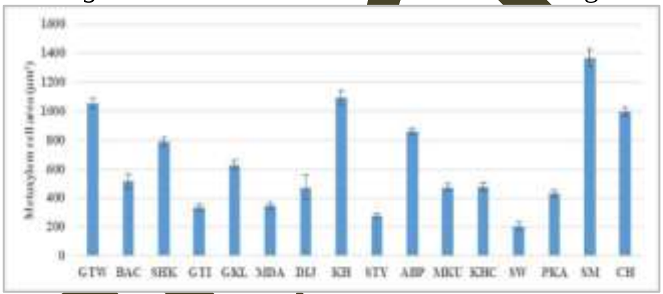
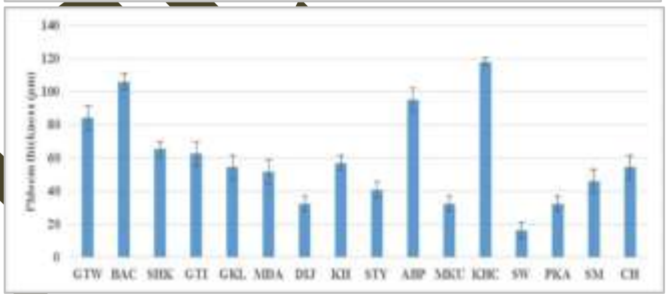


Figure 10

Leaf anatomical characteristics (metaxylem cell area, phloem thickness and number of vascular bundles) of *Dalbergia sissoo* collected from the Faisalabad region





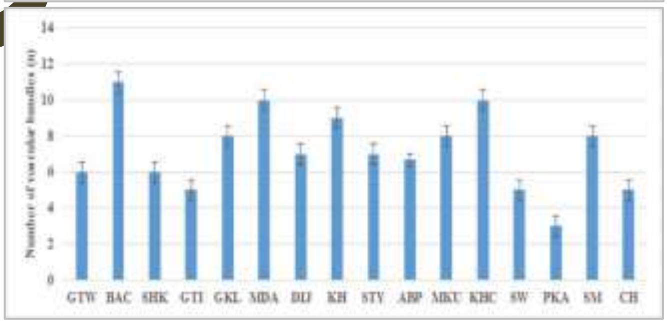
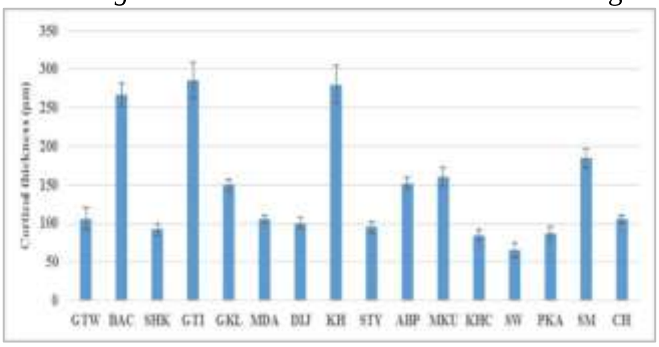


Figure 11

Leaf anatomical characteristics (cortical thickness, abaxial stomatal numbers and adaxial stomatal numbers) of *Dalbergia sissoo* collected from the Faisalabad region



