



Research Article

Euphorbia helioscopia L.: A Morphological and Anatomical Study of Wild Populations in Faisalabad

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Abstract | The family Euphorbiaceae ranks as the sixth largest among the Anthophyta class of plants and has 340 genera and 9000 species and is found in many parts of the world. Plants of this family exhibit various habits, including annuals, perennial herbs, shrubs, and trees. The distinguishing trait of plants in this family is the production of milky latex due to their succulent nature, enabling them to withstand or withstand water scarcity conditions. Various ecotypes of this species were gathered from distinct regions within the Faisalabad area. Various species of the *Euphorbia helioscopia* genus were randomly collected for morpho-anatomical examinations of their roots, stems, and leaves. The freehand sectioning technique was employed to perform section cutting of roots, stems, and leaves. Slides were prepared for long-term use after undergoing the staining process. Furthermore, the data was processed by using ANOVA technique. The primary objective of this investigation is to analyze, categorize, and confirm the existing classification of these taxa. This study presented information regarding the morphological and anatomical adaptations of *Euphorbia helioscopia* in response to different environmental changes. Anatomical modifications, such as enhanced thicknesses of root epidermis and endodermis, increased stem area, and greater thickness and cell area of the cortical region, have a notable impact in various habitats. Ultimately, *Euphorbia helioscopia* exhibited distinct alterations in its physical structural characteristics, which indicate its ability to thrive in diverse environments.

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Keywords | *Euphorbia helioscopia*, Anatomical, Morphological characteristic, Faisalabad, Pakistan



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Introduction

Pakistan's landscape exhibits distinct environmental changes, with frigid alpine regions in the north

and scorching areas near the southern coastline of the Arabian Sea (Sha et al., 2024). Except for a few desert places that receive less than 123 millimeters of precipitation each year, the climate is mostly

characterized by arid, semi-arid, and subtropical conditions (Naorem *et al.*, 2023). From June to September, the southwest monsoon produces copious precipitation (Saikranthi and Chiranjeevi, 2022). Temperature variations are primarily observed at high altitudes and just before the monsoon season. Nevertheless, in plain places, temperatures can rise to as high as 35°C to 40°C (Karki *et al.*, 2020). Deserts are characterized by high temperatures of 53°C and minimal precipitation (González-Pinilla *et al.*, 2021). In northern locales, the majority of the year is characterized by relatively low temperatures, with winter temperatures typically falling below freezing (Hansen *et al.*, 2014).

The family Euphorbiaceae, also called the spurge family, is a large family of flowering plants with 300 genera and around 7,500 species (Islam *et al.*, 2019). Most spurges are herbs, but some, especially in the tropics, are shrubs or trees. Some are succulent and resemble cacti (Alcántara-Rodríguez *et al.*, 2021). The majority of the plants in this family are herbs, shrubs, and trees, and one distinctive feature of the Euphorbiaceae family is the production of milky fluid from the stem and leaves (Althobaiti, 2023). This family occurs mainly in the tropics, with the majority of the species in the Indo-Malayan region and tropical America.

The members of the Euphorbiaceae family hold great economic importance for our nation due to its importance in the food and medicinal sector. Additionally, the members of these family used as ornamental plants and for industrial purpose specially in paints industry (Lubbe and Veerpooti, 2011). *Euphorbia helioscopia* L., known as “Zeqi” in China, is a globally distributed medicinal plant that has been extensively utilized for many years in the treatment of various ailments such as edema, phlegm and cough, malaria, dysentery, scab, tuberculous fistula, osteomyelitis, and cancer (Yang *et al.*, 2021). In various regions of Faisalabad, a prominent city in Pakistan, trees are highly beneficial in multiple ways (Rahman *et al.*, 2022). These include their contribution to timber production, provision of food and animal feed, creation of hedges, enhancement of landscapes, and formation of boundaries. They can be found in the regions of Jaranwala, Manawala, Gatwala, Samundri, and Sahianwala, as well as in the entire southern region of Pakistan (Khan and Hassan, 2003). This species is distributed in several regions of Pakistan

and West Africa (Zahra *et al.*, 2014). The primary objective of this investigation is to analyze, categorize, and confirm the existing classification of these taxa. Additionally, to conduct detailed morphological and anatomical studies to identify unique characteristics that could support or refute the existing classification.

