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ENVIRONMENTAL HETEROGENEITY DRIVES SITE-SPECIFIC MORPHO-ANATOMICAL VARIATION IN *DIGITARIA CILIARIS* (RETZ.) PERS. ACROSS DIVERSE HABITATS

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Abstract

Environmental heterogeneity influences plant growth and trait expression across natural habitats, particularly where soil moisture, fertility, salinity, and climatic conditions vary over short geographical distances. In this study, morphological and root anatomical variation in *Digitaria ciliaris* obtained from fourteen habitats was studied. The sites represented various ecological zones in Pakistan such as montane habitats, agricultural lowland habitats, Pothohar Plateau habitats, Salt Range habitats and Soon Valley habitats. One-way ANOVA, correlation analysis, principal component analysis, redundancy analysis and heatmap clustering were employed to evaluate the physicochemical properties of soil, morphological traits of plants, and root anatomical characters. Significant differences in soil moisture, organic matter, pH, electrical conductivity, and concentrations of major ions between sites were observed. Populations growing in relatively fertile and moisture-rich habitats had greater shoot and root biomass, longer shoots and roots, and large leaf area. On the other hand, the populations growing in soils with high pH, electrical conductivity, sodium, and chloride had less morphological growth. Root anatomical characters also varied among habitats. Tissue thickness of epidermis, cortex, and sclerenchyma was correlated to the edaphically stressful habitats, while aerenchyma development was mostly seen in moisture-rich habitats. The multivariate analyses discriminated the populations based on fertility, salinity, and moisture gradients, showing a relationship between soil and plant characters. In conclusion, *D. ciliaris* exhibited site-related morphological and root anatomical variability in ecologically heterogeneous habitats.

Key words: Phenotypic plasticity; Ecological adaptation; Soil edaphic gradients; Root anatomical variation

Abbreviations: Species: *Digitaria Ciliaris*= *D. Ciliaris.* **Sites:** KBR = Kala Bagh Road, KHR = Kahuta Road, MZR = Muzaffarabad Road, CMT= Chamyati, BBN= Bhurban, KUA = Khura, JHR = Jahlar, PDR= Padhrar, AMD= Ahmedabad, KKR= Kalar Khar, JWA = Jhongy Wala, CHK= Chakri, UAF= University of Agriculture Faisalabad, FSD= Faisalabad. **Soil Physiochemical Characteristics:** MC= Moisture contents, SP= Saturation percentage, pH= Potential hydrogen, ECe= Electric conductivity, OM= Organic matter, Na^+= Sodium, K^+= Potassium, Ca^{2+}= Calcium, Mg^{2+}= Magnesium, Cl^{-1}= Chloride, P= Phosphorous. **Morphological attributes:** SFW= Shoot Fresh weight, RFW=Root Fresh weight, SDW= Shoot Dry weight, RDW= Root Dry weight, SL= Shoot Length, RL= Root Length, LA= Leaf Area. **Root anatomical attributes:** RET= Root Epidermal Thickness, RDT= Root Endodermal Thickness, RCT= Root Sclerenchyma Thickness, RST= Root Steller Region Thickness, RMA= Root Metaxylem Area, RCA= Root Cortical cell Area, RPA= Root Pith cell Area; RLA= Root Phloem cell Area, RAA= Root Aerenchyma tissue cell Area

Introduction

Environmental heterogeneity refers to spatial and temporal variation in abiotic and biotic conditions across natural habitats, including differences in soil moisture, salinity, pH, nutrient availability, elevation, rainfall, and temperature. Such heterogeneity is an important factor shaping plant growth and trait expression. In grasses, these gradients are especially relevant because root architecture, biomass allocation, and anatomical traits respond strongly to edaphic conditions such as water availability, aeration, and ionic stress (Stein *et al*., 2014). Field-observed trait variation across heterogeneous habitats may reflect phenotypic plasticity, genetic differentiation among populations, or both. Therefore, studies of natural populations are useful for identifying soil-trait associations, although such patterns should be interpreted cautiously unless supported by controlled experimental or molecular evidence (Pérez-Sánchez *et al*., 2023).

Abiotic stresses such as salinity, drought, waterlogging, temperature extremes, and nutrient limitation can restrict plant growth by altering water uptake, ion balance, biomass accumulation, and root development (Mir *et al*., 2022). Plants exposed to stressful environments often show coordinated morphological and anatomical changes, including reduced shoot growth, modified root development, thickened epidermal and sclerenchyma tissues, altered vascular organization, and aerenchyma formation under low-aeration or moisture-supported conditions (Fatima *et al*., 2018). These structural adjustments are particularly important in grasses because root anatomical traits can influence water transport, mechanical protection, ion regulation, and oxygen diffusion under contrasting soil environments (Fatima *et al*., 2021).

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Pakistan contains strong climatic and edaphic gradients, ranging from humid montane habitats to semi-arid and salt-affected landscapes. These gradients provide a natural framework for examining how widespread grasses vary across contrasting environments (Abid *et al*., 2016; Hodkinson, 2018; Ullah *et al.*, 2025). The family Poaceae is one of the most ecologically successful angiosperm families, with approximately 11,000-12,000 species and 750-770 genera worldwide (Soreng *et al.,* 2017; Sadia *et al*., 2025). Grasses occupy diverse habitats, including subalpine, xerophytic, agricultural, and aquatic environments, and many species possess traits such as C_4 photosynthesis that improve water and nitrogen-use efficiency under warm and stressful conditions (Khan, 2012; Singh *et al.,* 2011).

The genus *Digitaria* includes terrestrial grasses with ruderal characteristics and broad ecological distribution across cultivated fields, no-till systems, roadsides, waste places, and other disturbed habitats (Jones *et al*., 2021). *Digitaria ciliaris* (Retz.) Pers., commonly known as southern crabgrass, is a widely distributed species that occurs across tropical and temperate regions (Chauhan & Johnson, 2008). In Pakistan, it has been recorded from irrigated fields to degraded road margins and flood-prone areas. Its prostrate growth habit, rooting at nodes, high seed production, and partial self-pollination may contribute to rapid establishment across heterogeneous environments. Species of *Digitaria* can also tolerate marginal soils, drought, and flooding, making the genus relevant for studying grass responses to environmental stress (Ayenan *et al*., 2018).

While *D. ciliaris* has a widespread geographic range, the patterns of morphological and root anatomical traits associated with *D. ciliaris* in different types of Pakistani soil are poorly known. Thus, the current research explored soil physical and chemical variations, morphological growth characteristics, and root anatomical characteristics of naturally-occurring populations of *D. ciliaris* found at fourteen different ecological locations in Pakistan. The hypotheses for the research were as follows: (1) Populations located in fertile and moisture-rich environments will display better growth morphologically; (2) Populations located in saline/alkaline or dry conditions will display poorer growth but better root anatomical differentiation; and (3) Multivariate analysis would allow populations to be separated according to soil fertility, salinity, and moisture levels. Since the experiment involved naturally occurring field populations, differences in the studied traits were considered to be soil environment-specific in-situ phenotypic variations.

Materials and Methods

Collection sites and sampling procedure: In present research a stratified ecological sampling approach was used to ensure that the major habitat differences where *D. ciliaris* inhabits in the selected geographical areas of Pakistan are reflected in the sampling. Four habitat strata were identified: (i) lowland agricultural habitats, (ii) Salt Range and Soon Valley semi-arid or saline habitats, (iii) transitional habitats of Pothohar Plateau, and (iv) Himalayan/ Azad Jammu and Kashmir montane habitats. The sampling sites were selected considering the habitat factors such as presence of *D. ciliaris*, ecology, physiochemical conditions of the soil, moisture, altitude, temperature, humidity, salinity, and other environmental variations as described in Table 1. These sites included Kala Bagh road (KBR; 32.966°N, 71.553°E), on the western bank of the Indus river at the salt range margin (Ahmad *et al*., 2009); Kahuta road (KHR; 33.35° N, 73.23°E), at 624m on the eastern Pothohar Plateau; Muzaffarabad road (MZR; 34.359°N, 73.47°E), a mountainous Himalayan zone drained by the Jhelum and Neelum rivers; Chamyati (CMT; 34.0653°N, 73.5443°E), in Bagh District, Azad Jammu and Kashmir; Bhurban (BBN; 33.9554°N, 73.4519°E), at approximately 2291m within the forested Murree zone of the Outer Himalayas; Khura (KUA; 32.3147°N, 72.1348°E), a xerophytic site in Khushab district dominated by vegetations such as *Acacia modesta*, *Olea ferruginea*, and *Dodonaea viscosa* (Gratzfeld & Khan, 2015); Jahlar (JHR; 32.5021°N, 72.0913°E), within the ecologically significant jhalar lake; Padhrar (PDR; 32.6558°N, 72.4909°E), near Soon Sakesar, Ahmedabad (AMD; 32.6184°N, 72.2793°E), a settlement in Soon Valley; Kallar Khar (KKR; 32.7769°N, 72.7068°E), at 554 m elevation with sandy loam soils of pH 7.7 to 7.8 derived from sandstone and limestone (Mumtaz *et al*., 2021); Jhongy Wala (JWA; 32.5000°N, 72.0000°E), within the rugged terrain of the Salt Range; Chakri (CHK; 33.2921°N, 72.7600°E), at the transitional zone between the Pothohar Plateau and the salt range; the University of Agriculture Faisalabad Botanical Garden (UAF; 31.4294°N, 73.0750°E); and Faisalabad (FSD; 31.4273°N, 73.1166°E, 186m), a lowland agricultural district in central Punjab, Pakistan (Parveen *et al*., 2012). A map of Pakistan showing the *D. ciliaris* collection sites and representative habitat views is presented in Fig. 1.

