Journal of Agriculture and Veterinary Science
ISSN: 2959-1198 (Print), 2959-1201 (Online)

Research Article

Distribution, Morphoanatomical and Physiological Adaptations in *Eulaliopsis binata* in the Salt Range, Chakwal, Pakistan

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ABSTRACT

This study provides a detailed examination of the distribution, morphoanatomical, and physiological adaptations of *Eulaliopsis binata* in the Salt Range of Chakwal, Pakistan. Five sites with varying salt levels, including Kallar Kahar Lake, Khushab Road (Kallar Kahar), Bhuchal Kalan, Miani, and Chakwal Road (referred to as sites 1, 2, 3, 4, and 5, respectively), were selected for plant and soil sample collection and analysis. The study identified key physicochemical properties of the soil that influenced plant growth and adaptive strategies. Plant growth parameters such as shoot length, fresh and dry biomass were observed to be higher at site 2 due to optimal soil conditions. Anatomical studies of root, stem, and leaf tissues revealed structural adaptations crucial for the resilience of plants against saline conditions. A more developed epidermis was observed at Site 1 (19.5 µm), providing better structural support and water transport under dry and saline conditions. The smallest metaxylem was observed at Site 4, indicating favorable conditions that do not require highly adaptive structures. Evaluation of physiological parameters, such as total chlorophyll content, showed differences among the sites. The highest total chlorophyll content was recorded at Site 2 (2.79 mg/g), suggesting a greater potential for biomass accumulation and highlighting strong adaptive capabilities of *E. binata* to saline environments.

Keywords: *Eulaliopsis binata*, Salt Range, Morphoanatomical, Physiological, pH, Electrical Conductivity.

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Article History
Received: March 22, 2025
Accepted: May 14, 2025
Published: June 19, 2025

INTRODUCTION

Eulaliopsis binata belongs to the *Poaceae* family, a highly successful family from an evolutionary standpoint with 11000–12000 species and about 750–770 genera (Kellogg, 2015; Soreng et al., 2017). *Poaceae* (*Gramineae*) is the fifth largest family after *Asteraceae*, *Orchidaceae*, *Fabaceae,* and *Rubiaceae* (Hodkinson, 2018). Plants in Poaceae are the most successful angiosperms on Earth, able to thrive in every climate, phytogeographical zone, and easily accessible habitat. *E. binate* (EB), a perennial grass also known as Babui grass or Sabai grass, belongs to the family Poaceae. It is extensively distributed in Pakistan, India, southern and central China, and is well-known for its ability to conserve water and improve soil quality (Yao et al., 2004). It can even grow in soils with low fertility (Ahmad et al., 2017). It is grown almost everywhere in India and has many applications in the paper-making industry and folk remedies for papillae (tongue projections) and internal injuries (Pande et al., 2007; Qu et al., 2008; Zou et al., 2013). According to research, EB is effective against wounds, cuts, and has noteworthy healing properties (Jyotsana et al., 2013).

https://doi.org/10.55627/agrivet.004.02.1306

The “Healthy Life Live” provided a medical dictionary stating that EB has reported diuretic and stone prevention properties (Kumar et al., 2018). The EB fiber plant is usually grown on unused and waste lands where it aids in the soil conservation of these areas (Guna et al., 2019).

The Salt Range has a specific ecosystem with various environmental stresses; therefore, the indigenous grasses are thought to have developed exceptional morphological characteristics to overcome severe salinity levels. These morphological attributes are indicators of specific environmental conditions, and these mechanisms of adaptation are important to avoid or tolerate environmental stresses, specifically elevated salt levels and excessive dryness, which are basic characteristics of the Salt Range (Farooq et al., 2015). Soils affected by natural salts must have accumulated significant morphoanatomical and physiological tolerances over millions of years under the constant selective pressures of their environment, such as increased salinification and desiccation (Hameed & Ashraf, 2008).

Many ecologically important ecosystems of semi-arid and arid zones are facing the severe problem of increasing soil salinity (Nawaz et al., 2010; Tarolli et al., 2024). Apparently, soils affected by natural salts must have accumulated enormous morpho-anatomical and physiological tolerances during millions of years under the constant selective pressures of their environment, such as increased salinification and desiccation (Hameed & Ashraf, 2008; Navarro-Torre et al., 2023). In general, plants exposed to stress environments tend to have changed anatomy and physiology that would support their survival and high performance under such conditions. For bearing the salt stress, many adaptations, tolerance mechanisms and avoidance behaviors of plants are necessary to understand to cope with the desertification and soil erosion problems (Monteverdi et al., 2008; Navarro-Torre et al., 2023). This research aims to study the morphological, anatomical, and physiological adaptations of EB in several regions of the Salt Range, Pakistan.