Plant anatomical investigations are employed with other systematic lines of evidence in Plant Taxonomy to achieve advantageous taxonomic decision-making (Lardos *et al.*, 2024). The reason for this is that anatomical traits are both consistent and preserved and therefore, they are used as taxonomic characteristics in the field of plant systematics and taxonomic research (Nibbelink and Tomescu, 2022). The structure of roots, trichomes, stems, stomata, epidermis, wood, nodes, sclereids, fibers, cambium, and leaves is used in biosystematics and taxonomic investigations to classify plants, ascertain genetic connections, and settle taxonomic disagreements (Mustafa *et al.*, 2021). Our study will pave the path for the researchers to do further experiments in order to understand the mechanisms and anatomy of the euphorbiaceae family from different regions.

Materials and Methods

Collection and identification of plant samples

Euphorbia helioscopia L an indigenous plant, has been gathered from several physiologically unique areas. A variety of plant species was chosen from eight separate geographical regions. The collection was carried out according to the habitat and ecology of the chosen species. *Euphorbia helioscopia* L was collected from various ecological regions in Faisalabad, including Jaranwal, Manawala, Gatwala, Samundri, Sahianwala, Old Botanical Garden, New Botanical Garden, and Khurianwal.

Section cutting

The roots, stems, and leaves were meticulously dissected into little fragments using an aluminum blade. To prevent dehydration, the sections were stored in petri dishes filled with water. The slides were examined under a light microscope to determine their eligibility for staining. To attain favorable results, the most appropriate elements were converted into ceramic plates through staining (Kiernan, 2000).

Staining

The highest quality sections were preserved in a

solution containing 30% alcohol for a duration of 12 to 15 minutes. Subsequently, the piece was immersed in a solution consisting of 50% and 70% alcohol for 15 minutes. The dried alcohol sections were subjected to a brief exposure of approximately 15 minutes to a small amount of safranin by following (Ruzin, 1999). The samples were pigmented or dyed. The additional strain was subsequently eliminated from the sections by introducing them to 95% alcohol. These portions had a duration of approximately five minutes. A single drop of quick green was applied to the sections, and because of its reactivity, it was promptly eliminated. Fast green was fully eradicated using 100% ethyl alcohol after being administered three or four times. Administer a small amount of xylene solution at the conclusion (Bancroft, 1996).

Preparation of slides

Pictures of the glass were captured to create presentation slides. The slides were permanently covered with Canada balsam using a needle. The components were positioned on slides that had been coated with Canada balsam, and subsequently, they were moved and covered with a cover slip. Anatomical analysis of the slides was conducted using a light microscope. The photographs were created to have elaborate and precise details.

Morphological and anatomical characteristics

The shoot and root lengths were determined using a meter ruler. The leaf count was determined using manual enumeration. The measurement of the leaf area was conducted. The electrical balance was used to measure shoot weight, fresh weight, and root fresh weight. The shoot, root, and dry weight were measured using an electrical balance. The root becomes desiccated. Furthermore, For example, the study of the structure of roots Diameter of the root and thickness of the epidermis the thickness of the sclerenchyma tissue Surface area of the epidermal cells Cortical thickness Area of the cortex cell Thickness of the endoderm. The vascular bundle refers to a cluster of specialized tissues that transport fluids and nutrients throughout a plant. Encompassing the structure of plant stems Radius of the stem Thickness of the epidermis Surface area of the epidermal cells Quantification of vascular bundles the phloem area contains a metaxylem.

Statistical analysis

All the results were analyzed by ANOVA (Analysis

of Variance) using software statistics 8.1 (Steel *et al.*, 1997).