Plant sampling: Plant and rhizosphere soil samples of *Digitaria ciliaris* were collected from fourteen ecologically distinct sites in Pakistan during field trips performed in spring 2024 and spring 2025. The AJK and mountainous sites were sampled in spring 2024 while other challenging sites including the semi-arid and salt-affected sites were sampled in spring 2025. The plant and soil samples were collected during the growing season of *D. ciliaris* at each site in order to collect the vegetation of similar age for morphological and root anatomical study. Three biological plant samples were collected from each of the fourteenth ecologically distinct sites to make up a total of 42 biological plant samples. Such high sampling intensity was chosen to combine a wide ecological range of study with destructive nature of root anatomical analysis. Every site was considered as fixed ecological sampling units while the three biological plant samples were taken as estimates of within-site variability in morphological and anatomical parameters. Plants were collected manually by a 15 cm earth auger in order to extract the root system intact for anatomical study. The adherent soil and debris were carefully washed off on site in order to avoid damaging the roots. Plant fresh weights were measured immediately after collection using a digital top-loading scale. Individual plant samples were packed into labeled plastic zip-lock bags and preserved under refrigerated conditions prior to analysis of morphological and root anatomical traits.

Table 1. Description of sampling sites and environmental characteristics of *Digitaria ciliaris* habitats.

Site	Coordinates	Habitat type	Related plant species	Mean annual Temp (°C)	Elevation (m asl)	GHI ($kWhm^{-2}d^{-1}$)	Rainfall mm yr^{-1}	Humidity (%)
KBR	32.966° N 71.553° E	Salt Range, sedimentary rocks	*Cynodon dactylon*, *Dichanthium annulatum*, *Sporobolus ioclados* (Ahmad *et al*., 2009)	26	220	5.1	685	46
KHR	33.35°N 73.23°E	Potohar sub-tropical region	*Cynodon dactylon*, *Cymbopogon jwarancusa*, *Aristida mutabilis*, *Sorghum halepense* (Riaz *et al*., 2011)	25	624	4.8	1400	55
MZR	34.359° N 73.4710° E	Mountainous habitat	*Saccharum spontaneum*, *Sorghum halepense*, *Arundo donax* (Siddiqui *et al*., 2010)	18	793	4.7	1308	57
CMT	34.0653° N 73.5443° E	Mountainous AJK mixed forest	*Heteropogon contortus*, *Chrysopogon serrulatus*, *Arundo donax* (Awan *et al*., 2021)	18	1528	4.2	1400	60
BBN	33.9554° N 73.4519° E	Tranquil area	*Cymbopogon jwarancusa*, *Aristida mutabilis*, *Heteropogon contortus* (Siddiqui *et al*., 2010)	20	2291	4.8	1616	63
KUA	32.3147° N 72.1348° E	Salt Range, Xerophytic	*Cymbopogon jwarancusa*, *Aristida adscensionis*, *Chrysopogon serrulatus* (Gratzfeld & Khan, 2015)	28	838	5	520	46
JHR	32.5021° N 72.0913° E	Salt Range, jahlar lake	*Chrysopogon serrulatus*, *Dactyloctenium scindicum*, *Sporobolus ioclados* (Gratzfeld & Khan, 2015)	33	828	5.1	410	47
PDR	32.6558° N 72.4909° E	Salt Range foothill regions	*Chrysopogon serrulatus*, *Cymbopogon jwarancusa*, *Digitaria sanguinalis* (Ahmad *et al*., 2009)	32	580	5	395	47
AMD	32.6184° N 72.2793° E	soon valley, grassland areas	*Dichanthium foveolatum*, *Sporobolus ioclados*, *Eragrostis papposa*, (Ahmad *et al*., 2009)	31	250	4.8	724	52
KKR	32.7769° N 72.7068° E	Salt Range, Sandy loam plateau region	*Dichanthium annulatum*, *Cenchrus pennisetiformis*, *Eragrostis poaeoides* (Chaudhary *et al*., 2001)	29	554	5.1	500	44
JWA	32.5000° N 72.0000° E	Rugged Salt Range	*Chrysopogon serrulatus*, *Cymbopogon jwarancusa*, *Aristida adscensionis*, (Hameed *et al*., 2013)	28	540	4.6	524	55
CHK	33.2921° N 72.7600° E	subtropical dry evergreen forest	*Cynodon dactylon*, *Dichanthium annulatum*, *Cenchrus pennisetiformis*, (Chaudhary *et al*., 2001)	24	508	4.8	989	61
UAF	31.4294° N, 73.0750° E	Irrigated urban land areas	*Sporobolus diandrus, Dichanthium annulatum* (Hameed *et al*., 2011)	29	184	4.7	602	64
FSD	31.4273° N 73.1166° E	Agricultural landscape	*Cynodon dactylon, Cenchrus pennisetiformis, Dichanthium annulatum*, (Hameed *et al*., 2011)	31	186	5.2	530	48

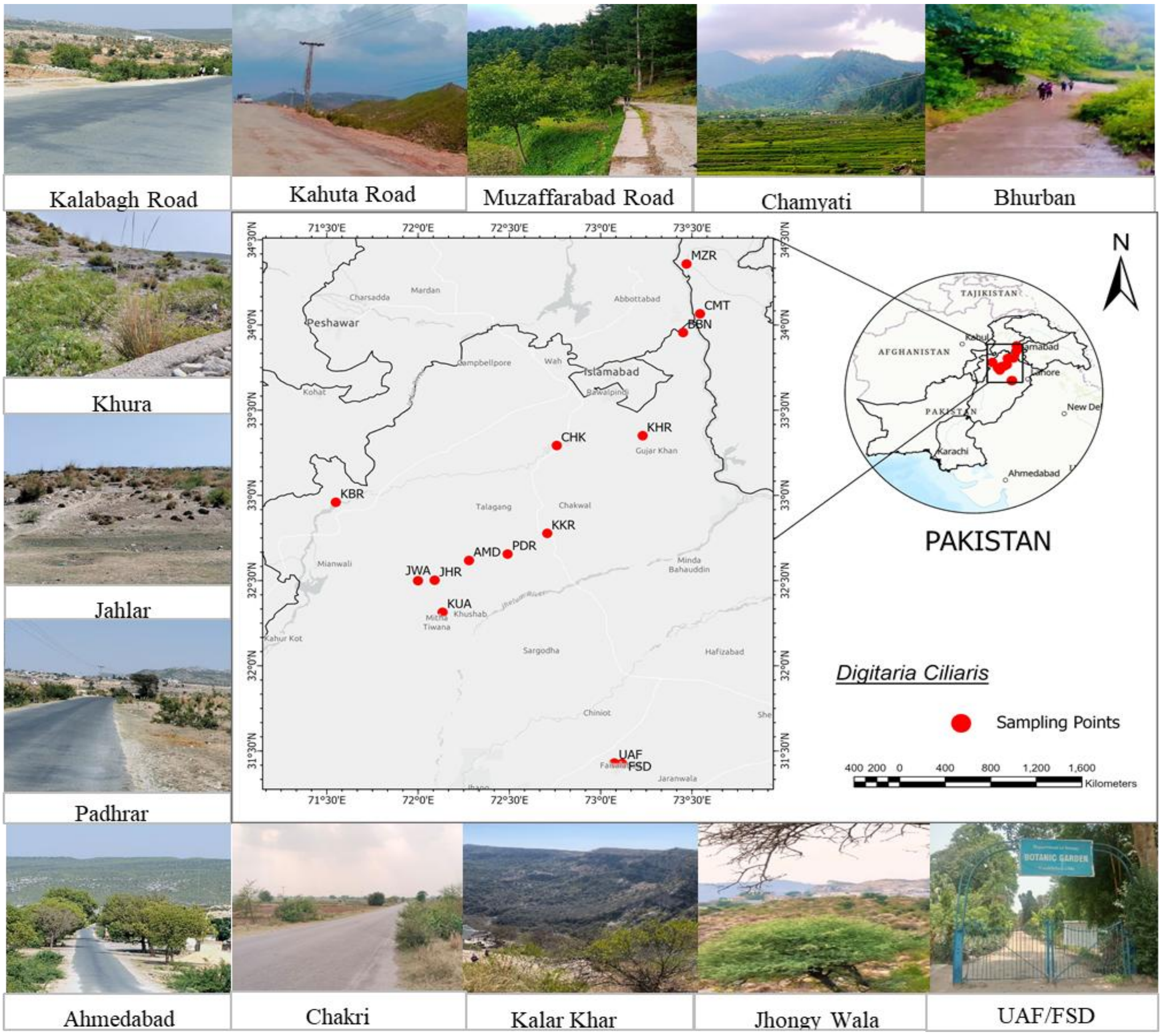


Fig. 1. Map of the Pakistan showing collection sites of *Digitaria ciliaris* with pictorial.