MATERIALS AND METHODS

Study area

District Chakwal is located at the beginning of the Potohar plateau and the Salt Range. It is a barani district classified as dry subtropical zone (Naseer et al., 2017). Five different sites (Kallar Kahar Lake, Khushab Road, Kallar Kahar, Bhuchal Kalan, Miani, Chakwal Road) were randomly selected from District Chakwal and named as sites 1, 2, 3, 4, and 5, respectively.

Sample collection

Samples of plants and top soil were collected and kept in labeled air tight bags until further use. The physiochemical properties of the collected soil samples were examined in the "Soil and Water Testing Lab" in Sargodha, Punjab, Pakistan.

Morphological parameters

Morphological parameters such as root length and shoot length were measured using an inch tape while root and shoot fresh and dry weights were measured using an electric balance.

Anatomical parameters

The cortex area, epidermal thickness, sclerification thickness and metaxylem cell area of root were measured to study anatomical adaptations in grass.

Sample preservation

For the examination of anatomical structures, root and stem samples were preserved for 6 days in 70% ethanol, while leaf specimens were preserved using a 30% glacial acetic acid and 70 % ethanol solution for a week.

Sectioning technique

All the sections were dipped in distilled water, fitted in potato, and cut using the freehand sectioning technique with platinum blades. The cut sections were dehydrated in different concentrations (30%, 50%, 70%, and 95%) of ethanol, each for ten minutes. Staining was done following the standard protocol by Johansen (1940) using safranin and fast green dyes. The slides were prepared and observed under a microscope.

Physiological parameters

The methodology by Arnon (1949) was used to quantify the content of chlorophyll a, carotenoids and chlorophyll b in freshly chopped leaves (150 mg) of grass from all the study sites. They were preserved in an 80% acetone solution and kept at 4˚C in the dark for 24 hours to extract all possible chlorophyll content. A spectrophotometer was used to measure the absorbance of the supernatant for chlorophyll a at 663 nm, carotenoids at 480nm, and chlorophyll b at 645 nm.

Statistical analysis

Analysis of variance was conducted for the entire data set on morphoanatomical and physiological adaptations, and the results were analyzed using Minitab software. Tukey's HSD test was used to compare all the mean values.

RESULTS AND DISCUSSION

Soil physio-chemical properties

Variations in key soil parameters such as pH, electrical conductivity, saturation percentage, organic matter, available phosphorus, and potassium concentrations can be influenced by geographical conditions and site-specific characteristics. The lowest pH value was observed at Site 4 (Miani) (7.6), which can have significant impacts on nutrient availability for plants, as shown in Table 1. Site 1 had higher pH levels, which reduced the availability of nutrients such as phosphorus and potassium. Kabata-Pendias (2000) described how an increase in soil pH can lead to a reduction in the absorption of micronutrients in the soil. The highest electrical conductivity (EC) value was recorded at site 4 (2.15 mS/cm), as shown in Table 1. According to Corwin et al. (2003), EC can affect plant growth by influencing pH, salinity levels, and soil saturation percentage. Consistent with these findings, our study also found that a higher EC value at Site 4 indicated a higher concentration of soluble salts, possibly due to irrigation or natural salinization processes. Lower EC values at Sites 1 and 5 suggested less saline conditions, creating more favorable conditions for plant growth.