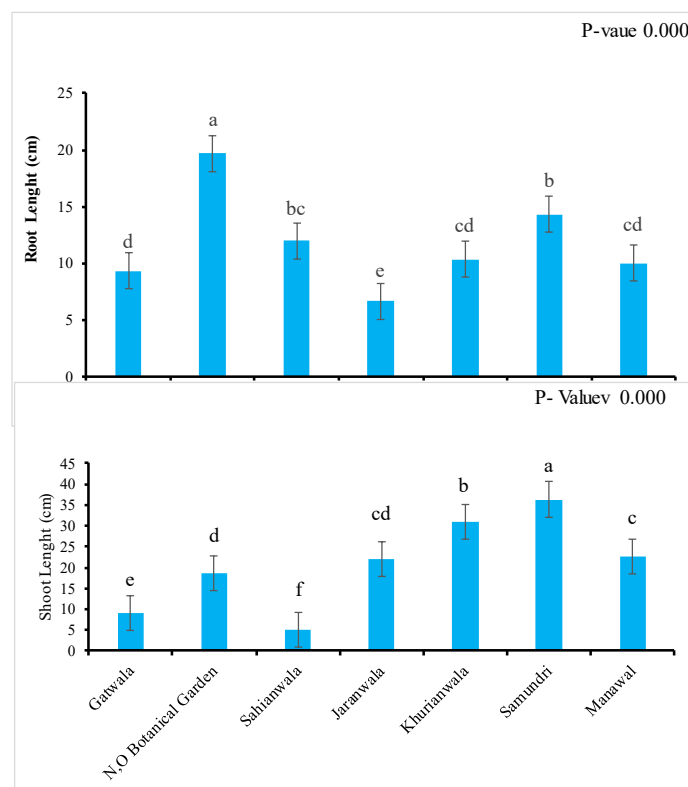


Figure 1: The root and shoot length of *Euphorbia helioscopia* from different regions of Faisalabad.

Results and Discussion

According to Figures 1, 2, root length of *Euphorbia helioscopia* showed no significant variation on data gathered from Jaranwala. The longest root length was seen in the Jaranwala exhibit. The minimum root length of *Euphorbia helioscopia* was documented in Khurianwala. The shoot length of *Euphorbia helioscopia* exhibited significant variation. The data gathered from Khurianwala and Jaranwala indicated that the maximum shoot length was observed in these locations. *Euphorbia helioscopia* had the shortest shoot length in Manawala. The root fresh weight of *Euphorbia helioscopia* did not show any significant variation. However, the samples taken from Jaranwala exhibited the highest root fresh weight. The root fresh weight of *Euphorbia helioscopia* was at its lowest in the Old Botanical Garden and Sahianwala. The shoot fresh weight of *Euphorbia helioscopia* exhibited significant variation. The highest shoot fresh weight was seen in the samples taken from Jaranwala. The shoot fresh weight of *Euphorbia helioscopia* was the lowest in Manawala. The total number of leaves of *Euphorbia helioscopia* obtained from Gatwala exhibited the highest count, and this variation was not statistically significant. The

minimum number of leaves of *Euphorbia helioscopia* was observed in Sahianwala. The root dry weight of *Euphorbia helioscopia* showed no significant variation. The highest root dry weight was seen in the samples collected from the Samundri exhibit. The minimum root dry weight of *Euphorbia helioscopia* was recorded in the Old Botanical Garden. The shoot dry weight of *Euphorbia helioscopia* exhibited significant variation. The data gathered from Jaranwala indicated that the greatest shoot dry weight was observed. The shoot dry weight of *Euphorbia helioscopia* was the lowest in the Manawala and New Garden locations. The leaf area of *Euphorbia helioscopia* exhibited significant variation conducted in Jaranwala. The highest leaf area was seen in this study. The leaf area of *Euphorbia helioscopia* was at its lowest in Manawala and the Old Botanical Garden.

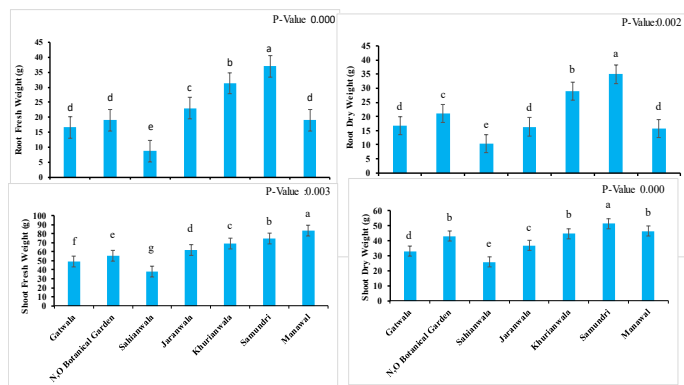


Figure 2: The root and shoot fresh and dry weight of *Euphorbian helioscopia* from different regions of Faisalabad.