Geographic and environmental data: Geographic parameters, including latitude, longitude, and elevation, were recorded for each site using a Garmin eTrex Venture HC GPS unit (Table 1). In situ slope measurements were taken using LD360 digital inclinometer and aspect measurement by means of magnetic compass. Annual precipitation and temperature information was extracted from Anon., (2025) regional observatories nearest to the study area. For FSD (Faisalabad) and UAF (University of Agriculture Faisalabad) observatory data was acquired from Faisalabad, for BBN (Bhurban), CMT (Chamyati) and MZR (Muzaffarabad Road) from stations of Murree/Muzaffarabad, KHR (Kahuta Road) and CHK (Chakri) from Islamabad/Rawalpindi observatories, while KBR (Kala Bagh Road), KUA (Khura), JHR (Jahlar), PDR (Padhrar), AMD (Ahmedabad), KKR (Kalar Khar) and JWA (Jhongy Wala) were considered to be Mianwali/Sargodha or Salt Range regional observatories. Since not all collection sites had a weather station at site level, climatic values may be considered only as estimations of the regional environment rather than precise measurement values for the plot itself. Annual solar radiation estimates were made on NASA POWER annual all-sky surface shortwave downward irradiance (ALLSKY_SFC_SW_DWN) for years 2022-2025 based on site coordinates (Anon., 2026). Habitat maps were created in QGIS by means of WGS 84 geographic coordinates and the map final layout is exported with site name and habitat pictures shown in Fig. 1.

$$\text{Mean GHI} = \frac{\text{GHI(2022)} + \text{GHI(2023)} + \text{GHI(2024)} + \text{GHI(2025)}}{4}$$

Assessment of soil physicochemical traits: Samples of rhizosphere soil were taken from the 15-25 cm soil zone around the plant roots of each plant sampled. The depth was chosen as the main excavated root-zone soil since it is the soil where the plants in the samples were gathered and has enough soil material for physicochemical analysis. The 15-25 cm soil depth was used as a standard rhizosphere sampling horizon for all sites, as this layer may be slightly different in depth for mature adventitious roots to be used for anatomical observations. Three soil samples, representative for the three biological plant replicates, were taken from each site. Air drying, gentle grinding and sieving were used to prepare soil samples for analysis of physicochemical parameters according to USDA

Handbook No. 60 (Richards, 1954). A saturation paste (1:2 by volume) was made using 200 g of air-dried soil samples to evaluate soil pH, electrical conductivity (ECe), saturation percentage (SP%), and ionic composition. Distilled water was sequentially mixed with the soil and carefully stirred until a homogeneous saturated paste was produced. Subsequently, saturation-paste extract samples were collected under partial vacuum at 2-4 atm. The saturation percentage was determined by subtracting the dry weight of the soil from the weight of the saturated paste. The soil pH and electrical conductivity (ECe) were determined utilizing a pH/ECe meter (WTW series, compact InoLAB pH/Cond 720, Washington, USA) with the saturated-paste extract. Organic matter was determined using the colorimetric method of Sims and Haby (1971), while sodium (Na^+) and potassium (K^+) were measured using a Jenway PFP-7 flame photometer. Calcium (Ca^{2+}) and magnesium (Mg^{2+}) were determined by atomic absorption spectrophotometry, chloride (Cl^-) was measured using a digital chloride ion meter, and available phosphorus was estimated by the colorimetric procedure described by Yoshida *et al*., (1971).

$$\text{Saturation percentage (\%)} = \frac{\text{Weight of water required to saturate the soil}}{\text{Oven dry weight of soil}} \times 100$$

Morphological characteristics: These plants were uprooted meticulously and thoroughly washed in tap water to make sure that no soil remained in order to observe their morphology. The fresh weight of the plants was determined immediately after uprooting, employing an electronic weighing balance (Model FA2004B; YK Scientific Instrument, China). To ascertain dry weight, samples were subjected to oven drying at 60°C for one week until a stable weight was attained, subsequent to which dry weights were measured using an electronic scale. Plant height was measured from the base to the stem apex, and inflorescence length was measured using a meter ruler. The leaf area was determined using the formula established by Schrader *et al*., (2021). The fresh and dry weights were used to calculate the plant water contents using the following formula:

Plant water contents (g plant) = Fresh weight of the plant (g plant) - oven dried weight (g plant)

Leaf area = (length × width × correction factor 0.75)

Anatomical attributes: Anatomical characteristics of root were studied on standardized transverse sections of mature adventitious roots. Three adventitious roots of similar diameter from each replicate plant were randomly chosen. The segments of the roots were cut 2-3 cm back from the root tip to ensure that there was no developmental variability between samples. The root segments were fixed in FAA fixative consisting of 5% formalin, 50% ethanol, 10% acetic acid, and 35% distilled water, then transferred into 75% ethanol solution for storage. Manual transverse sections were made using a double-edged razor blade. In order to avoid bias during sectioning, several sections were made from each root segment. Only those sections which were intact, circular or near circular, not oblique, thin, had intact epidermis, cortex, endodermis, stele, vascular tissues and aerenchyma were used for measurements. Sections which were torn, compressed, folded, had incomplete tissue boundaries, were oblique or had disrupted vascular region were not used for further study. The sections selected for anatomical analysis were dehydrated through ethanol series, stained with safranin and fast green according to Ruzin (1999). Then the slides were permanently mounted in DPX and observed using compound microscope. Minimum three good sections per biological replicate were photographed and anatomical measurements were made on the same areas using calibrated ocular micrometer. Thickness of epidermis, endodermis, sclerenchyma, and stele were measured at representative radial positions, while metaxylem, cortical cells, pith cells, phloem and aerenchyma areas were measured from well-defined tissue areas.

Statistical analysis

The data analyses were carried out using one-way ANOVA, where site was taken into consideration as the primary factor with completely randomized design, and each site had three biological replicates. The LSD test (at $p<0.05$) was employed to compare the mean values of the sites. Since there was little replication per site, the statistical results were also interpreted in combination with the direction of the effects, consistency of the traits, and multivariate pattern rather than just based on p-value. Correlation analyses, PCA, and heatmap clustering analysis were applied as supporting exploratory tools to analyze the relationship among soil physicochemical attributes, morphological traits, and anatomical traits of roots. Habitat group results were used only for descriptive purposes and not as a replacement for real site-level replication.

Results

Environmental heterogeneity across the diverse ecotypes: The fourteen sampling sites had widely different environmental conditions (Table 1). The mean maximum temperature was between 28°C and 42°C, while the mean minimum temperature was between 5°C and 31°C. The highest maximum temperature of 42°C was recorded at Kala Bagh Road (KBR), which is situated within the Salt Range with sedimentary rock formations and an arid thermal regime. The lowest minimum temperature was recorded at Chamyati (CMT), Azad Jammu and Kashmir (5°C) which shows the cooler climate of the montane climate in the region. The annual precipitation ranged significantly among the sampled habitats, reflecting the wide elevational and climatic variation in the study. The highest annual rainfall (1616 mm) was recorded at Bhurban (BBN), located in the Murree Hills, which may be influenced by the hills' orographic precipitation. However, in the Salt Range, Padhrar (PDR) had recorded the lowest annual rainfall of 395 mm, which is representative of the semi-arid climate of the region. Humidity varied greatly between different sites as well. Humidity ranged up to a maximum of 64% at the UAF because of vegetation and trees that provided for a relatively moist environment. Lower humidity values, however, were noticed at the dry Salt Range sites, such as Kalar Khar (KKR 44%) and KBR (46%), which indicates the drier environmental conditions of such habitats. Global Horizontal Irradiance (GHI) ranged from site to site depending on the exposure and vegetation cover. The maximum GHI is obtained at Faisalabad (FSD) with direct solar radiation received for most of the day, and the value is 5.2 kWh m^{-2} day^{-1}. In contrast, the lowest GHI value (4.2 kWh m^{-2} day^{-1}) was found at Chamyati, where high vegetation cover and a humid environment block the direct solar irradiance reaching the ground surface.

Environmental variability across the diverse habitat: Radar-chart grouping summarized the main habitat contrasts (Fig. 2). The sampling sites were classified into four ecological habitat groups based on environmental conditions and habitat descriptions. The two lowland agricultural sites FSD (Faisalabad) and UAF (University of Agriculture Faisalabad), were formed in group A, the seven Salt Range and Soon Valley sites, KBR (Kala Bagh Road), KUA (Khura), JHR (Jahlar), PDR (Padhrar), AMD (Ahmedabad), KKR (Kalar Khar), and JWA (Jhongy Wala) in group B, the two Pothohar Plateau sites KHR (Kahuta Road) and CHK (Chakri) in group C, and the three Himalayan and AJK Montane sites BBN (Bhurban), CMT (Chamyati), and MZR (Muzaffarabad Road) in group D. The mean environmental profiles of four habitat groups of *Digitaria ciliaris* across six axes (temperature, elevation, GHI, rainfall, humidity, and soil pH) are compared here using this format, wherein red, blue, purple, and green polygons refer to Groups A, B, C, and D. If a polygon is higher on any axis, then this group has a higher mean value for the given axis. Group D had the highest mean elevation (1,537 m asl), the highest rainfall (1,441 mm yr^{-1}), and the highest humidity (60%); and the lowest temperature (18.7°C), GHI (4.57 kWh m^{-2} day^{-1}), and soil pH (6.93). In this case, a large polygon was produced on the elevation and rainfall axes and a contracted profile on temperature and GHI. Group B displayed an opposite trend, with the highest mean temperature (29.6°C), lowest rainfall (537 mm yr^{-1}), lowest humidity (48.1%), and highest pH (7.64). The polygon produced by this group was contracted on the elevation and rainfall axes but elongated on the temperature and pH axes. Group A presented a polygon that has the highest GHI (4.95 kWh m^{-2} day^{-1}) but the lowest elevation (185 m asl). This represents the flat, high-irradiance, and agriculturally managed lowland landscape at FSD and UAF. Group C occupied a truly intermediate position on all six axes and was most prominent in radar as a moderately sized polygon overlapping both Group B on temperature and Group D on rainfall, reasonably reflecting its geographical position in the transitional zone between the semi-arid lowlands and the Himalayan foothills.

