

Ecological adaptations of *Justicia adhatoda* L. against environmental constraints: Strategies for survival and sustainability

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Abstract

Sixteen ecologically distinct ecotypes of *Justicia adhatoda* (L.) Pers. were studied to explore key attributes of its widespread distribution in heterogeneous environments. Ecotypes of saline areas (KW, KL and KK) showed better vegetative growth (shoot fresh and dry weight and plant height) and accumulation of inorganic ions sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) as compared to all other ecotypes. Increased stem radii, scarification around parenchyma (pith and cortex) and increased metaxylem area were remarkable modifications to resist environmental changes. Notable stem modifications in dry mountains included the development of thicker collenchyma tissue and reduced protoxylem and vessel number. Moreover, longer shoots and increased shoot potassium in the KM population help to improve water conservation by reducing surface water loss and increasing storage capacity. Ecotypes from Mountain Valley exhibited the most distinct characteristics in growth, physiology and anatomy features, less shoot K^+ and Ca^{2+} contents, and increased epidermal cell, cortical region and protoxylem cell area. The populations along the roadside (CS, BU, MN and FD) had specific anatomical variations like epidermal thickening and intensive sclerification around cortical regions. It was concluded that different populations of *Justicia adhatoda* L. exhibited variations in morpho-physiology and anatomy in heterogeneous environments that may contribute to its distribution and diversification.

Introduction

Justicia adhatoda, commonly known as Malabar Nut, belongs to the Acanthaceae family, widely distributed across tropical and subtropical regions, including South Asia, Southeast Asia, and Africa, with adaptability to diverse climatic conditions[1, 2]. It can survive in diverse habitats, ranging from lowland plains to high-altitude environments. The plant is well known for its medicinal properties, particularly due to bioactive compounds like vasicine and vasicinone, which have significant pharmacological applications in treating respiratory ailments, inflammation, and microbial infections [3]. It is effective against tuberculosis and respiratory diseases, with potential candidates for drug development [4]. Beyond its medicinal value, *J. adhatoda* plays a crucial ecological role in stabilizing soil, phytoremediation, enhancing nitrogen contents in compost, supporting biodiversity and acting as a nectar source for pollinators[1].

Justicia adhatoda exhibits morphological and physiological adaptations to abiotic stress. It was found that xeric environments show increased root fresh and dry weight and root length in response to severe dryness. Anatomical changes such as thick epidermal layers and enhanced vascular structures contribute to water conservation and mechanical stability[5]. Despite its adaptability, *J. adhatoda* faces multiple environmental stressors that impact its survival potential, ecological distribution and sustainability. Water availability is a limiting factor for *J. adhatoda* in arid and semi-arid regions, necessitating morphological and physiological adjustments such as deep root systems and enhanced tissue water retention. Drought affects soil moisture, reducing nutrient availability and plant stress, which can diminish its physico-chemical efficiency[1].

Additionally, the ecological condition of soils is significantly crucial in the growth of *J. adhatoda*; contaminated or degraded soils can adversely affect its health and productivity. It exhibits a wide tolerance to varying soil pH and nutrient levels but is often challenged by salinity, erosion, and nutrient depletion, especially in degraded landscapes. Moreover, urbanization and industrial activities contribute to air and soil pollution, affecting plant health and growth. Soil contamination from pollutants can lead to reduced soil fertility, impacting the plant's ability to thrive and its role in ecosystem services like soil stabilization [6]. However, high pollution levels can overwhelm the plant's detoxification capacity, leading to reduced growth and medicinal value[2]. Irrespective of abiotic stresses imposed by environmental constraints, altitudinal gradients also significantly affect the plant's physiology and photochemistry with changes in chlorophyll concentration, osmoprotectant compounds (proline, glycine betaine), phenolic, flavonoids, sugars[7] and leaf structural adaptations like increased cuticle thickness and epidermal cells[8].

The present study aimed: (i) To understand different morpho-physiological and structural modifications of differently adapted natural populations of *Justicia adhatoda* collected from different environments (ii) To investigate the *Justicia adhatoda* response to varied environmental cues (iii) To explore conservation and sustainability approaches to ensure the long-term viability of the *J. adhatoda* population. It was hypothesised that the evolution of different modifications might help to investigate the distribution patterns of *Justicia adhatoda* in different environments. The present study will help explore the traits in *Justicia adhatoda* that make it the most successful and dominant plant species in that particular environment. Moreover, the plasticity in shoot morpho-physiological and anatomical attributes of *Justicia adhatoda* L. contributes to its ecological success in different environments. Moreover, the adaptive mechanisms of *J. adhatoda* will provide valuable insights into plant resilience, environmental sustainability and conservation strategies. However, understanding its responses to abiotic stressors can aid in developing climate resilience and ecological restoration efforts in degraded habitats. Identifying genetic variations across different environmental conditions may also contribute to selective breeding programs to enhance stress tolerance.

Methodology

Study Site and Collection

The Punjab province, geographically positioned in the southeast of Pakistan, covers latitudes 29.30 to 32.32 North, 73.55 to 76.50 East, with a height range of 180 to 500 meters. The climate varies from very hot to dry and cold extremes. Average annual rainfall is between 213 and 307mm, with a temperature range of 4°C to 45 °C. The dry sample of wild plant *Justicia adhatoda* was deposited in the herbarium of University Of Agriculture, Faisalabad; voucher number 224-21-04. *Justicia adhatoda* was identified by Prof. Dr. Mansoor Hameed (Retired Prof. UAF). Various ecotypes of the perennial *Justicia adhatoda* were collected from different habitats in Punjab (Pakistan) i.e., Dry mountains [PU (Phulgran), BR (Barakhu), KT (Katas), NW (Neela waha-n), AH (Ahmedabad), KS (Katha sagral), KM (Katha mountains)], Mountains valley [PD (Padhrar), JB (Jabba)], saline -areas [KW (Khewra), KL (Khabeki lake), KK (Kallar khar)],

Roadside [CS (Choa sidnshah), BU (Buchal), MN (Munara), FD (Faisalabad)] as shown in Fig. 1. The plant samples were collected during July 2020 to September 2020 at flowering stage Fig. 1.

Climate and geographical data

The data for average annual minimum and maximum temperature and precipitation were obtained from Meteorological Department substations situated in each district (Table 1). A GPS (Etrex 20 CAN310, Garmin, USA) was used for coordinates and elevation data. Annual precipitation varied from 375 mm annually to 1220 mm up to 876 m elevation. The minimum annual rainfall was recorded at the lowest elevation (185 m). The maximum average temperature dropped from 44 to 30.36 °C with 743 to 544 m elevation. Minimum annual temperature ranged between 5 and 20 °C up to 634-685m elevation Table 1.