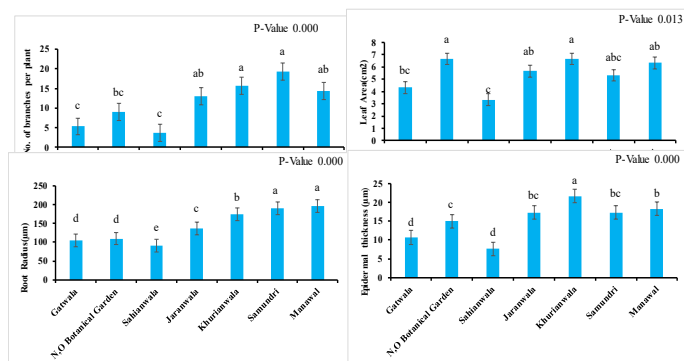


Figure 3: The root radius, number of branches leaf area and epidermal thickness of *Euphorbian helioscopia* from different regions of Faisalabad.

As displayed in Figures 3,4, the root radius of *Euphorbia helioscopia* showed no significant variation on samples taken from Samundri and Khurianwala. The highest root radius was observed in the samples from Khurianwala. The minimum root radius of *Euphorbia helioscopia* was reported in the Old Botanical Garden. The analysis of variance (ANOVA) conducted on the epidermis thickness of *Euphorbia helioscopia* root

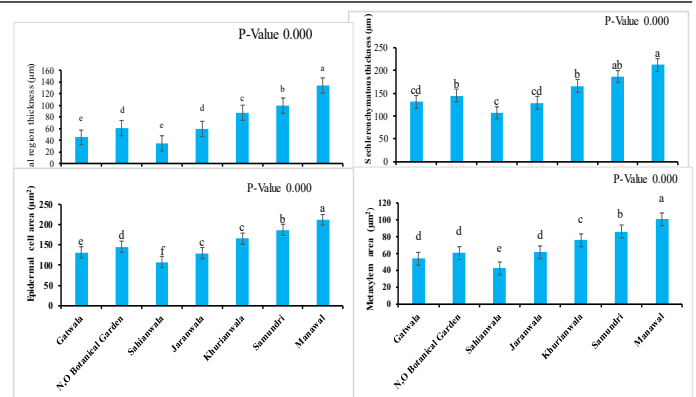


Figure 4: The epidermal cell area, epidermal and sclerenchymatous cells and metaxylem of *Euphorbian helioscopia* from different regions of Faisalabad.

gathered from the Old Botanical Garden revealed no significant variation. However, it was observed that the highest epidermal thickness was found in this sample. The *Euphorbia helioscopia* plants in Jaranwala has the thinnest epidermis. The sclerenchymatous thickness of the *Euphorbia helioscopia* root did not vary considerably on samples taken from Samundri. The maximum findings were observed. The thickness of the sclerenchyma tissue in *Euphorbia helioscopia* was at its lowest in New Garden. The cortical region thickness of the *Euphorbia helioscopia* root did not vary substantially. However, the samples taken from Jaranwala and Samundri exhibited the highest cortical region thickness. The lowest cortical region thickness of the *Euphorbia helioscopia* root was recorded in the New Garden. The endodermis thickness of the *Euphorbia helioscopia* root did not vary substantially. The data gathered from Manawala and Sahianwala indicated that the maximum endodermal thickness was observed. The endodermal thickness of *Euphorbia helioscopia* was at its lowest in the Old Botanical Garden and New Garden. The vascular bundle numbers of the *Euphorbia helioscopia* root did not show any significant variation. The samples collected from Jaranwala and Samundri exhibited the bigger vascular bundle area. The vascular bundle area of the root of *Euphorbia helioscopia* was found to be at least in the Gatwala region. The epidermal cell area of the *Euphorbia helioscopia* root exhibited significant variation. The data gathered from Samundri indicated that the greatest epidermal cell area was seen. The size of the epidermal cells of *Euphorbia helioscopia* populations was found to be the smallest in Jaranwala. The cortical cell area of the *Euphorbia helioscopia* root exhibited significant variation at Gatwala. The greatest cortical cell area was observed in this study. The cortical cell area of *Euphorbia helioscopia* was

found to be at its lowest in Sahianwala. The vascular bundle area of the *Euphorbia helioscopia* root showed a substantial variation. The samples taken from Jaranwala and Samundri had the highest vascular bundle area. The vascular bundle area of *Euphorbia helioscopia* was found to be at its lowest in Gatawala.