Soil physicochemical traits: Soil physicochemical traits varied significantly among sites (Fig. 3). The maximum soil pH was recorded at Kalar Khar (KKR, 7.9), and the minimum value at Chakri (CHK, 6.84). Similarly, the maximum ECe value was recorded at KKR (2.58 dS m^{-1}), which indicates hyper salinity, and the minimum EC value at Chamyati (CMT) was 0.81 dS m^{-1}. The ionic composition of soil was influenced markedly with respect to site, with a highly significant difference ($p<0.05$). Ionic contents of sodium (Na^+) and chloride (Cl^-) showed the highest values at KKR (89.00 and 58.00 mg/kg). Conversely, equal concentrations of ions were observed at Muzaffarabad Road (MZR), recording 45.33 and 24.67 mg/kg. Soil ionic contents like potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), and phosphorus (PO_4^{3-}) differed significantly. The maximum values were recorded at MZR (26.33, 43.33, 1.97, and 4.37 mg/kg), whereas the minimum values of the same ionic contents were shown at KKR (10.67, 25.00, 1.10 and 2.20 mg/kg). An extremely significant difference was found between the moisture content and saturation percentage of soil ($p < 0.05$). MC and SP showed maximum value at Kala Bagh Road (KBR, 38.4 and 47.00%) and minimum value at KKR (11.44 and 30.67%). Soil organic matter showed the highest values at Faisalabad (FSD, 3.20%) due to the agricultural land and the lowest at KBR (0.54%).

Morphological traits: Morphological traits of *Digitaria ciliaris* showed significant ($p<0.05$) differences among sampling sites (Fig. 4). Shoot fresh weight (SFW) and root fresh weight (RFW) had similar trends, with the highest at University of Agriculture Faisalabad (UAF) and the lowest at Faisalabad (FSD), (12.36, 11.87, 4.25 and 3.98 g). In contrast, it showed minimum values at KKR (Kalar Khar), followed by (PDR) Padhrar (6.95, 8.26, 2.47 and 2.70 g). Similarly, shoot dry weight (SDW) and root dry weight (RDW) showed the same morphological pattern, exhibiting maximum values at UAF. On the contrary, the minimum values at KKR are followed by PDR (1.48, 1.85, 0.82, and 0.89 g). Furthermore, shoot length (SL), root length (RL), and leaf area (LA) all exhibited a similar trend of maximum values at the UAF, followed by FSD. In contrast, minimum values were observed at KKR, followed by PDR (34.33 and 36 cm); (9 and 9.83 cm); and (2.4 and 2.53 cm^2) (Fig. 4).

Root anatomical attributes: Root anatomical traits varied among habitats (Table 2). Root epidermal thickness (RET), root endodermal thickness (RDT), root sclerenchyma thickness (RCT), root stelar region thickness (RST), root metaxylem area (RMA), root pith cell area (RPA), root phloem cell area (RLA) and root aerenchyma area (RAA) showed significant site-wise variation ($p<0.05$), whereas root cortical cell area (RCA) showed weaker or non-significant variation ($p>0.05$). The aerenchyma area of the root was found to be the maximum at CMT (1549.96 µm^2) and the minimum at FSD (106.5 µm^2). In particular, the aerenchyma tissues were missing at KBR, KUA, JHR, PDR, AMD, KKR and JWA. Plants from the salt range had the thickest root epidermal thickness at KKR (60.566 µm); larger root cortical cell area at KKR (68.464 µm^2); thicker root sclerenchyma thickness at KKR (66.623 µm); thicker root stelar region thickness at PDR (2144.06 µm); and larger root metaxylem area at JWA (625.69 µm^2). Conversely, collections from the Himalayan and montane regions had the thinnest root epidermal thickness at BBN (24.226 µm), smaller root cortical cell area at CMT (24.723 µm^2), thin root sclerenchyma thickness at CMT (24.226 µm), thickest root stelar region thickness at CMT (1053.86 µm) and smaller root metaxylem area at KUA (216.804 µm^2). Similarly, plants collected from mountainous regions and agricultural lands exhibit maximum root endodermal thickness at MZR (78.736 µm) and maximum root phloem cell area at KHR (39.937 µm^2). However, the thickness of the endodermal cells and the area of the phloem cells of the roots were found to be least for KKR (42.396 µm) and BBN (9.508 µm^2). Root micrographs of transverse sections are presented in Fig. 5.

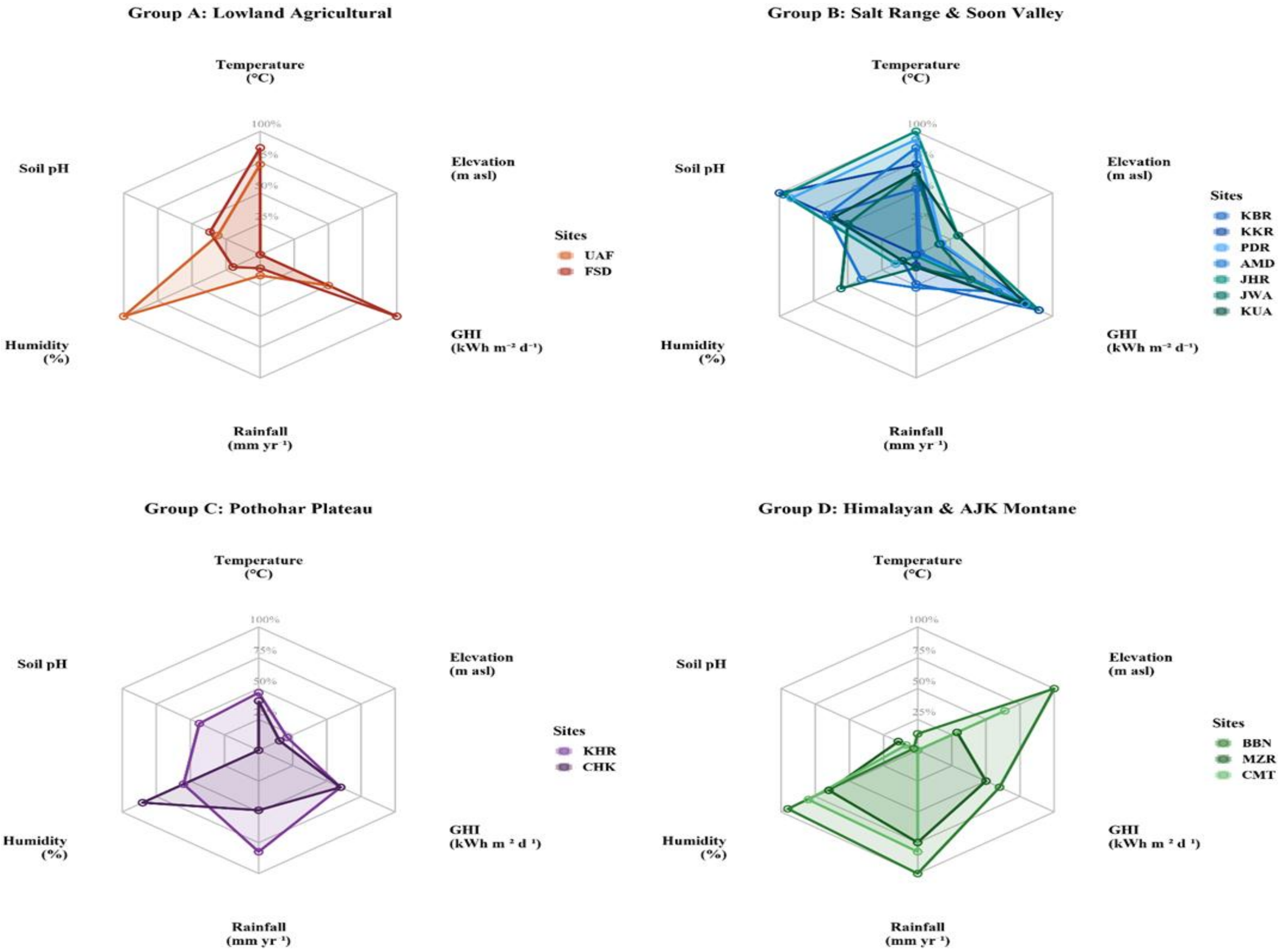


Fig. 2. Radar chart showing environmental variables of *Digitaria ciliaris* habitats.