Soil Physico-Chemical Attributes

Sixteen ecotypes of *Justicia adhatoda* were randomly uprooted using a soil auger at each habitat. Different soil samples were also collected to study soil attributes, i.e., organic matter percentage, pH, saturation percentage and ionic contents. The soil adhering to the rooting zone was collected from each sampling site at a 15-20 cm depth to determine soil-physicochemical traits. The soil was oven-dried at 70 °C, and 200 g of soil was used to calculate the saturation percentage. A pH metre (pH/Cond 720, WTW series InoLab, USA) was used to test pH in soil water isolated from saturation paste. The soil ionic contents of cations (Ca^{2+} , K^{+} , and Na^{+}) were analysed from the soil extract using a flame photometer (PFP-7, Jenway, UK). A titration method (Richard, 1954) was used to measure the chloride content. An atomic absorption spectrophotometer (AAAnalyst 300, PerkinElmer, USA) was used to analyze magnesium (Mg^{2+}). Soil phosphorus was analyzed by using a spectrophotometer at 470 nm (UV-1100) [9].

Morphological attributes

The morphological traits, i.e., fresh and dry weight of shoot, plant height, of each ecotype were calculated. Fresh weight was measured immediately after collection on a digital loading balance (ISO 9001, Household Electronic Co., Ltd., Guangdong, China). For dry weight, samples were oven-dried at 65 °C to measure a constant weight.

Shoot Ionic contents

The dried shoot (0.5 g) was crushed and left in a flask with 5 mL of concentrated H_2SO_4 overnight. Following [10] instructions, shoot samples were digested by adding H_2O_2 on a hot plate (at 350 °C) until adding H_2O_2 turned the solution colourless. A flame photometer (Model 410, Sherwood Scientific Ltd.,

Cambridge, UK) was used to determine the concentration of monovalent cations, Sodium (Na^+), Potassium (K^+) and divalent cations, Calcium (Ca^{2+}), Magnesium (Mg^{2+}) and shoot phosphorus.

Anatomical attributes

The plant shoot samples collected from the field were immediately fixed in FAA solution and then preserved in acetic alcohol solution (1:3 ratio). Transverse sections of plant materials (stem) were prepared by free-hand sectioning and then dehydrated using ethanol solutions [11]. Finally, safranin and fast green were used to stain internal tissues. Canada balsam was used to mount the sections on a slide so they could be made into permanent slides. The stained sections were photographed using a Nikon 104 stereo microscope with a Nikon FDX-35 camera. An ocular micrometre was used to measure various attributes, i.e., shoot epidermal thickness, cortex thickness, cell area, pith, sclerenchyma thickness, vascular bundle, met xylem, and phloem. The anatomical attributes of the stem are labelled and presented in Fig. 2.

Statistical analysis

The data was statistically analysed, with LSD values computed using COSTAT. Means were calculated using LSD ($p > 0.05$) to represent physiological and anatomical attributes. A one-way analysis of variance (ANOVA) was conducted to assess the effect of the site [12]. Principal Component Analysis (PCA) was performed using R Studio, utilising the FactoMineR and factoextra packages. Pearson's correlation analysis was done to correlate physicochemical and anatomical attributes of plants and soil physicochemical properties using the corrplot package in R Studio. Clustered heatmaps were drawn in R Studio (v 4.1.2) to visualize the associations between physio-anatomical traits of plants and soil physicochemical attributes in different adapted populations.

Results Soil chemical attributes

Soil physicochemical attributes of various habitats were significantly varied. The soil was usually sandy loam along roadside sampling sites, i.e., BU (Buchal), MN (Munara) and FD (Faisalabad), while sandy soil was observed in mountainous regions, i.e., BR (Barakhu), KT (Katas) and NW (Neela Wahn) (Table 1). The moisture contents vary (11%-6.08%) among all habitats of *Justicia adhatoda* ecotypes, where the maximum soil MC was recorded at MN and FD sites, and the minimum at the KK site. The maximum saturation percentage was observed in roadside (MN and FD) sites. Soil organic matter ranged from 1.76 to 2.9 %, where the maximum organic matter was noticed at JB (Jabba), MN (Munara) and BR (Barakhu) and the minimum at saline area KW (Khewra). Most of the habitats were characterised by alkaline pH ranging from 7.2 to 9.2 in ecotypes of the dry mountainous, mountainous valley and roadside sampling sites. In contrast, a slightly acidic pH (6.82) was observed in the soil of the saline area (KW). Greater variations exist in soil Na^+ contents; the ecotypes of saline areas, i.e., KK (Kallar kahar), showed

maximum sodium contents (32.3 mg/g) as compared to other sampling sites. The minimum sodium contents were observed at the roadside sites BU and CS. Variations exist according to the topographic factors of respective habitats. Chlorine contents also follow the same trends, but the minimum chlorine contents were found at FD site. The variations in potassium contents follow saline areas > dry mountains > mountain valley > roadside. The calcium contents range from 12-30 mg/g at KW and JB sites. The magnesium and phosphorus contents vary significantly; the maximum Mg and P contents were found at KK site (Figure 3a, 3b).

Morphological attributes

Various ecotypes of *Justicia adhatoda* responded differently to soil types in terms of their growth attributes. The shoot fresh weight to dry weight ratio was maximum at BR (Barakhu) followed by PD (Padhrar), and the minimum ratio was found at the KK (Kallar Khar) ecotype. The shoot fresh weight varied according to heterogeneity in respective habitats; the maximum shoot fresh and dry weight was recorded at the KK site, while the ecotype of KM (Katha Mountains) showed the minimum shoot fresh and dry weight. The maximum shoot length was recorded at KW, followed by CS (Choa Sidan Shah) and PD (Padhrar), while the minimum was observed in the KK (Kallar Khar) ecotype (Figure 4).

Physiological attributes

The shoot ionic contents differed significantly in various habitats. Among saline habitats, the maximum S-Na (27 mg Kg⁻¹) was recorded at KK (Kallar Khar) site and the minimum S-Na (12.16 mg Kg⁻¹) at BU and MN (Buchal and Munara) of the roadside sampling site. Meanwhile, the ecotypes of dry mountain sampling habitats KM (Katha mountains) showed S-Na (23 mg Kg⁻¹). The highest S-K (23.33 mg Kg⁻¹) was recorded in saline areas (KK, KL) sites, followed by KS and KM sites at dry mountains, but the ecotypes of CS and BU showed minimum S-K (6.8 mg kg⁻¹). Fewer variations were recorded in calcium contents among all habitats except dry mountains. The S-Mg varied between 14.2 and 21.5 mg Kg⁻¹. The maximum value of S-P was observed in ecotype pf KM (Katha Mountains), while KK sites showed less S-P content (Figure 5).