The highest saturation percentage was observed at Site 5 (36%), indicating a greater soil moisture retention capacity and enhanced plant growth, as supported by Munns and Tester (2008). Lower saturation percentages at Site 1 (25%) and Site 3 (24%) suggested that these soils retain less water, which can impact plant productivity, as demonstrated by Feng et al. (2012). Site 3, with the highest organic matter content (1.95%), showed greater nutrient availability. These results are consistent with Murphy (2015), who showed that increased organic matter in soil can lead to improved soil saturation and nutrient cycling, as supported by Gerke (2022), who emphasized the crucial role of organic matter in managing soil fertility. The concentration of available phosphorus at Site 4 (3.6 mg/kg) indicated high phosphorus levels, which can create a favorable environment for crops, as demonstrated by Malhotra et al. (2018). In contrast, the phosphorus concentration at Site 3 (2.1 mg/kg) can affect plant growth negatively. The potassium concentration at Site 1 was significantly high (210 mg/kg) as explained by Wang et al. (2013), highlighting the importance of potassium that potassium is involved in water regulation, enzyme activation, and overall health of the plant. Lower potassium levels at Site 4 (152 mg/kg) may have implications for plant growth.

Table 1. Soil physicochemical properties of different habitats in Salt Range.

	Site 1	Site 2	Site 3	Site 4	Site 5
Coordinates	32.773017''N 72.715353''E	32.745765''N 72.687364''E	32.723847''N 72.677008''E	32.737705''N 72.681628''E	32.804319''N 72.732477''E
EC ($mScm^{-1}$)	0.78d	0.85c	1.17b	2.15a	0.84c
pH	8.3a	8.1ab	7.8cd	7.6d	8bc
Organic matter (%)	0.98e	1.18c	1.95a	1.68b	1.11d
Available Phosphorus ($mgkg^{-1}$)	2.9b	2.8b	2.1c	3.6a	2.3c
Available Potassium ($mgkg^{-1}$)	210a	186b	170c	152e	156d
Saturation (%)	25c	30b	24c	26c	36a

Means followed by the same letter within each row are not significantly different at $p < 0.05$

Morphological attributes

High adaptability is demonstrated by the morphological characteristics of *E. binata* with the wide range of environmental conditions of the sampled sites. The roots of the species are fibrous and branched, generally ranging from 8 cm to 18 cm. Such an extensive system of roots is important for nutrient and water absorption, especially in sandy or nutrient-poor sites, as described by White (2019). The presence of cotton-like material surrounding the roots indicates a symbiotic relationship with mycorrhizal fungi for better nutrient uptake under adverse soil conditions. Site 2 had the maximum mean root length (18.23 cm) while Site 5 had the minimum root length (8.3 cm**;** Table 2). The differences may be attributed to variations in moisture content, organic matter, and soil compaction. Similarly, Ostonen et al. (2007) declared that root length is a marker of variations in soil properties and environmental conditions.

Shoot length varies significantly from site to site, with Site 1 showing the maximum shoot length of 64.83 cm. The order of shoot length indicates that natural environmental factors, such as soil fertility and moisture, determine the growth intensity of EB at different locations. As Munns and Tester (2008) described in their study, moisture content affects the growth rate of plants, especially shoot growth.

The size of the leaves, length, and width differed significantly, with Site 2 having the longest leaves at 56.67 cm and Site 5 having the shortest at 25.6 cm. The length and structure of the leaves are associated with the nutrient profile and the water content in the soil. In nutrient-rich soils, the leaves develop robust structures to improve photosynthetic efficiency and overall plant vigor. Leaf parameters can also show variations in the presence of higher levels of salts (CERİTOĞLU et al., 2020). Leaf width varied little among the sites, indicating stability across the growth sites. This implies that although there are differences in growth conditions, this characteristic is stable for EB.

The fresh and dry weights of the plants showed a significant difference between sites, with the highest values obtained from Site 1, averaging 9.36 g for fresh weight and 3.57 g for dry weight. The observations of root and shoot lengths suggest that plant biomass is related to favorable conditions, like soil. Site 4 recorded the lowest fresh and dry weights, possibly due to soil degradation or nutrient deficiencies affecting the general health condition of the plant, as supported by Andriolo et al. (2005).

Anatomical attributes

Anatomical features of the EB root display considerable variation among different sites, indicating the species' adaptability to varied environmental conditions. The greatest thickness of the root epidermis was recorded in Site 1, indicating a need for increased absorption of water and nutrients, as described by Fatima et al. (2021). While Site 3 had the least thickness, which may imply lesser environmental stress or possibly different soil composition. The maximum root pith cell area was shown by plants at Site 3, and the minimum at Site 2 (Table 2), as observed by Nawaz et al. (2013) that plants facing stress showed more pith area.

Table 2. Morphoanatomical and physiological parameters of *E. binate*.