Principal component analysis: Principal component analysis (PCA) was used to examine the variation in soil physicochemical traits, morphological attributes, root anatomical characters, and *Digitaria ciliaris* populations (Fig. 6). The first two principal components explained 70.48% of the total variation, with PC1 contributing 58.68% and PC2 contributing 11.80%. The first principal component (PC1) was the leading principal component of the fertility-salinity gradient. Morphological characteristics (such as fresh shoot weight, dry shoot weight, fresh root weight, dry root weight, shoot length, root length, leaf area) were clustered on the positive side of PC1 along with organic matter, Mg^{2+} and other moisture-nutrient-related variables. Sites such as UAF, FSD, MZR, CMT, CHK, and KHR were located on the positive side of PC1, showing their association with favorable morphological performance and relatively good edaphic conditions. On the other hand, sites such as KKR, PDR, JHR, AMD, KUA, and JWA were located mostly on the negative side of PC1 near pH, ECe, Na^+, Cl^-, and several root anatomical characteristics (such as RET, RCT, RCA, RST, and RPA). It indicates that alkaline or saline locations were associated with poor morphological growth and root anatomical differentiation. PC2 separated populations on the basis of moisture-related variables, organic matter, saturation percentage, and root anatomical differentiation. Sites like UAF, FSD, and KBR were found near the upper part of the biplot, while CMT, MZR, BBN, KUA, and AMD were found near the lower part of the biplot where Ca^{2+}, K^+, and RAA dominated.

Correlation of soil physicochemical properties with Morpho-Anatomical traits: Correlation analysis revealed that there is correlation among the physicochemical properties of the soils, morphological traits, and root anatomical characters of *Digitaria ciliaris* (Fig. 7). Cyan color depicts positive correlations in the correlogram, while magenta represents negative correlations and asterisk represents the significance levels at $p \leq 0.05$. MC% in the soils is positively correlated with SP%, while pH, ECe, Na^+, and Cl^- are positively correlated with each other which showed that the higher concentration of salts in the soils makes the soils more alkaline and conductive. Salinity/alkalinity related variables are negatively correlated with organic matter, P, K^+, Ca^{2+}, and Mg^{2+}. The morphological traits such as SFW, SDW, RFW, RDW, SL, RL, and LA have positive associations with one another and are also positively correlated with organic matter and other nutrients-related variables. However, the growth-related traits have shown negative correlations with pH, ECe, Na^+, and Cl^- which indicate that the plants perform poorly under the saline and alkaline soils. The root anatomical traits reveal a different kind of association pattern. The structural traits of the roots such as RET, RDT, RCA, RCT, RPA, RMA, and RLA are positively correlated with each other, while RST shows positive correlation with salinity- and alkalinity-related variables. RAA has shown positive correlations with the growth-related traits and fertility-related variables and negative correlations with salinity-related variables.

Table 2. Root anatomical attributes of *Digitaria ciliaris* collected across diverse habitats.

Sites	RPA (μm^2)	RST (μm)	RMA (μm^2)	RLA (μm^2)	RET (μm)	RDT (μm)	RCT (μm)	RCA (μm^2)	RAA (μm^2)
KBR	36.13 ± 8.29^{bc}	1913.90 ± 57.84^{bc}	$496.36 \pm 45.33^{a-c}$	30.42 ± 3.80^{ab}	60.56 ± 6.06^{a}	66.62 ± 6.06^{ab}	54.51 ± 10.50^{ab}	38.03 ± 10.07^{ab}	0^{e}
KHR	74.16 ± 5.71^{a}	2131.94 ± 84.24^{a}	$363.24 \pm 26.45^{c-e}$	39.93 ± 5.71^{a}	42.39 ± 6.06^{ab}	60.56 ± 6.06^{ab}	42.39 ± 6.06^{ab}	49.44 ± 1.90^{ab}	952.79 ± 59.45^{b}
MZR	30.42 ± 3.80^{bc}	1084.14 ± 21.86^{f}	572.43 ± 49.39^{ab}	30.42 ± 3.80^{ab}	30.28 ± 6.06^{ab}	78.73 ± 6.06^{a}	42.39 ± 6.06^{ab}	41.83 ± 3.80^{ab}	1182.91 ± 105.34^{b}
CMT	15.21 ± 3.80^{c}	1053.86 ± 27.78^{f}	$473.54 \pm 14.37^{a-c}$	30.42 ± 3.80^{ab}	30.28 ± 6.06^{ab}	48.45 ± 6.06^{ab}	24.22 ± 6.06^{b}	24.72 ± 5.03^{b}	1549.96 ± 134.54^{a}
BBN	17.11 ± 5.71^{bc}	1423.31 ± 32.08^{de}	245.33 ± 32.48^{de}	9.50 ± 1.90^{b}	24.22 ± 6.06^{b}	42.39 ± 6.06^{b}	30.28 ± 6.06^{ab}	49.44 ± 10.07^{ab}	$224.41 \pm 31.17^{c-e}$
KUA	15.21 ± 3.80^{c}	1489.94 ± 31.50^{d}	216.80 ± 22.84^{e}	15.21 ± 3.80^{b}	36.34 ± 10.50^{ab}	48.45 ± 6.06^{ab}	30.28 ± 6.06^{ab}	32.33 ± 11.58^{ab}	0^{e}
JHR	81.77 ± 6.86^{a}	2125.89 ± 31.50^{a}	$363.24 \pm 1.90^{c-e}$	15.21 ± 3.80^{b}	36.34 ± 10.50^{ab}	42.39 ± 6.06^{b}	42.39 ± 6.06^{ab}	32.33 ± 11.58^{ab}	0^{e}
PDR	41.83 ± 9.52^{b}	2144.06 ± 31.50^{a}	$414.59 \pm 56.09^{b-d}$	30.42 ± 3.80^{ab}	48.45 ± 6.06^{ab}	48.45 ± 6.06^{ab}	48.45 ± 6.06^{ab}	58.95 ± 5.03^{ab}	0^{e}
AMD	22.82 ± 6.59^{bc}	2144.06 ± 31.50^{a}	$397.47 \pm 28.81^{b-e}$	13.31 ± 5.03^{b}	30.28 ± 6.06^{ab}	48.45 ± 6.06^{ab}	30.28 ± 6.06^{ab}	38.03 ± 3.08^{ab}	0^{e}
KKR	22.82 ± 6.59^{bc}	2041.1 ± 32.86^{ab}	258.64 ± 24.97^{de}	9.50 ± 1.90^{b}	60.56 ± 6.06^{a}	42.39 ± 6.06^{b}	66.62 ± 12.12^{a}	68.46 ± 9.89^{a}	0^{e}
JWA	81.77 ± 6.86^{a}	1871.51 ± 37.86^{c}	625.69 ± 34.64^{a}	28.52 ± 5.71^{ab}	42.39 ± 6.06^{ab}	42.39 ± 6.06^{b}	60.56 ± 6.06^{ab}	55.15 ± 6.86^{ab}	0^{e}
CHK	34.23 ± 0.00^{bc}	1320.35 ± 33.76^{e}	$334.71 \pm 27.65^{c-e}$	30.42 ± 3.80^{ab}	42.39 ± 6.06^{ab}	60.56 ± 6.06^{ab}	36.34 ± 10.50^{ab}	47.54 ± 15.58^{ab}	424.09 ± 13.32^{c}
UAF	89.38 ± 1.90^{a}	1417.26 ± 27.78^{de}	$382.26 \pm 42.10^{c-e}$	24.72 ± 5.03^{ab}	42.39 ± 6.06^{ab}	60.56 ± 6.06^{ab}	30.28 ± 12.12^{ab}	45.64 ± 5.71^{ab}	266.25 ± 25.18^{cd}
FSD	36.13 ± 8.29^{bc}	1314.29 ± 32.08^{e}	$477.35 \pm 45.73^{a-c}$	15.21 ± 3.08^{b}	30.28 ± 6.06^{ab}	48.45 ± 6.06^{ab}	42.39 ± 6.06^{ab}	34.23 ± 9.89^{ab}	106.50 ± 7.61^{de}

Values are given as mean ± SE (n = 3). Different letters (a, b, c, d) in the same column for each root anatomical trait represent significant differences ($p<0.05$) among sites based on the LSD post-hoc test. There was no significant difference between means sharing same letter

Heatmap: The heatmap revealed distinct clusters of *Digitaria ciliaris* populations according to the soil physicochemical properties, morphological traits and root anatomical characters (Fig. 8). The colors red and blue represent values above and below the trait mean, respectively, based on z-score scaling. Traits associated with morphology of *Digitaria ciliaris*, such as SL, SDW, SFW, RFW, RDW, LA, and RL, form a cluster and have relatively high values primarily at UAF and FSD and moderately high values at MZR and CMT. At the same time, PDR and KKR show low values of morphological growth traits. Root anatomical traits, such as RST, RCA, RCT, RET, RPA, RMA, and RLA, showed relatively high values at KKR, PDR, JWA, and KHR in spite of low morphological traits. RAA showed high relative values mostly at CMT and MZR. Soil characteristics also form separate clusters: fertility and moisture related parameters such as Ca^{2+}, Mg^{2+}, K^{+}, P, MC%, SP%, and OM showed high values mostly at UAF, FSD, MZR, and CMT, while salinity and alkalinity related parameters such as pH, Na^{+}, Cl^{-}, and ECe had high values mostly at KKR and PDR. Thus, the heat map is consistent with PCA, and correlation analysis results in terms of clustering populations of *Digitaria ciliaris* with strong growth conditions and fertility of soil and populations with high salinity and alkalinity and more developed roots.