Stem anatomical attributes

Stem epidermal cell area was maximum (175.62 μm^2) at KK site of saline areas. The minimum stem epidermal cell area was maximum (175.62 μm^2) at KK site of saline areas. The minimum (84.65 μm^2) area was recorded at JB site of the mountain valley region. Similarly, stem epidermal thickness was highest at KK site, followed by KW site, while the minimum thickness was recorded at FD site of the roadside. The heterogeneous environment showed variations in stem cortical cell area and thickness. Cortical cell area varied significantly in different habitats. Saline areas showed the maximum cortical cell

area at KS site ($676.43 \mu\text{m}^2$) and the minimum ($219.69 \mu\text{m}^2$) cortical cell area was recorded at KL site of saline areas. Cortical region thickness (CRT) varied in the following order: saline area > dry mountains > road sides > mountain valley ($112\text{-}186.26 \mu\text{m}$) (Table 2, Figure 6). The stem pith area increased with increasing moisture deficit. The maximum ($476 \mu\text{m}^2$) pith area was recorded at KW site of saline areas, while the BU site of roadside habitats showed reduced ($169 \mu\text{m}^2$) pith area (Table 3, Figure 6). Stems of saline areas showed wider stem radii than stems collected from other habitats.

Ecotypes of saline areas showed maximum stem sclerenchyma thickness, while dry mountain regions showed less variation in stem sclerenchyma thickness. The collenchyma thickness was maximum in ecotypes of saline areas, but BU and MN showed minimum stem collenchyma thickness. The stem metaxylem area increased significantly among different habitats. Increased metaxylem area was recorded at KK and KW sites of saline areas and BR site of the dry mountains region. Whereas, variations in protoxylem area ranged from $94\text{-}437 \mu\text{m}^2$. Meanwhile, ecotypes of the saline areas showed a minimum vascular bundle area compared to other habitats. The maximum vascular bundle area was recorded at FD site. The highest vessel number (175) was observed at the KK site, while JB site showed a lower number (87) of vessels. Whereas, the maximum phloem thickness was observed at the KW site, and the thin phloem was observed at PD site (Table 2, Figure 6).

Multivariate analysis PCA

The principal component analysis shows a major contribution of PC_1 (42.2%). Ecotypes of four different environments showed different engine values. The SL, SDW, SFW showed significant contributions and are affected by soil P and pH. The population of the saline environment (KL, KW and KK) showed negative engine values. The plant ionic contents SK, SNa, and SCa were affected by soil Mg^{2+} , Cl, Na, and K^+ at NW, KS and M-KT. Soil MC, SP, Ca^{2+} and OM affect SPh at PD and PU sites. The principal component analysis shows anatomical attributes where PC_1 major contributes (56.6%) and PC_2 shows a minor contribution (11.6%). Soil attributes strongly affected plant anatomical characters (S ScT, PCA, SVN, SMA, S.CRT, SDA, SPHT, S.ChT) at BR, PU, KL, NW, KS, KL and AH sites, while at other habitats, i.e., JB, MN, FD, BU and PD, SVA was affected by soil OM, Ca, MC, SP, SPA (Figure 7).

Correlation

Person correlations presented soil physico-chemical, plant morpho-physiological and anatomical attributes. The growth parameters SL, SFW, and SDW are not correlated with soil OM, SP, Ca, pH and P. The ionic contents, S-Na, S-K, and S-Ca, positively correlated with K^+ , Na and Cl. In contrast, S-Mg showed a negative correlation to some extent with soil and plant parameters. pH was strongly negatively correlated with SA and SR. Among anatomical attributes, SPA and SVA are negatively correlated with soil Na^+ , Cl^- and K^+ and positively correlated with soil OM, Ca, SP and MC. SDA, SA, SR, S.CRT, SPHT, SDT, SChT, SMA, and SVN positively correlated with soil attributes (Figure 8).

Heat maps

The clustered heatmap represents the association of soil physicochemical properties with stem morpho-physiological attributes. There was a positive association of S-K, SFD, S-Ca, and S-Na with soil K^+ , Na^+ , Cl and Mg^{+2} at NW, KT, BR, KS and AH and the negative association was observed at CS, JB, PD, FD, BU, and MN sites. At the same time, the ecotype of *Justicia adhatoda* collected from CS, JB, PD, FD, and BU showed SDW and SFW a positive association with soil parameters (ECe, P and pH). However, ecotypes collected from mountainous and saline habitats were negatively associated with soil MC, OM, Ca^{2+} , pH, and P on SFW, S-Mg, SL, and SPh. These parameters are positively related to other habitats, i.e., roadsides and mountain valleys (Figure 9). Moreover, the anatomical attributes are differently associated with various soil physicochemical attributes. Soil Mg, K^+ , Na, and Cl are positively associated with S ScT, SVN, SMA, S. ChT, SDT, SDA, SR, SA and S.CRT and the negative association was observed among soil Cl, Mg, K^+ and Na, with S ScT, SVM, SMA, S ChT, SPhT, SDT at roadside and mountain valley habitats (Figure 9).

Discussion

The present study found significant differences in morpho-anatomical and physiological aspects among ecotypes, which were linked to the environmental conditions of their respective habitats. The Salt Range contains geographically essential lakes. The most important are Khabeki Lake and Kalar Kahar Lake. These lakes have hyper-saline waters due to salts accumulated from rock weathering[13]. The ecotypes of hypersaline lakes have greater potential to grow better in saline environments [14, 15]. Most plant species retain green stems in saline environments[16]. The present study revealed that the ecotypes of *Justicia adhatoda* adapted to saline regions (KK, KL and KW) had a fresher and drier weight than the other ecotypes, indicating salinity tolerance. This increase in biomass is referred to as the accumulation of inorganic ions for enhanced turgor management. It was also found that a similar rise in inorganic ions, sodium (Na^+) and potassium (K^+) in these ecotypes may also play a crucial role in growth and biomass production by maintaining internal cell osmotica, which improves division and elongation processes occurring in cells [17]. It was suggested earlier that the increased sodium (Na^+) content with increasing potassium (K^+) and calcium (Ca^{2+}) contents may neutralize sodium toxicity. The present study also revealed that the KK ecotype showed maximum Na^+ contents as compared to other sites of saline areas with reduced uptake of K^+ ions and increased uptake of Ca^{2+} ions that minimizes Na^+ ion toxicity in saline environment [14].