Parameters	Site 1	Site 2	Site 3	Site 4	Site 5
Morphology					
Leaf length (cm)	54.47a	56.6a	43.3b	35.87b	25.6c
Shoot length (cm)	64.8a	63.7a	52.83b	44.7c	32.7d
Root length (cm)	0.28c	0.51a	0.40b	0.25cd	0.19d
Leaf width (cm)	0.43a	0.5a	0.46a	0.4a	0.43a
Plant fresh weight (g)	9.36a	6.5b	5.4bc	3.04d	4.8c
Plant dry weight (g)	3.57a	2.29b	2.18b	0.89c	1.21c
Root anatomy					
Epidermal thickness(µm)	14.5a	6.5b	15a	7.8b	8.5b
Metaxylem area(µm^2)	699.33b	622.2c	1179.3a	600c	1148a
Pith cell area(µm^2)	156c	125d	289a	140cd	183.3b
Stem anatomy					
Epidermal thickness(µm)	19.5a	19a	16ab	13.3b	15b
Vascular bundle area(µm^2)	5180a	5103a	3030c	2594.3d	4053.3b
Metaxylem area(µm^2)	290.67b	285.17b	353a	190.17d	250.17d
Leaf anatomy					
Epidermal thickness(µm)	12.5bc	11.5bc	14b	10c	18a
Vascular bundle area(µm^2)	5180a	5103a	3030c	2594.3d	4053.3b
Metaxylem area(µm^2)	305.5a	240b	153.33d	104.5e	175c
Chlorophyll content (mg/g)					
Chlorophyll a	1.4b	1.57a	1.64a	1.57a	1.65a
Chlorophyll b	0.15d	1.11a	0.95b	0.71c	0.16d
Total chlorophyll	2.12b	2.72a	2.56a	1.87b	1.38c
Carotenoids	0.033c	0.055a	0.050a	0.047ab	0.035bc

Means followed by the same letter within each row are not significantly different at $p < 0.05$ (n = 15)

Similarly, in the case of the stem, more developed epidermis and metaxylem parts were observed at Site 1, providing better water transport conditions under harsher factors while the smallest were observed at Site 3, probably due to more favorable conditions as Mansoor et al. (2019) described that plants possessed maximum metaxylem for better storage and survival. The record depicts that the highest leaf metaxylem and vascular bundle areas occur at Site 1, essential for the proper flow of nutrients and water, as Fatima et al. (2018) elaborated on the significance of thickened epidermal layers in leaves required for proficient water storage. In contrast, the lowest values are recorded at Site 4, indicating not as high a demand for the said process.

The total chlorophyll content peaked at Site 2 at 2.72 mg/g, indicating a higher potential for greater biomass accumulation, which can affect morphological traits such as plant height and leaf are as according to the findings of Dale and Causton (1992) showing a relationship between higher levels of chlorophyll a/b and more light absorption by leaves. The carotenoid content also demonstrated a significant difference, with the maximum average recorded at Site 2 (Table 2). Carotenoids can play a critical role in photoprotection and assist in moderating oxidative stress, which can influence the morphological phenotypes of the leaf, such as thicker cuticles or altered leaf size that may limit the loss caused by transpiration.