Discussion

The environmental heterogeneity is an ecological context to explain the observed plant morphology and root anatomy in the field. In present study, *Digitaria ciliaris* populations were collected from a variety of contrasting habitats in Pakistan such as lowland agricultural sites, Pothohar plateau sites, Salt range/Soon valley sites and Himalayan/AJK montane sites. These sites varied in temperature, precipitation, topographic height, light exposure, soil pH, soil moisture levels, and salinity, which established varying ecological conditions for plant establishment and growth. Similar environmental gradients have been reported to influence plant distribution, functional trait expression, and community structure across heterogeneous landscapes (Pérez-Sánchez *et al*., 2023). The lowland agricultural sites (UAF and FSD) were somewhat fertile and cultivated, while the Salt Range and Soon Valley sites (KKR, PDR, KBR, JWA, and KUA) were warmer, drier, and more alkaline or salt-affected. Montane habitats (CMT, MZR, BBN) were cooler and more moist than other habitats. The variability in microenvironments can affect plant functional traits, particularly for species living in variable or stressful habitats, and these differences are likely to be important (Westerband *et al*., 2021; Malik *et al*., 2024). Grasses are especially important, as biomass allocation, root growth, and anatomical structure are greatly affected by local edaphic conditions such as water availability, aeration, salinity, and nutrient status. The site-associated variation of *D. ciliaris* within UAF, FSD, KKR, PDR, CMT, MZR and BBN is thus considered to be a field-level response pattern related to environmental heterogeneity.

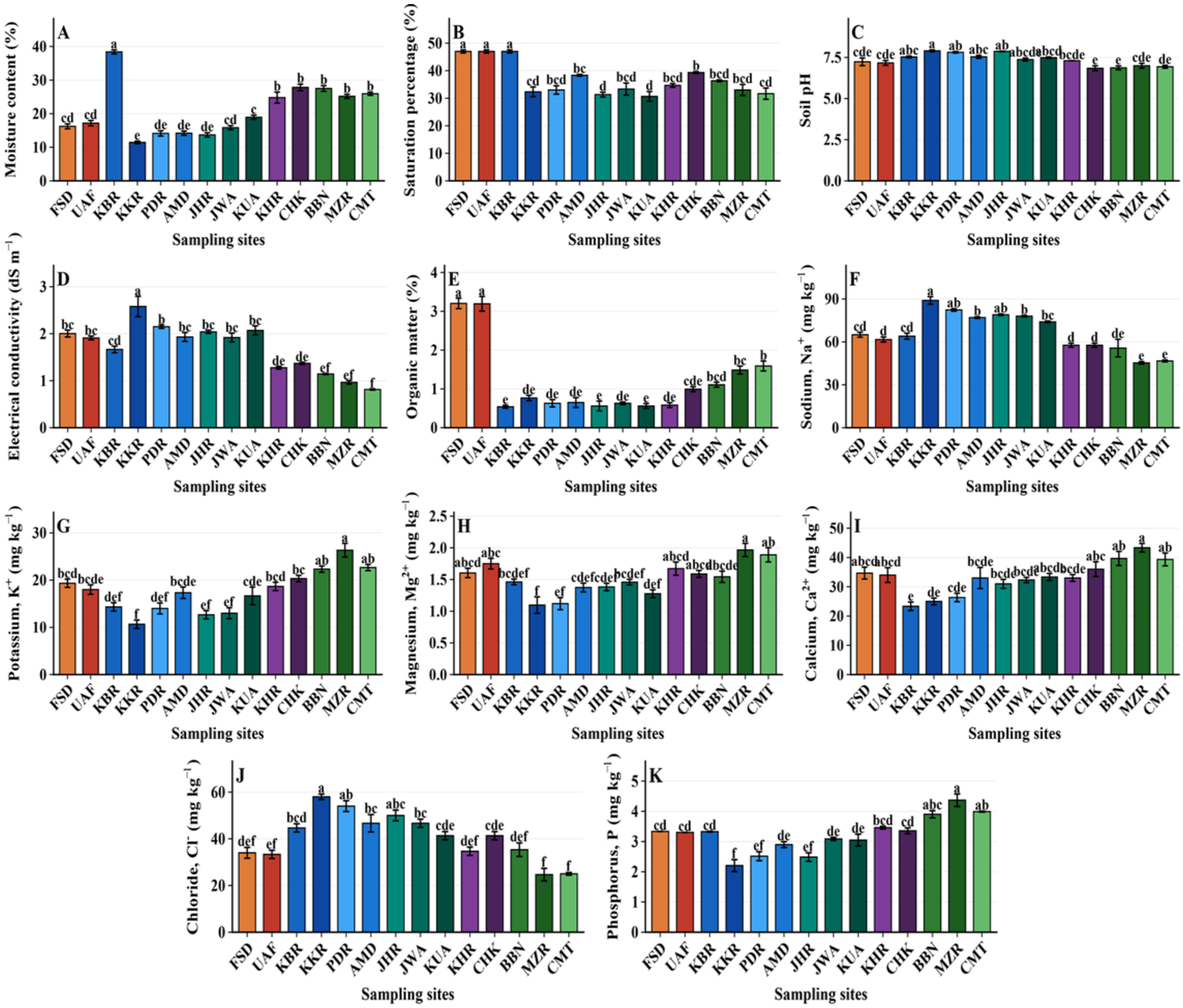


Fig. 3. Soil physicochemical traits of *Digitaria ciliaris* habitats.

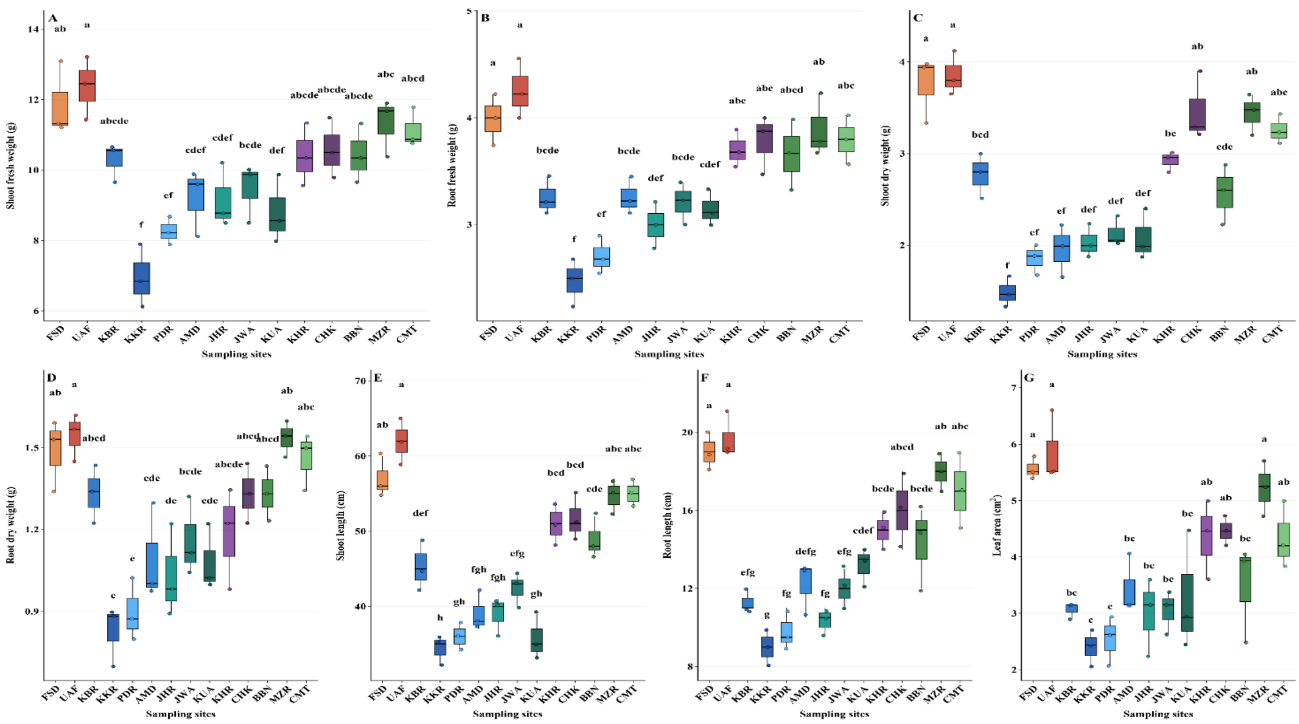
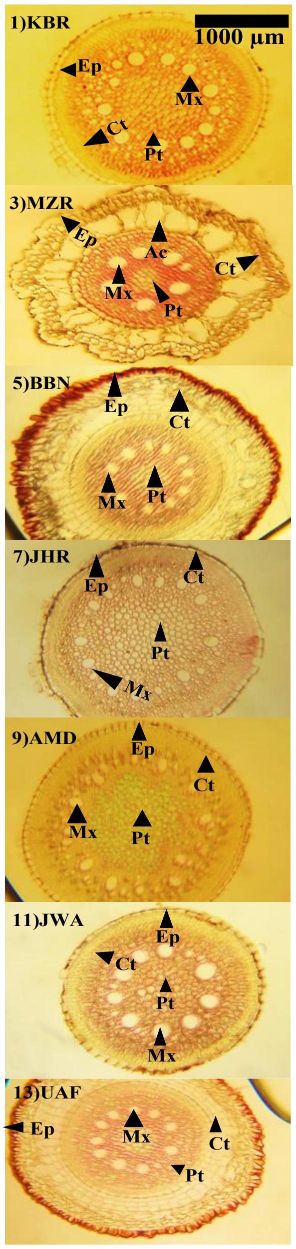
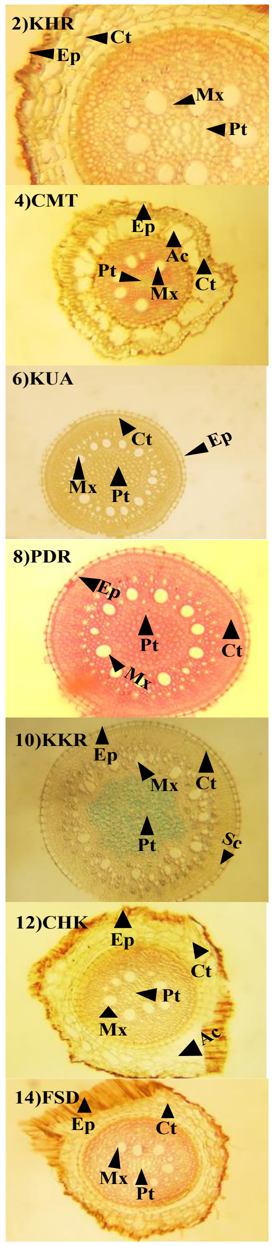


Fig. 4. Boxplot for morphological traits of *Digitaria ciliaris* collected across diverse habitats.