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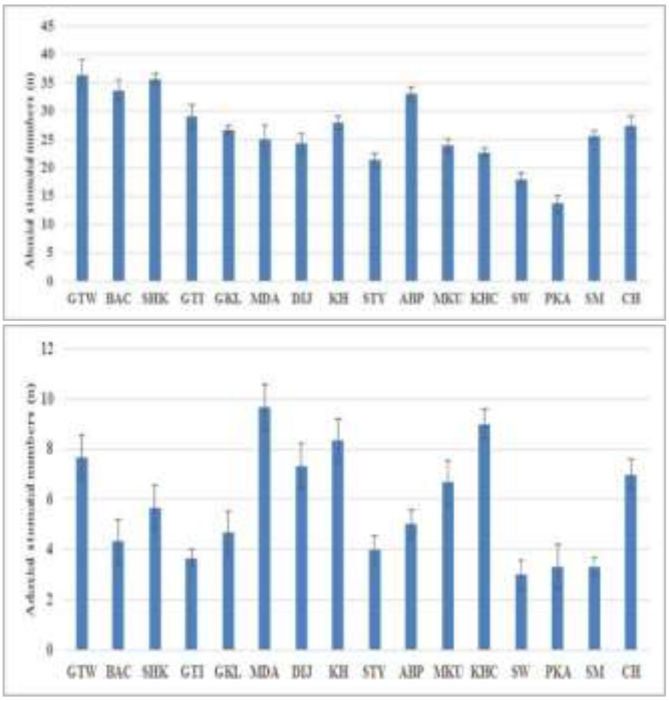
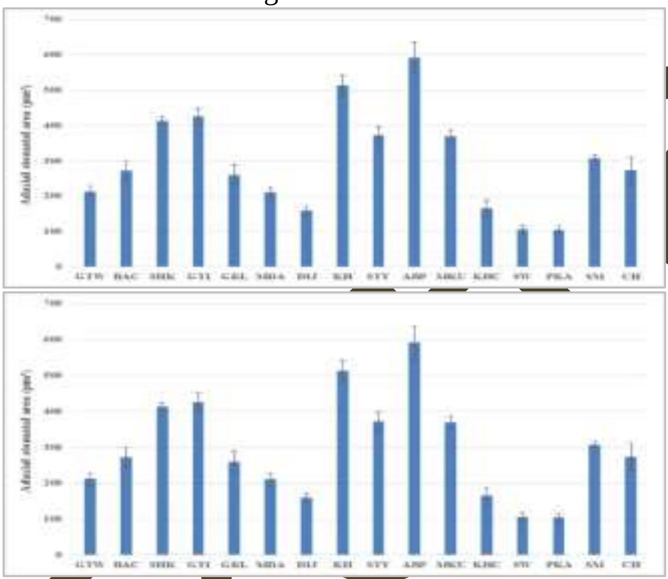


Figure 12

Leaf anatomical characteristics (adaxial stomatal area and abaxial stomatal area) of *Dalbergia sissoo* collected from the Faisalabad region



DISCUSSION

One of the most important member of the Fabaceae family is *Dalbergia sissoo* commonly known as tahli, sisu, jag, shisham and iruguduyam in different regions of the world. Shisham is one of the main tree species frequently suggested for planting programs in arid areas for the preservation of water and soil as well as to produce firewood. Low nitrogen soil is also suitable for this plant. It is useful for afforestation and reforestation in various regions of the world due to its flexibility, hardiness, nitrogen fixing ability and multifunctional nature. *D. sissoo* also act as a shelter belt.

Plants with different anatomical modifications are more likely to survive under varied environmental conditions. Plant specific anatomical adaptations in the leaves, roots and stem are essential for surviving in harsh climates. The anatomy of the plants is significantly impacted by environmental conditions. Anatomical features act as an indicator of how plants have adapted to environmental conditions (11). Each plant has unique adaptations in its anatomy, and they are extremely susceptible to environmental conditions. As a result, adaptations in the anatomy are useful in identifying various plant species and assessing their ability to withstand abiotic and biotic stress.

In the present study, *D. sissoo* plant samples were collected from several sites in the Faisalabad region and examined for their anatomical modifications. Reduced numbers and area of the stomata on the adaxial and abaxial surface of the leaf are important strategies to prevent excessive water loss through transpiration when water availability is low (1). Leaf samples from Sahianwala and Pakka Anna ecotypes showed a reduction in the size and numbers of stomata. A great number of earlier reports are in relative accordance with the present study (21).

Stem radius decrease in waterlogged areas. In the present study, stem radius was the maximum in the Gokhowal ecotype and it was the minimum in the Pakka Anna ecotype. Studies on wheat are in relative accordance with the present study (16). Saline conditions reduce the number of vascular bundles and disorganized the metaxylem and protoxylem (3). The numbers of vascular bundles in the stem and leaf were the minimum in the Pakka Anna and Sahianwala ecotypes.