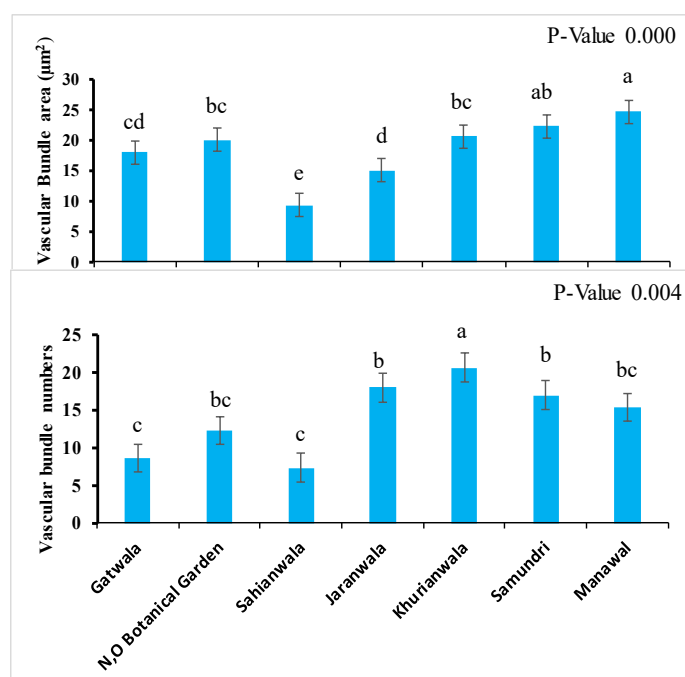


Figure 5: The vascular bundle number and vascular area of *Euphorbia helioscopia* from different regions of Faisalabad

As seen in Figure 5, the stems radius of *Euphorbia helioscopia* revealed no significant variation. The data gathered from Samundri and Sahianwala indicated that the maximum stem radius was observed. The minimum stem radius of *Euphorbia helioscopia* populations was measured in Gatwala. The epidermal thickness of the *Euphorbia helioscopia* stem did not vary substantially from the Old Botanical Garden. However, the highest epidermal thickness was observed in this study. The thickness of the outer layer of *Euphorbia helioscopia* was at its lowest in the New Garden. The vascular bundle of the stem of *Euphorbia helioscopia* showed no significant variation. The samples taken from Jaranwala had the highest number of vascular bundles. The vascular bundle count of *Euphorbia helioscopia* was at its lowest in the New Garden. The epidermal cell area of the *Euphorbia helioscopia* stem exhibited significant variation. The samples taken from Sahianwala displayed the highest epidermal cell area. The smallest epidermal cell area of *Euphorbia helioscopia* ecotypes was observed in the Gatwala and Jaranwala regions. The vascular bundle area of the *Euphorbia helioscopia* stem exhibited significant variation on data obtained from the Old

Botanical Garden. The largest vascular bundle area was observed in this study. The vascular bundle area of *Euphorbia helioscopia* was at its lowest in the Gatwala and Samundri regions. The phloem area of the *Euphorbia helioscopia* stem exhibited significant variation. The data gathered from Sahianwala indicated that the greatest phloem area was seen. The phloem area of *Euphorbia helioscopia* was found to be the smallest in the regions of Samundri and Gatwala. The metaxylem area of the stem of *Euphorbia helioscopia* showed significant variation. The data gathered from the Old Botanical Garden indicated that the greatest metaxylem area was observed. The metaxylem area of *Euphorbia helioscopia* was the smallest in Sahianwala.