1) Kala Bagh Road (KBR): Thik-walled epidermis (Ep), loosely arranged cortex (Ct), central stele with metaxylem vessels (Mx), and thin-walled parenchymatous pith (Pt)

2) Kahuta Road (KHR): Thin-walled epidermis (Ep), loosely arranged cortex (Ct), central stele with metaxylem vessels (Mx), and thin-walled parenchymatous pith (Pt)

3)Muzaffarabad Road (MZR): Thin-walled epidermis (Ep), broad loosely arranged cortex (Ct), prominent aerenchyma with large air spaces (Ac), central stele with metaxylem vessels (Mx), and thin-walled parenchymatous pith (Pt)

4) Chamyati (CMT): Thin-walled epidermis (Ep), broad loosely arranged cortex (Ct), prominent aerenchyma with large air spaces (Ac), central stele with metaxylem vessels (Mx), and thin-walled parenchymatous pith (Pt)

5) Bhurban (BBN): Thin-walled epidermis (Ep), loosely arranged cortex (Ct), central stele with metaxylem vessels (Mx), and thin-walled parenchymatous pith (Pt)

6) Khura (KUA): Thik-walled epidermis (Ep), loosely arranged cortex (Ct), central stele with metaxylem vessels (Mx), and thin-walled parenchymatous pith (Pt)

7) Jahlar (JHR): Thik-walled epidermis (Ep), loosely arranged cortex (Ct), central stele with metaxylem vessels (Mx), and thin-walled parenchymatous pith (Pt)

8) Padhrar (PDR): Thik-walled epidermis (Ep), loosely arranged cortex (Ct), central stele with metaxylem vessels (Mx), and thin-walled parenchymatous pith (Pt)

9) Ahmedabad (AMD): Thick Thik-walled epidermis (Ep), loosely arranged cortex (Ct), central stele with metaxylem vessels (Mx), and thin-walled parenchymatous pith (Pt)

10) Kalar Khar (KKR): Thick-walled epidermis (Ep), well-developed sclerenchyma (Sc), loosely arranged cortex (Ct), central stele with metaxylem vessels (Mx), and thin-walled parenchymatous pith (Pt)

11) Jhongy Wala (JWA): Thik-walled epidermis (Ep), loosely arranged cortex (Ct), central stele with metaxylem vessels (Mx), and thin-walled parenchymatous pith (Pt)

12) Chakri (CHK): Thin-walled epidermis (Ep), broad loosely arranged cortex (Ct), prominent aerenchyma with large air spaces (Ac), central stele with metaxylem vessels (Mx), and thin-walled parenchymatous pith (Pt)

13) University of Agriculture Faisalabad (UAF): Thin-walled epidermis (Ep), loosely arranged cortex (Ct), central stele with metaxylem vessels (Mx), and thin-walled parenchymatous pith (Pt)

14) Faisalabad (FSD): Thin-walled epidermis (Ep), loosely arranged cortex (Ct), central stele with metaxylem vessels (Mx), and thin-walled parenchymatous pith (Pt)

Fig. 5. Representative root transverse sections of *Digitaria ciliaris* populations collected from ecologically diverse habitats of Pakistan. Labels indicate major root tissues used for measurement.

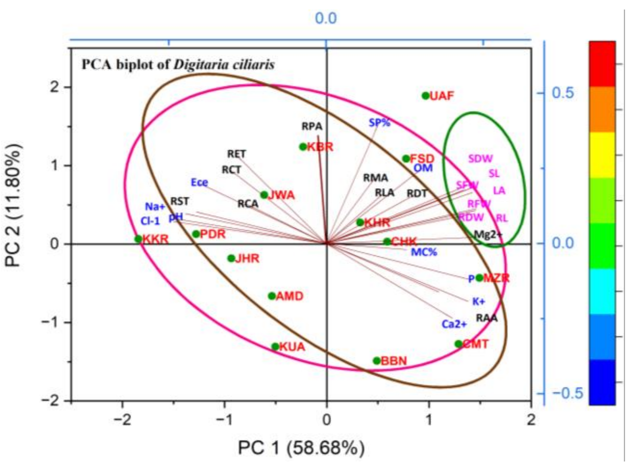


Fig. 6. Principal component analysis (PCA) showing relationship among soil and morpho-anatomical attributes of *Digitaria ciliaris* collected across diverse habitats.

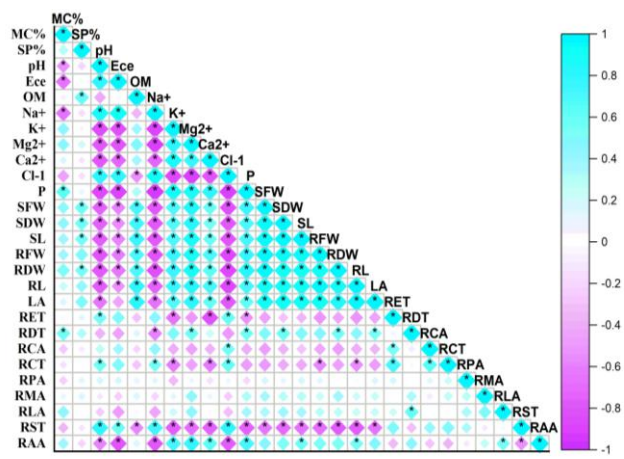


Fig. 7. Correlation analysis showing relationship among soil and morpho-anatomical attributes of *Digitaria ciliaris* collected across diverse habitats.

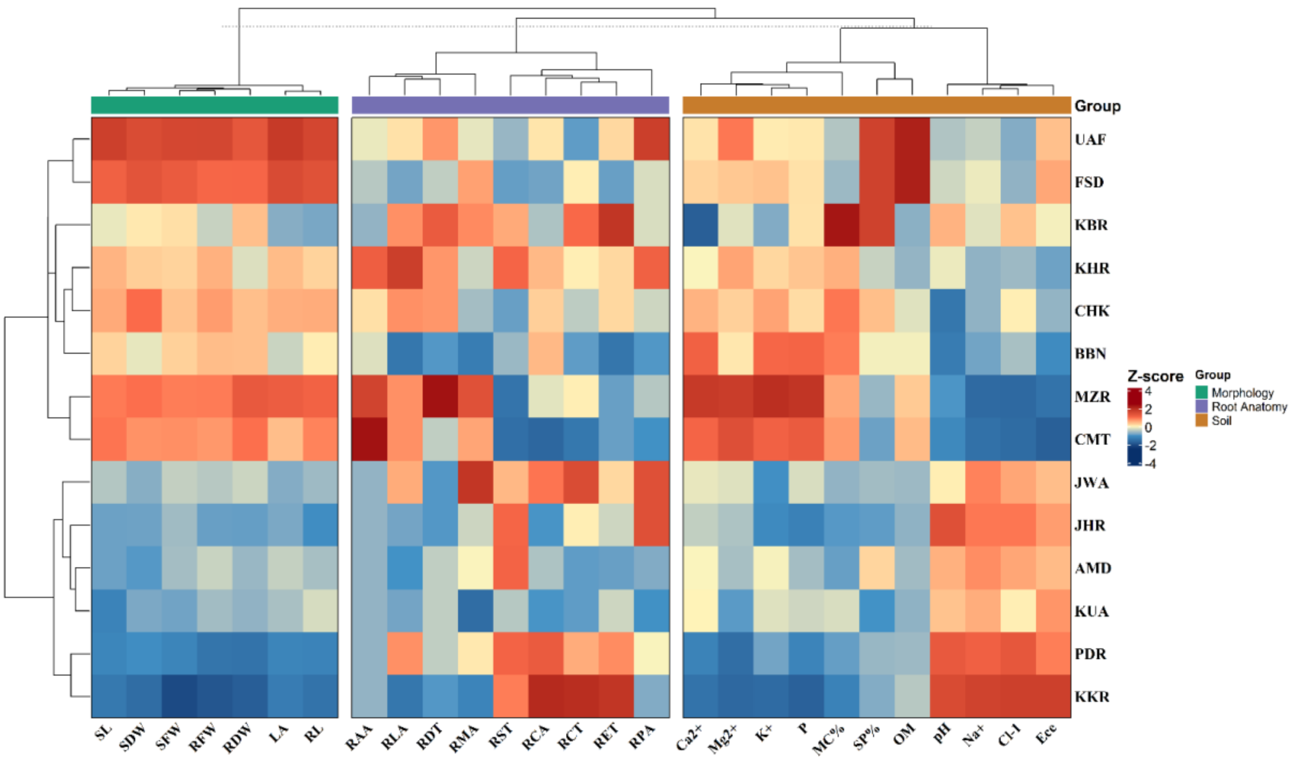


Fig. 8. Clustered heatmaps representing the morpho-anatomical attributes of *Digitaria ciliaris* across diverse habitats.