However, anatomically, there was an increase in stem epidermal cell area and thickness, reduced cortical area, increased sclerenchyma, chlorenchyma and parenchyma thickness and increased metaxylem and phloem area, as well as vessel numbers in KK and KW ecotypes. All these modifications help plants protect themselves from surface injury under extreme water loss in heterogeneous environments, thereby enhancing the survival of plants[18]. These modifications also play an important role in increasing water potential to cope with abiotic stresses[15].

Another ecotype (KBL) collected from saline areas showed physio-anatomical distinct traits that enabled it to survive in a saline environment. Among physicochemical attributes, this ecotype showed increased sodium and calcium contents, helping to maintain ionic homeostasis under saline stress[19]. Anatomically, these ecotypes respond differently to other ecotypes of saline areas by encountering reduced stem epidermal area, cortical cell and thickness, and reduced vascular bundle area. All these features play an important role in osmotic adjustment to hydrate plant tissues to support plants[14] mechanically.

Plant growth is an important criterion for studying plants' responses in different environments. The ecotypes collected from dry mountainous habitats, i.e., PU, BR, KT, NW, AH, KS, KM) showed various responses under heterogeneous environments. These ecotypes showed a reduction in growth parameters. Growth suppression is due to the conversion of normal growth metabolism to stress metabolism. Moreover, the reduction of growth in dry environments is directly linked to lower photosynthetic activity and limited ion uptake[20], as reported earlier in *Desmostachya bipinnate*. Various plant species, such as *Cynodon dactylon* in dry mountains (NWN), exhibited the maximum shoot length. This represents the principal attribute among all growth features because it provides better mineral redistribution within the plant body, which significantly contributes to survival in heterogeneous environments. Moreover, stems with increased vascular bundles help in maximum water conduction and translocation of essential metabolites. Irrespective of that, the stem also protects plants from solar irradiance and keeps a check on the regulation of transpiration and photosynthetic process at drier top hills[21, 22]. The present study also suggested the ecotypes of *Justicia adhatoda* adapted to dry mountainous region (NW) had long shoots increased epidermal cell area and thickness, increased vascular bundle area, increased vascular bundle number, metaxylem and protoxylem area as compared to other ecotypes of mountainous region, these modifications help to prevent plant from mechanical injury and also play a key role in improving water conservation by reducing surface water loss and increasing storage capacity. The mountain range (KAM) population of various monocots contained a high concentration of inorganic ions (shoot K^+ and Ca^{2+}). K^+ is involved in the activation of photosynthetic enzymes, regulation of cell turgidity and maintenance of hydrostatic pressure, whereas calcium (Ca^{2+}) is involved in signal transduction and cell osmosis[23, 24]. The recent research on *Justicia adhatoda* also suggested that the ecotype of *Justicia adhatoda* in dry mountains (KM) has high potassium and calcium in shoots compared to other habitats of the mountain range. These features contribute to the survival of *J. adhatoda* in dry habitats. Water conservation plays are critical for plants growing in dry habitats, and it can be accomplished by either preventing water from passing through the plant surface, such as a thick epidermis and cuticle[25], or by storing more water in parenchyma tissues[26]. Moreover, efficient water translocation also helps conserve water by reducing water loss. Sclerification is also a distinctive trait [27] that provides mechanical strength and prevents tissues from collapsing in dry habitats. It was also reported in *Cenchrus ciliaris* that the stem area is the most critical structural attribute, and it mainly depends on the parenchymatous region. The present study showed that the stem area of *Justicia adhatoda* increased in dry mountainous habitats (PU, BR, KT, NW, AH, KS, KM). The other prominent feature of dicot is, an increase in succulence through the cortical region (Mansoor

et al., 2019) to conserve water for long period in dry environments in plant tissues [28], and therefore ecotypes with increased storage parenchyma can better tolerate water deficiency problems [29].

It was studied earlier that low potassium uptake efficiency is low in soils due to the physicochemical similarity of sodium (Na^+) with potassium (K^+). The presence of sodium (Na^+) in soil and groundwater affects plant potassium (K^+) absorption, negatively impacting enzymatic activities, cellular turgor and protein biosynthesis [30, 31]. Other anatomical modifications, deposition in the epidermal wall and long epidermal cells enable the plants to prevent transpiration via the epidermis [31, 32]. The ecotypes of *Justicia adhatoda* from Soon Valley (PD, JB) showed the most distinct characters in growth, physiological and anatomical features, less shoot K^+ and Ca^{2+} contents, and increased epidermal cell, cortical cell area, and protoxylem cell area. These modifications are critical responses of plants in heterogeneous environments to restrict water movement outside the plant surface. The population along the roadside (CS, BU, MN and FD) had particular anatomical variations like epidermal thickening and intensive sclerification around the cortical region. These attributes are important in preventing desiccation by providing surface protection [33]. They also provide mechanical strength to soft tissues and avoid evapotranspiration [34].

Conclusion

It is concluded that the morpho-physiological and structural adaptations in naturally occurring populations of *Justicia adhatoda* have evolved independently over an extended evolutionary period. Modifications in growth characteristics (such as shoot length, shoot fresh and dry weight), micro-structural features (including epidermal, mechanical, vascular, and storage tissues) and functional traits (such as shoot ionic content) contribute to the successful colonisation of *Justicia adhatoda* in diverse and heterogeneous environments.

Declarations

Declaration of competing interests

The authors have declared that no competing interests exist.

Clinical Trial Number

Clinical trial number is not applicable.'

Declaration statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethics statement

This study was conducted in accordance with the Declaration of Helsinki and all relevant ethical guidelines. All procedures involving plant collection and analysis were approved by the appropriate institutional committee at the Department of Botany, University of Agriculture Faisalabad.

Consent to Participate

Not applicable

Consent to Publish

Not applicable

Author Contribution Statement

ZN, MSA, MH conceived and designed the study and led field surveys in the arid regions of Punjab, Pakistan. SB and SF collected plant and soil samples and performed morphophysiological, biochemical, and anatomical assessments. SM contributed to biochemical analyses and data interpretation. AES assisted with laboratory experiments and data collection. IAA contributed to the evaluation of soil properties and environmental data. All authors participated in data analysis, interpreted the results, contributed to manuscript writing, and approved the final version for submission.

Data Availability Statements

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interest Statement

It is declared that there is no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Tables

Tables 1 to 3 are available in the Supplementary Files section.