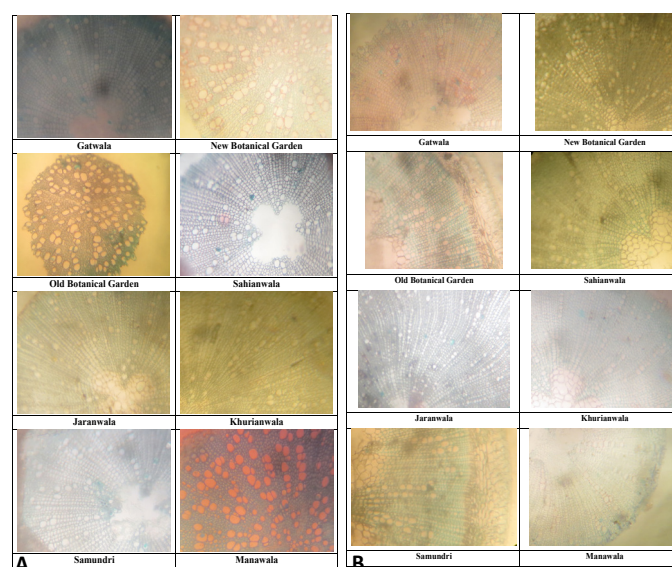


Figure 6: Microscopic image of Root (a) and Stem (b) anatomical characteristics, respectively, including Radius, Epidermal thickness, Vascular bundle numbers, Epidermal cell area, Vascular bundle area, Phloem, area, Metaxylem area of *E. helioscopia* collected from different habitats of Faisalabad.

As noted in Figure 6, the lamina thickness of *Euphorbia helioscopia* leaf did not vary substantially on samples taken from Manawala. However, the highest lamina thickness was observed in these samples. The lamina thickness of *Euphorbia helioscopia* was at its lowest in Gatwala. The midrib thickness of *Euphorbia helioscopia* leaves revealed no significant variation on samples taken from Manawala. The highest midrib thickness was seen in these samples. The midrib thickness of *Euphorbia helioscopia* was at its lowest in Sahianwala. The vascular bundle numbers of *Euphorbia helioscopia* leaves obtained from Sahianwala revealed no significant variation. However, these leaves exhibited the largest vascular bundle area. The vascular bundle count of *Euphorbia helioscopia* was at its lowest in the Gatwala region. The mesophyll thickness of

Euphorbia helioscopia did not vary substantially on samples obtained from Manawala. The maximum mesophyll thickness was seen in these samples. In Gatwala, the mesophyll thickness of *Euphorbia helioscopia* was found to be at its lowest. The vascular bundle area of the *Euphorbia helioscopia* leaf exhibited a considerable variation. The data gathered from Sahianwala and Samundri indicated that the highest vascular bundle area was seen. The vascular bundle area of *Euphorbia helioscopia* was found to be the smallest in the Gatwala and Manawala regions. The phloem area of the *Euphorbia helioscopia* leaf did not vary substantially. The samples taken from Samundri and Jaranwala exhibited the highest phloem area. The phloem region of *Euphorbia helioscopia* exhibited the lowest extent in New Garden. The metaxylem area of *Euphorbia helioscopia* exhibited significant variation. The samples obtained from Jaranwala and Samundri displayed the highest metaxylem area. The metaxylem area of *Euphorbia helioscopia* was smallest in New Garden and Sahianwala.

Environmental heterogeneity, which governs species variety through factors such as habitat, biotic interactions, productivity, and water supplies, is a key aspect of Pakistan's terrain (Majeed *et al.*, 2022). Plants possess a wide range of genetic diversity, which enables them to withstand and adapt to various environmental situations. The entire population of *Euphorbia helioscopia* exhibited characteristic changes in their anatomy and morphology in reaction to various environmental variables (Riaz *et al.*, 2022). Hence, despite the dynamic nature of the environment, adaptation can facilitate long-term survival. Samples of *Euphorbia helioscopia* were collected from different locations in Faisalabad (Butnariu *et al.*, 2023). The main problem arising from a deficiency of minerals in arid regions worldwide is the deposition of these elements on land through irrigation or drainage water, leading to desertification. As a result, farmers face the challenge of cultivating crops in saline soil, resulting in significantly reduced yields (Abdelhak, 2022).