Soil physicochemical traits formed the direct local environmental filters responsible for variation in morphological and root anatomical characteristics in *D. ciliaris*. The fourteen studied sites demonstrated distinct variation in soil moisture, saturation percentage, organic matter, pH, ECe, and major ions, distinguishing more favorable sites from more stressful edaphic conditions. Soil moisture and saturation percentage were indicative of differences in root zone water availability and water holding capacity in soils, which play a crucial role in plant establishment and growth in different habitats (Brady & Weil, 2016). Sites characterized by higher levels of moisture and saturation percentage could provide a more stable water supply for roots and facilitate plant biomass production. Alkalinity and soil salinity, indicated by the higher soil pH, ECe, Na^+, and Cl^-, were critical environmental filters, allowing separating sites like KKR, PDR, JHR, and KBR from relatively neutral or less salty sites like CHK, BBN, CMT, and MZR. High soil pH, ECe, Na^+, and Cl^- suggest higher concentration of salts in the rhizosphere and their negative effect on plant growth because of osmotic stress, ion toxicity, nutrient imbalance, and decreased water availability (Hussain *et al.,* 2019a). These harsh environmental conditions cause the allocation of more resources by plants to their maintenance, ion regulation, and protection rather than biomass formation (Nalina *et al*., 2021). It is possible to explain the poor morphological development in populations occurring at sites with saline or alkaline soils. Analogous soil-induced restrictions have been reported for other grass species and halophytes living in arid and salt-affected environments, where the level of salinity and high pH determine the growth, ion regulation, and structure (Hameed *et al*., 2011; Mansoor *et al*., 2019). Organic matter and nutrient related variables showed the opposite tendencies compared to salinity-related environmental filters. The higher levels of soil organic matter at UAF, FSD, CMT, and BBN imply comparatively higher soil fertility, better aggregation, and more water and nutrient storage capacity. Organic matter plays a vital role in nutrient turnover and soil fertility and promotes the availability of nutrients necessary for root and shoot growth (Bashir *et al*., 2021). The positive relationship between organic matter, P, K^+, Ca^{2+}, and Mg^{2+} with enhanced morphological growth can be considered as evidence of the importance of nutrient availability for biomass accumulation, root elongation, shoot growth, and leaf expansion in *D. ciliaris*. At the same time, low soil fertility combined with high soil pH and salt ion concentration at sites like KKR and PDR might provide a more limiting edaphic environment.

The morphological traits had one of the most distinct responses to the fertility-moisture gradient and to the salinity-alkalinity gradient. Populations growing in favorable habitats such as UAF, FSD, MZR, and CMT had more biomass, larger shoots, longer roots, and increased leaf area compared to those growing in unfavorable habitats. Favorable habitats possessed higher organic matter, moisture status, and more nutrients like P, K^+, Ca^{2+}, and Mg^{2+} compared to the less favorable habitats. These conditions favor vegetative growth through improved nutrient acquisition, water availability, photosynthesis, and carbon partitioning to shoots and roots. On the contrary, populations growing in unfavorable habitats had lower biomass, shorter shoots and roots, and decreased leaf area. Unfavorable habitats possess high pH, ECe, Na^+, and Cl^- content, implying that salinity and alkalinity acted as limiting factors in growth. Decreased morphological growth under salinity or alkalinity is a common plant response to salt-affected soils, whereby high ECe, salt-ion content can decrease soil water potential, decrease water uptake, cause disturbance in nutrient balance, and increases the energy demand for ion regulation (Hussain *et al.*, 2019b). Plants usually respond to this situation by decreasing their leaf expansion, shoot elongation, and biomass accumulation as a strategy of conservational growth. Reduced root growth further limits the ability to obtain water and nutrients, which results in decreased shoot biomass and leaf area formation (Calleja-Cabrera *et al.*, 2020). Similar growth restrictions under salinity have been reported in salt-tolerant grasses where survival under unfavorable environmental conditions is mostly related to reduced external growth but enhanced defensive and regulatory attributes (Hameed *et al.*, 2011). Hence, low growth in morphological traits at KKR, PDR, and KHR reflects the combined effect of salinity, alkalinity, and reduced availability of resources.

The analysis of root anatomical traits presented a partially different pattern compared with external morphology, indicating that below-ground structural features were closely related to edaphic conditions at each habitat. Populations sampled at the sites KKR, KBR, PDR, and KHR demonstrated high values of the selected structural traits such as root epidermal thickness (RET), root sclerenchyma thickness (RCT), root cortical cell area (RCA), root stelar region thickness (RST), and root metaxylem area (RMA). Those features may be responsible for mechanical strength, protection of internal structures, control of radial water transport, and limitation of ion penetration under saline, alkaline, or dry soil conditions. Under salt-affected conditions, high RET and RCT values could reduce uncontrolled water loss and increase the resistance of the outer layer to stress, while variability in root endodermal thickness (RDT) and RST might affect the transport of water and solutes into the vascular cylinder. The variability in RST and RMA indicates the possible adjustment of root hydraulic architecture in different habitats. The higher development of stelar or metaxylem layers may promote water transport in certain cases, but the changes could be accompanied by the trade-off between efficiency of transport and protection of roots against stress-related hydraulic dysfunctions. Consequently, the increased anatomical features of some stress-associated sites could demonstrate the adaptation of roots with the structural rather than overall growth strategies under unfavorable soil conditions. Contrary to other structural features analyzed above, the root aerenchyma area (RAA) had a partially different pattern. High values of RAA were mainly found in the moisture-supported sites such as CMT, MZR, and KHR, while aerenchyma was poorly developed or absent in a number of Soon Valley and Salt Range sites. The pattern could be explained by the role of aerenchyma in the improvement of internal oxygen transport under the conditions of high humidity and compacted soil (Pedersen *et al.*, 2021; Yamauchi *et al.*, 2021). Aerenchyma formation is usually typical of flooding or hypoxic environments for many grasses and plants growing in wetlands (Thomas *et al.*, 2005). The absence of aerenchyma in drier and salt-affected sites should be considered carefully as the characteristic of field-anatomical features rather than the result of oxygen stress.

The multivariate approaches gave a cohesive account of the soil-trait association between different *D. ciliaris* populations. PCA showed distinct separation among the different populations based on fertility-salinity and moisture contrasts, while correlation and heatmap clustering demonstrated that growth-associated traits were strongly correlated with organic matter, nutrients, and moisture-dependent factors (Brady & Weil, 2016; Bashir *et al.*, 2021). On the other hand, pH, ECe, Na^+, and Cl^- showed correlations with poor morphological performance and higher variation in chosen root anatomy traits. All this shows that there was a correlation between soil heterogeneity and one individual trait but rather that of growth and root morphology organization (Hussain *et al.*, 2019a). The separation of morphological traits from some of the anatomy traits implies that growth under external conditions and underground anatomy are affected by soil stress in different ways. Hence, according to multivariate approaches, fertile and moisture soils enhance vegetative performance while salinity and alkaline soils limit growth and promote root anatomy differentiation in *D. ciliaris*.

Conclusion

This study revealed that soil heterogeneity is related to the site-specific variation in morphological and root anatomical characteristics in *Digitaria ciliaris* in diverse ecosystems of Pakistan. The populations of *D. ciliaris* from fertile and moisture-rich areas exhibited higher root and shoot biomass production, long roots and shoots, and large leaf surface area, while the populations from areas that were affected by saline conditions, high alkalinity or low soil moisture content exhibited reduced morphological characteristics and increased root anatomical differentiation. It was evident that soil fertility, salinity, alkalinity, and moisture availability constitute key ecological filters related to field performance and root anatomical features of *D. ciliaris*. At the same time, the results of the study demonstrated the importance of using root anatomical features, specifically epidermis, sclerenchyma, stele, metaxylem, and aerenchyma characters, to analyze grass response to heterogeneous environments. However, it should be noted that due to the field nature of collected populations, the above-mentioned differences should be considered as an in-situ phenotypic variation of species rather than phenotypic plasticity or genetic differentiation. Further investigations should consider increased within-site replication, controlled environmental conditions, depth-wise soil sampling, and molecular analysis of population structure.

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Author's Contribution: Muhammad Shakeel, Methodology, Formal analysis, Writing original draft. Muhammad Sajid Aqeel Ahmad, Project conceptualization and administration. Farooq Ahmad, writing-reviewing and editing. Adnan Younis, methodology, data curation. All authors read and approved the final manuscript.

Declaration of AI or AI Assisted Services: It is declared that the authors did not use any AI or AI Assisted services for language editing of this manuscript.

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