Figures

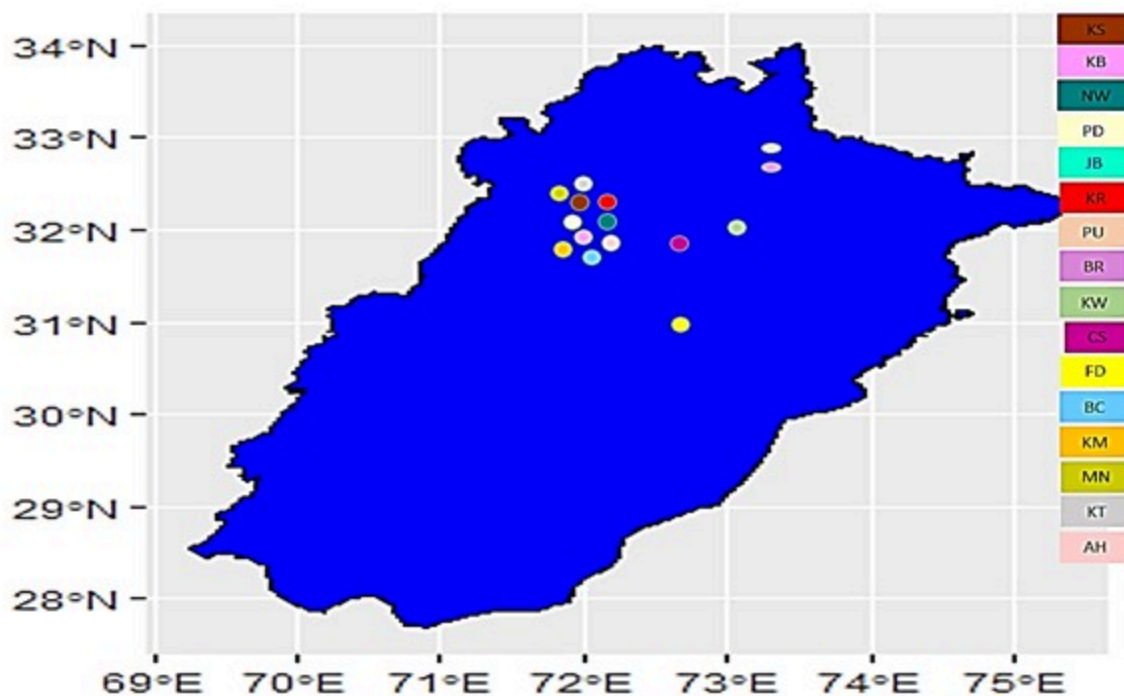


Figure 1

Map of Punjab, Pakistan representing; Habitats: [Phulgran (PU), Barakhu (BR), Katas (KT), Neelawahn (NW), Ahmadabad (AH), Katha Sargral (KS) and Katha mountains (KM), Mountains Valley [Padhrar (PD) and Jabba (JB), Saline areas [Khewra (KW), Khabeki lake (KL) and Kallar khar(KK), Roadside [Choa sidan shah (CS), Buchal (BU), Munara (MN) and Faisalabad (FD).

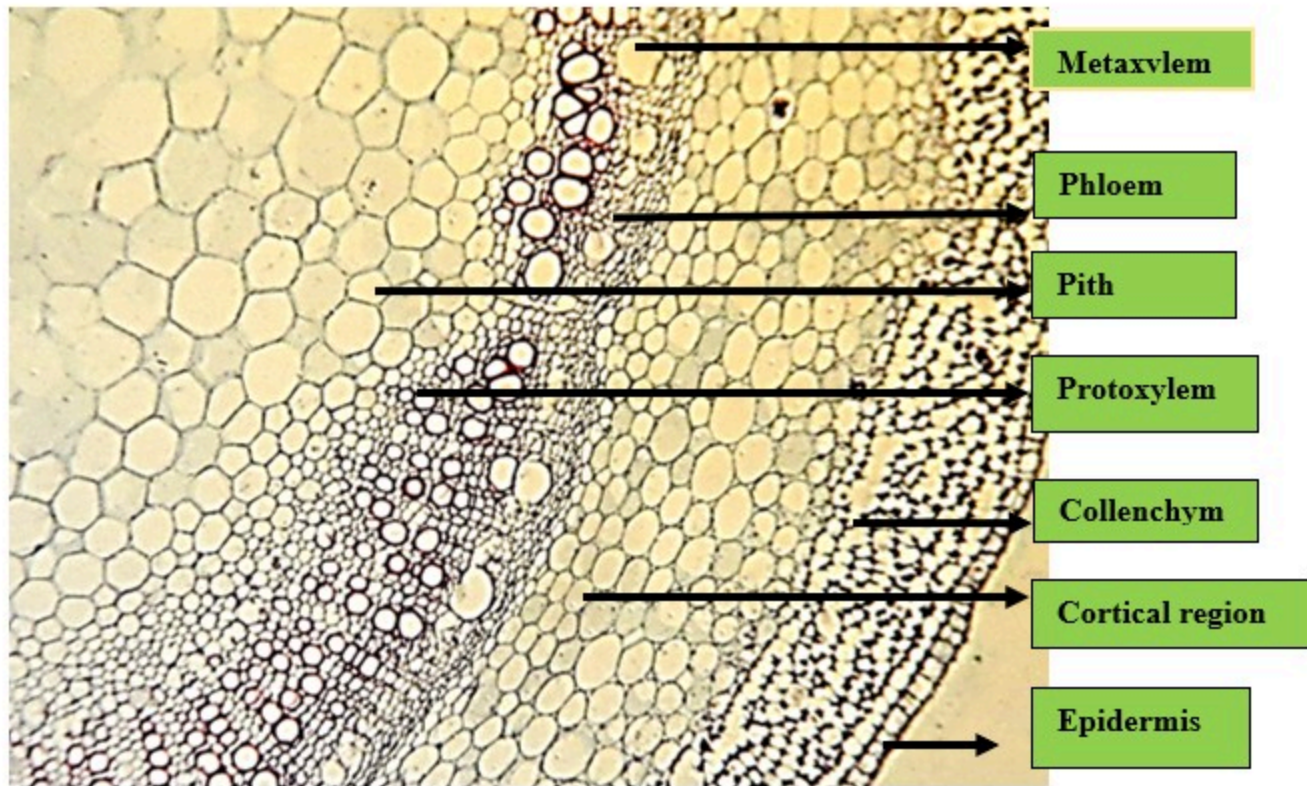


Figure 2

Measurement details of the stem anatomical attributes of *Justicia adhatoda* L.