Epidermal thickness of the stem increase in water stressed conditions of drought (4). In the present study, epidermal thickness of the stem was the maximum in the Khurianwala ecotype while it was the minimum in the Pakka Anna and Sahianwala ecotypes. Pakka Anna and Sahianwala both are saline regions and the reduction in the thickness of the epidermis of the stem in saline environments was also confirmed by the previous researchers (27).

Thick cuticle and thick epidermis are essential to stop the water loss from the surface of leaves in water stressed areas (20). The adaxial epidermal thickness of the leaf was the maximum in the plant samples collected from Pakka Anna and Satiana and it was the minimum in the Shahkot ecotype. Physiological drought caused by salinity increased the epidermal thickness. The abaxial epidermal thickness of the leaf was the maximum in the plant samples collected from the Gatti, Abbaspur and Satiana.

Plants that can tolerate salinity have reduced lamina and midrib thickness (16), which may help to maintain turgor and water contents in the leaves (17). In the present study, lamina thickness was the maximum in the Satiana and Bawa Chak ecotypes, while midrib thickness was the maximum in the plant samples collected from

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Dijkot and Samundri and both were the minimum in the salinity effected Pakka Anna and Sahianwala ecotype. Increased cortical cell area of leaf is important strategy of plants in pollution affected areas (18). Cortical cell area in the leaf was the maximum in the plant samples collected from Shahkot and it was the minimum in the Bawa Chak and Sahianwala ecotypes.

Stress factors disturb the development of sclerenchyma fibers in the leaves of the Poaceae family members (6). The plants need to increase the thickness of sclerenchyma in leaves with increasing salinity because it provides rigidity to the parts of the plants. Despite giving plant organs mechanical strength, sclerenchyma is a xerophytic feature that has been observed to minimize wilting damages. In the present study, sclerenchyma thickness was the maximum in the Pakka Anna ecotype, while it was the minimum in the Gatti ecotype.

Metaxylem cell area was the maximum in the Samundri ecotype leaves and Abbaspur ecotype stem, while it was the minimum in the leaves and stem of the Sahianwala and Pakka Anna ecotypes. Phloem thickness was the maximum in the Kohala Chak ecotype leaves and Gutwala ecotype stem, while it was the minimum in the stem and leaves of the Sahianwala ecotype. Phloem thickness and metaxylem cell area of the stem and leaf of plants decrease in highly salinized areas. This has been reinforced by the early researchers (19).

Cortex in stem and leaves is used to store and transport nutrients and its thickness is greatly reduced in waterlogged conditions (26). In the present study, the cortical thickness of the stem was the maximum in the Bawa Chak ecotype and it was the minimum in the Sahianwala and Kohala Chak ecotypes. While cortical thickness of the leaf was the maximum in the Gatti ecotype and it was the minimum in the Pakka Anna and Sahianwala ecotypes.

CONCLUSION

The study reveals significant anatomical adaptations of *Dalbergia sissoo* in response to environmental stressors such as salinity, drought, waterlogging, and pollution. Variations in stomatal density, epidermal thickness, vascular structure, and sclerenchyma provide key insights into how the plant copes with stress conditions. These anatomical modifications, which differ across various ecotypes, enhance the plant's ability to conserve water and withstand abiotic stress, making *D. sissoo* a valuable species for afforestation and reforestation efforts in challenging environments.

ABBREVIATIONS

GTW- Gutwala, BAC-Bawa Chak, SHK-Shahkot, GTI-Gatti, GKL-Gokhowal, MDA-Madina Abad, DIJ-Dijkot, KH-Khurianwala, STY-Satiana, ABP-Abbaspur, MKU-Makkuana, KHC-Kohala Chak, SW-Sahianwala, PKA-Pakka Anna, SM-Samundri, CH-Chicha

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