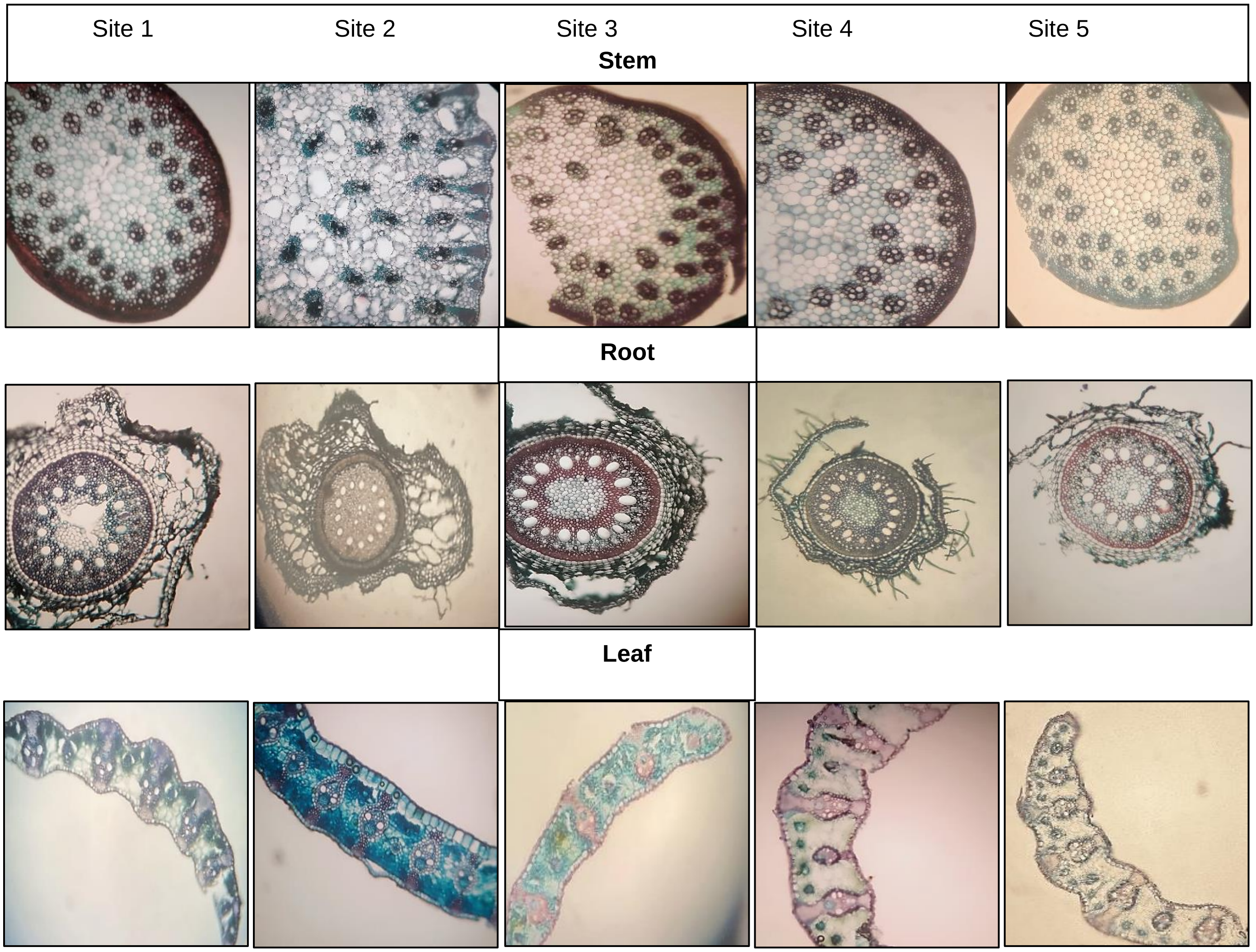

Figure 1. Transverse sections of the stem, root, and leaf of *E. binata* collected from five sites in the Salt Range.

CONCLUSION

E. binata across different sites in Chakwal, Pakistan, exhibited significantly varying soil properties and plant growth responses among the sites, with Site 2 being identified as the most favorable for *Eulaliopsis binata*. Soil analysis clearly indicated that the pH level at site 1 was higher, limiting the availability of certain nutrients for the plants. In site 4, electrical conductivity increased, indicating higher salinity levels, which negatively impacted plant growth and development. An anatomical study revealed adaptations to saline environments in site 2, such as thicker epidermal layers and vascular bundles, providing better protection and transport of water and nutrients. These characteristics make the site 2 plants suitable for ecological purposes in rehabilitating saline-affected areas through land reclamation and restoration. Physiological analysis also showed differences in chlorophyll concentration among the sites, with site 2 having the highest chlorophyll content, indicating higher photosynthetic activity and adaptive capabilities. Site 1 exhibited the lowest pigment contents overall. These findings highlight the importance of Site 2 for the growth and ecological functions of *Eulaliopsis binata* in saline condition.

AUTHOR CONTRIBUTIONS

Conceptualization, A. Sajad, and M. Ayaz.; Methodology, A. Sajjad, M. Ayaz and N. Akram.; Formal Analysis, N. Ullah, M. Ayaz and A. I. Khan.; Writing Original Draft Preparation, A. Sajjad.; Writing Review & Editing, M. Ayaz, N. Ullah, A. I. Khan. and N. Akram.

COMPETING OF INTEREST

The authors declare no competing interests.

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