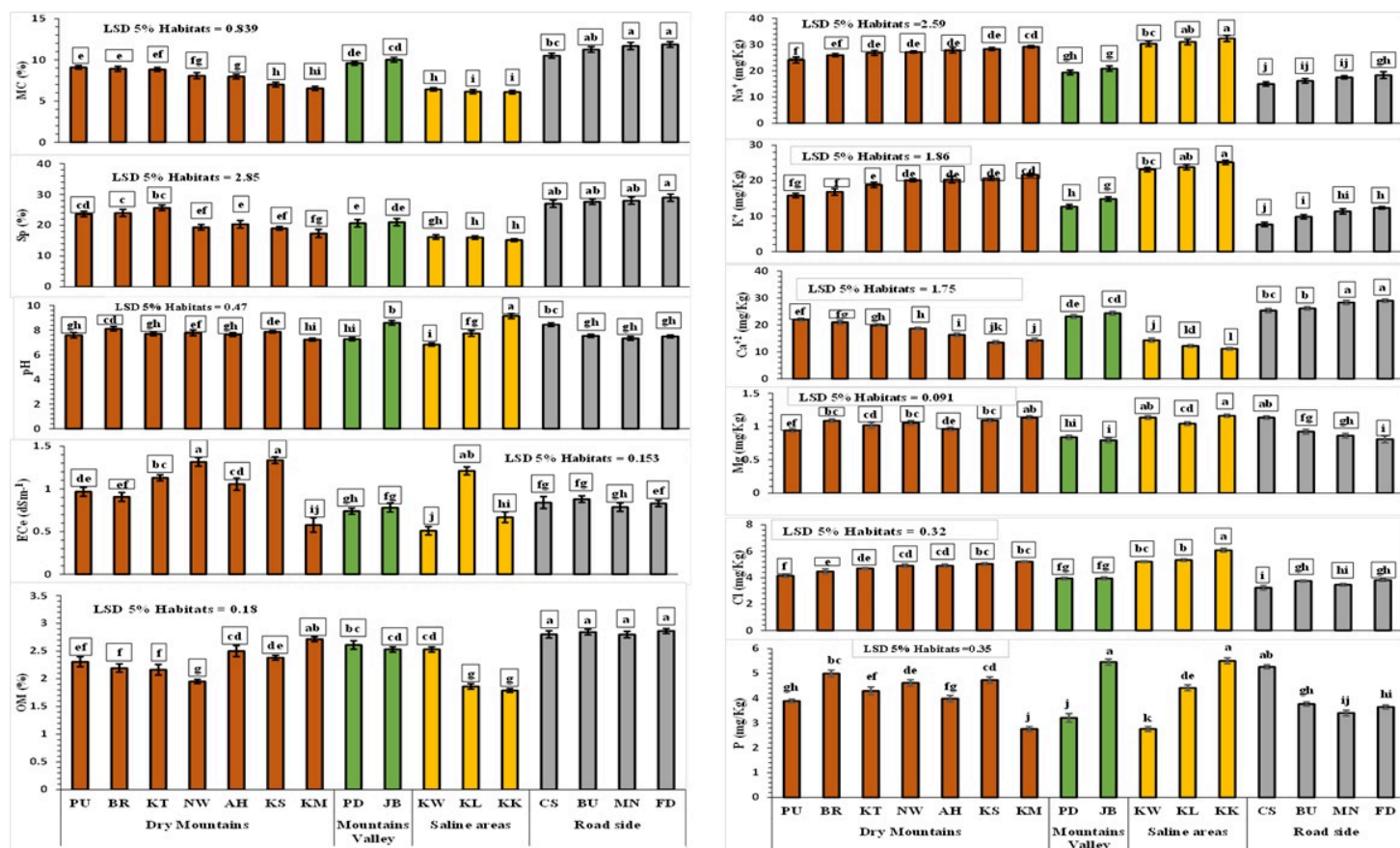


Figure 3

(a): Soil physico-chemical attributes of different habitats in Punjab. Habitats were categorized as **Dry Mountains** [Phulgran (PU), Barakhu (BR), Katas (KT), Neelawahn (NW), Ahmadabad (AH), Katha Sargral (KS) and Katha mountains (KM), **Mountains Valley** [Padhrar (PD) and Jabba (JB), **Saline areas** [Khewra (KW), Khabeki lake (KL) and Kallar khar (KK), **Roadside** [Choa sidan shah (CS), Buchal (BU), Munara (MN) and Faisalabad (FD). **Soil physicochemical attributes:** Soil moisture content (MC; %), Saturation percentage (SP; %), Soil pH (pH), Soil electric conductivity (ECe; dS m^{-1}) and Soil organic matter (OM; %).

(b): Soil physico-chemical attributes of different habitats in Punjab. Habitats were categorized as **Dry Mountains** [Phulgran (PU), Barakhu (BR), Katas (KT), Neelawahn (NW), Ahmadabad (AH), Katha Sargral (KS) and Katha mountains (KM), **Mountains Valley** [Padhrar (PD) and Jabba (JB), **Saline areas** [Khewra (KW), Khabeki lake (KL) and Kallar khar (KK), **Roadside** [Choa sidan shah (CS), Buchal (BU), Munara (MN) and Faisalabad (FD). **Soil physico-chemical attributes:** Soil Sodium (Na^+ ; mg/kg), Potassium (K^+ ; mg/kg), Calcium (Ca^{2+} ; mg/kg), Magnesium (Mg^{2+} ; mg/kg), Chlorine (Cl; mg/kg), and Phosphorous (P; mg/kg).