Glenn *et al.* (2022) species that possess the ability to tolerate both high salt levels and drought circumstances usually have a thick outer layer of cells called the epidermis. This thick epidermis serves as an effective barrier against water loss from the surface of the leaves during periods of water scarcity. Basharat *et al.* (2024) suggested that, when subjected to water stress, the ecotype originating from a habitat affected

by drought exhibited a distinct and noteworthy modification in the structure of its stems. The metaxylem vessels experienced deformation, resulting in a reduction in vessel area. This adaptation is crucial for enhancing water conduction in situations of water scarcity. The domain was initiated by (Iqbal *et al.*, 2022). The habitats were characterized by an increase in epidermal thickness, inner and outer phloem, sclerenchyma fibers, lamina thickness, orientation, density, and size of stomata on the surface of leaves. With a decrease in stomatal density and area, especially at varying levels, the ecotype from the salt range demonstrated greater suitability compared to the ecotype from Faisalabad. This could be vital during a physiological drought since it has the potential to decrease water loss through the surfaces of leaves. The study concludes that epidermal traits are crucial for taxonomy analysis. *Euphorbia* species exhibit adaptability to several environmental circumstances, including salinity, drought, and waterlogging (Yang *et al.*, 2013). To enhance their ability to absorb ions and water while maintaining mechanical stability and succulence, large parenchymatous tissues must contain all of these properties and the majority of the population of *Euphorbia* have anatomical and functional modifications in different areas, which help them to survive in different environmental conditions as stated by (Iftikhar *et al.*, 2021).

The study of morphology, anatomy, and structure of the *E. hirta* and *E. helioscopia* species to determine how they reacted to different environmental conditions was the main goal of this study. Anatomical and functional changes in *E. helioscopia* are more responsive to environmental variety. They can survive in different environmental conditions by anatomical changes, including epidermal thickness, endodermis thickness in roots, collenchyma thickness, sclerenchymatous bundles, midrib and lamina thickness of leaves, deep or vast vascular bundles, increased pith area, orientation of stomata on both adaxial and abaxial surfaces of leaves, metaxylem area, and phloem area (Haq *et al.*, 2021). Zhang *et al.* (2023) statement, as a result of the worsening environmental conditions, the *Euphorbia* population developed denser foliage, which also played a part in storing water indirectly. Plant tissues are protected from desiccation due to the presence of a dense layer of collenchyma beneath the epidermis. *E. helioscopia* was introduced to a dry environment, and all of their populations showed anatomical changes increase in epidermis thickness

reduced metaxylem area, sclerifications of endodermis, increase in stellar regions and growth characteristics (Rahat *et al.*, 2023). *E. helioscopia* plant has been found to contain a diverse array of secondary metabolites, such as alkaloids, flavonoids, cardiac glycosides, polysaccharides, saponins, tannins, and triterpenoids. The presence of these secondary metabolites indicates that the plant *Euphorbia helioscopia* has the potential to be employed in the future for discovering various biological properties, such as antibacterial activity, due to the undesirable side effects of synthetic antibiotics (Riberio, 2020).

Conclusions and Recommendations

The *Euphorbiaceae* family, the sixth largest Anthophyta class of plants, has 340 genera and 9000 species worldwide. They produce milky latex due to their succulent nature, allowing them to withstand water scarcity. A study in the Faisalabad area examined the morpho-anatomical characteristics of *Euphorbia helioscopia* species, revealing significant adaptations in response to environmental changes. These modifications include enhanced root epidermis and endodermis thicknesses, increased stem area, and greater cortical region thickness, indicating their ability to thrive in diverse environments. Further investigation is needed to assess these qualities by applying adverse conditions.

Novelty Statement

The *Euphorbiaceae*'s modifications include enhanced root epidermis and endodermis thicknesses, increased stem area, and greater cortical region thickness, indicating their ability to thrive in diverse environments.

Author's Contribution

Shahid Ali Khan: Conduct experiment and data collection.

Farooq Ahmad: Supervise the whole experiment as project head.

Fatima Urooj, Husnain Ishaq, Yan Tongyu, Asima Shabbir: Statistical analysis.

Tahira Batool, Aqsa Bibi: Helped with relevant literature.

Muhammad Bilal, Javeria Tariq, Ansa Asghar, Yang Yan: Data collection.

Amrat Eman: Initial drafting and finalizing the MS.

Samreen Nazeer: Reviewed final draft of MS.

Conflict of interest

The authors have declared no conflict of interest.

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