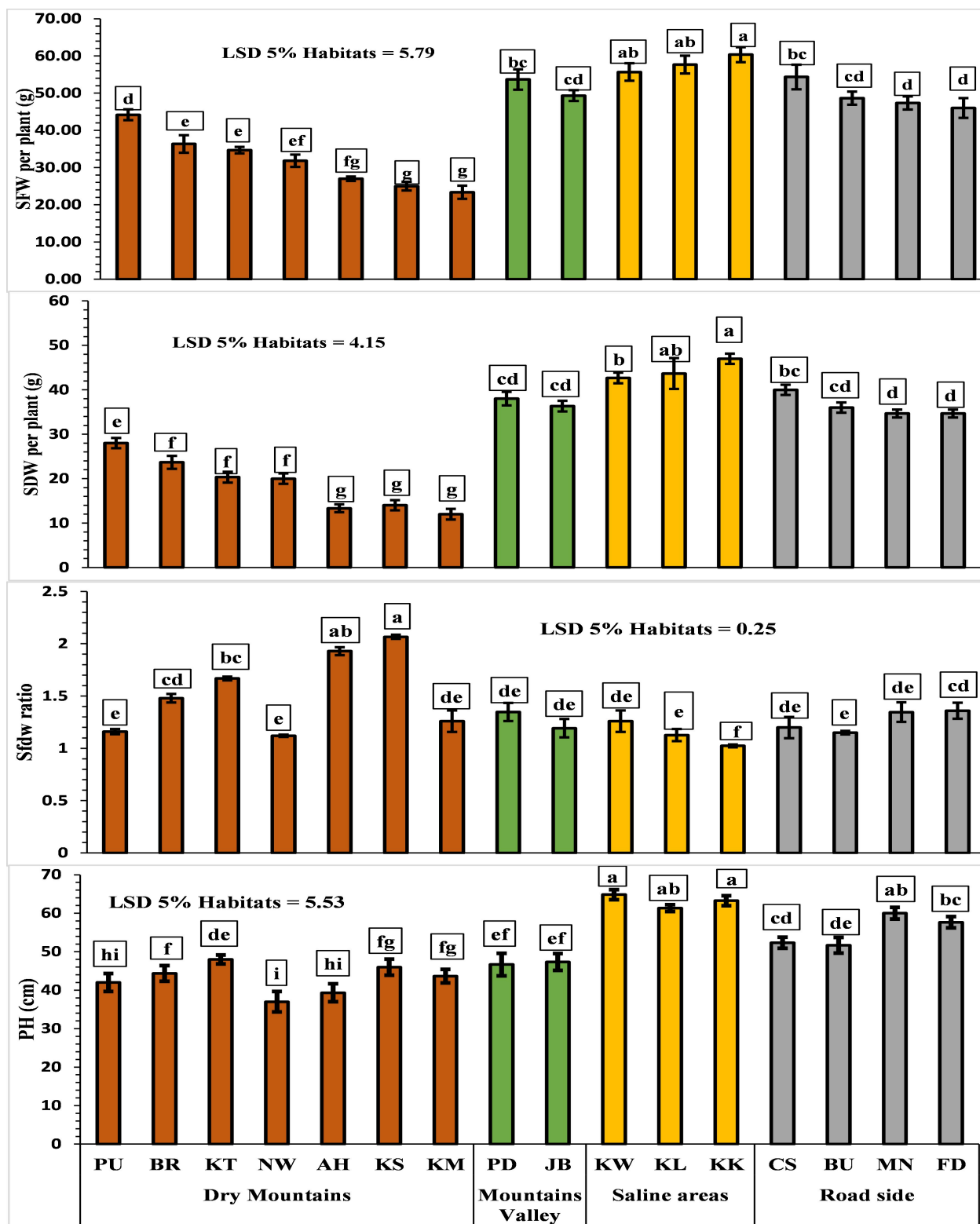


Figure 4

Morphological attributes of *Justicia adhatoda* L. collected from different habitats of Punjab. Habitats were categorized as Dry Mountains [Phulgran (PU), Barakhu (BR), Katas (KT), Neelawahn (NW), Ahmadabad (AH), Katha Sargral (KS) and Katha mountains (KM), Mountains Valley [Padhrar (PD) and Jabba (JB), Saline areas [Khewra (KW), Khabeki lake (KL) and Kallar khar (KK), Roadside [Choa sidan

shah (CS), Buchal (BU), Munara (MN) and Faisalabad (FD). Stem morphological attributes: Shoot fresh weight (SFW), Shoot dry weight (SDW), Shoot fresh to dry weight ratio (Sfdw), Plant height (PH).

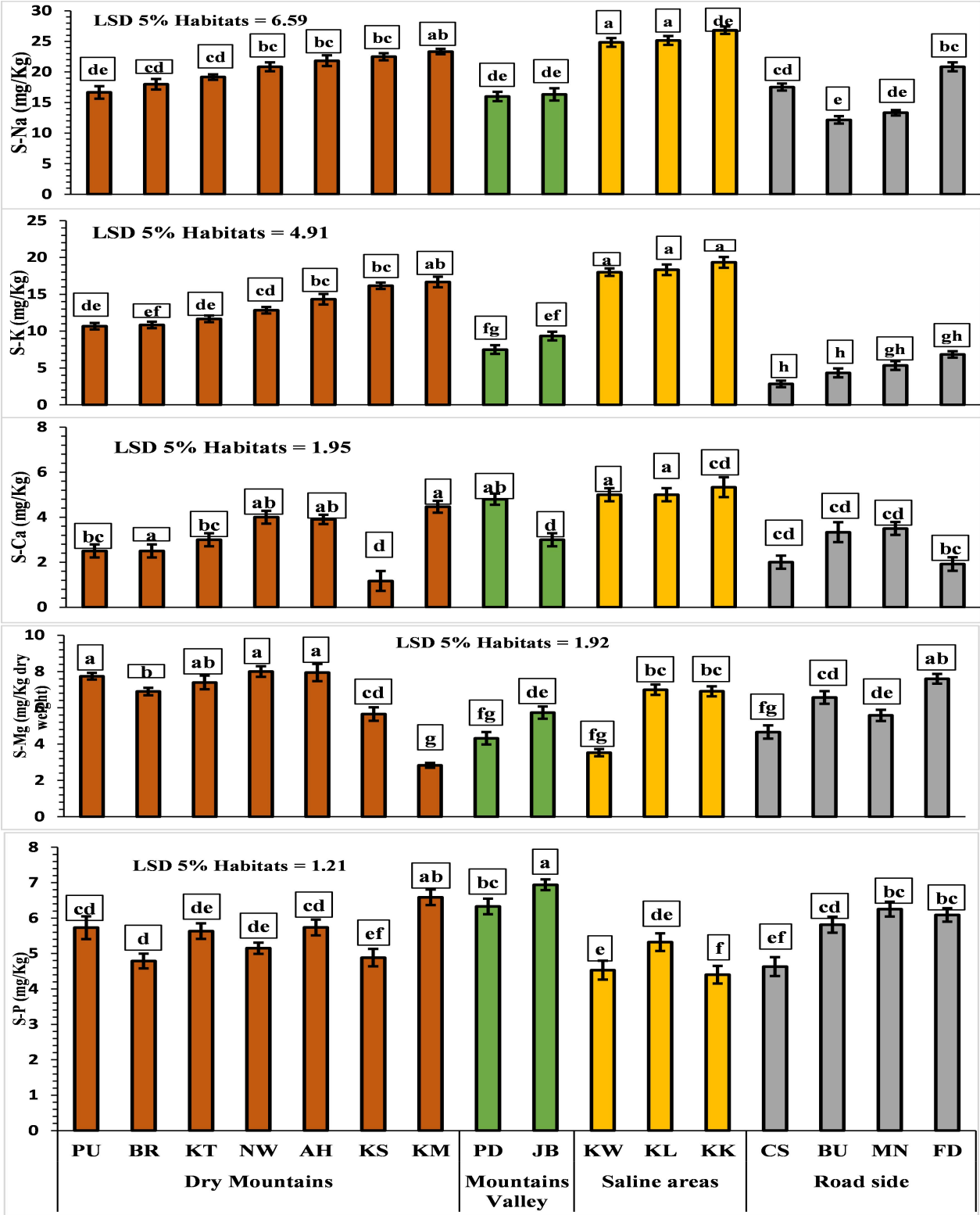


Figure 5

Ionic contents of *Justicia adhatoda* L. collected from different habitats of Punjab. Habitats were categorized as Dry Mountains [Phulgran (PU), Barakhu (BR), Katas (KT), Neelawahn (NW), Ahmadabad (AH), Katha Sargral (KS) and Katha mountains (KM), Mountains Valley [Padhrar (PD) and Jabba (JB),

Saline areas [Khewra (KW), Khabeki lake (KL) and Kallar khar(KK), **Roadside** [Choa sidan shah (CS), Buchal (BU), Munara (MN) and Faisalabad (FD). **Shoot ionic contents:** Shoot sodium (S-Na), Shoot potassium (S-K), Shoot calcium (S-Ca), Shoot magnesium (S-Mg) and Shoot phosphorus (S-P).

Image not available with this version

Figure 6

Root transverse sections of *Justicia adhatoda* L. collected from different habitats of Punjab, Pakistan. Habitats were categorized as Dry Mountains [Phulgran(PU), Barakhu(BR), Katas (KT), Neelawahn (NW), Ahmadabad (AH), Katha Sargral (KS) and Katha mountains (KM), **Mountains Valley** [Padhrar(PD) and Jabba (JB), **Saline areas** [Khewra (KW), Khabeki lake(KL) and Kallar khar (KK), **Roadside** [Choa sidan shah (CS), Buchal (BU), Munara (MN) and Faisalabad (FD).

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Figure 7

Principal correspondence analysis representing (a) Shoot morphological attributes, Shoot ionic contents, and (b) Shoot anatomical attributes plotted against soil physicochemical attributes of *Justicia adhatoda* L. collected from different dry habitats. Soil physicochemical attributes: Soil moisture content (MC), saturation percentage (SP), soil pH (pH), soil electric conductivity (ECe), soil organic matter (OM), Sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg) and Chlorine (Cl). **Habitats were categorized as Dry Mountains** [Phulgran (PU), Barakhu (BR), Katas (KT), Neelawahn (NW), Ahmadabad (AH), Katha Sargral (KS) and Katha mountains (KM), **Mountains Valley** [Padhrar (PD) and Jabba (JB),

Saline areas [Khewra (KW), Khabeki lake (KL) and Kallar khar(KK), Roadside [Choa sidan shah (CS), Buchal (BU), Munara (MN) and Faisalabad (FD). **Stem morphological attributes:** Shoot fresh weight (SFW), Shoot dry weight (SDW), Shoot fresh to dry weight ratio (Sfdw), Shoot Length (SL). **Shoot ionic contents:** Shoot sodium (S-Na), Shoot potassium (S-K), Shoot calcium (S-Ca), Shoot phosphorus (S-P) and Shoot magnesium (S-Mg). **Stem epidermal cell area (SDA), Stem epidermal thickness (SDT), Stem Cortical cell area (SCA), Stem protoxylem cell area (SPA), Stem metaxylem cell area (SMA), Stem vessel number (SVN), Stem vascular bundle area (SVA), Stem phloem thickness (SPhT), Stem cortical thickness (S.CRT), Stem sclerenchyma thickness (S ScT), Stem area (SA), Stem radius (SR), Stem collenchyma thickness (S ChT), Pith cell area (PCA), Xylem to phloem ratio (XPR)**

Image not available with this version

Figure 8

Persons correlation representing (a) Shoot morphological attributes, Shoot ionic contents and (b) Shoot anatomical attributes plotted against soil physicochemical attributes of *Justicia adhatoda* L. collected from different dry habitats. Soil physicochemical attributes: Soil moisture content (MC), saturation percentage (SP), soil pH (pH), soil electric conductivity (ECe), soil organic matter (OM), Sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg) and Chlorine (Cl). **Habitats were categorized as Dry Mountains** [Phulgran (PU), Barakhu (BR), Katas (KT), Neelawahn (NW), Ahmadabad (AH), Katha Sargral (KS) and Katha mountains (KM), **Mountains Valley** [Padhrar (PD) and Jabba (JB), **Saline areas** [Khewra (KW), Khabeki lake (KL) and Kallar khar(KK), Roadside [Choa sidan shah (CS), Buchal (BU), Munara (MN) and Faisalabad (FD). **Stem morphological attributes:** Shoot fresh weight (SFW), Shoot dry weight (SDW), Shoot fresh to dry weight ratio (Sfdw), Shoot Length (SL). **Shoot ionic contents:** Shoot sodium (S-Na), Shoot potassium (S-K), Shoot calcium (S-Ca), Shoot phosphorus (S-P) and Shoot magnesium (S-Mg). **Stem epidermal cell area (SDA), Stem epidermal thickness (SDT), Stem Cortical cell area (SCA), Stem protoxylem cell area (SPA), Stem metaxylem cell area (SMA), Stem vessel number (SVN), Stem vascular bundle area (SVA), Stem phloem thickness (SPhT), Stem cortical thickness (S.CRT), Stem sclerenchyma thickness (SvScT), Stem area (SA), Stem radius (SR), Stem collenchyma thickness (S ChT), Pith cell area (PCA), Xylem to phloem ratio (XPR).**

Image not available with this version

Figure 9

Clustered Heatmap representing (a) Shoot morphological attributes, Shoot ionic contents and (b) Shoot anatomical attributes plotted against soil physicochemical attributes of *Justicia adhatoda* L. collected from different dry habitats. **Soil physicochemical attributes:** Soil moisture content (MC), saturation percentage (SP), soil pH (pH), soil electric conductivity (ECe), soil organic matter (OM), Sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg) and Chlorine (Cl). **Habitats were categorized as Dry Mountains** [Phulgran(PU), Barakhu(BR), Katas (KT), Neelawahn (NW), Ahmadabad (AH), Katha Sargral (KS) and Katha mountains (KM), **Mountains Valley** [Padhrar(PD) and Jabba (JB), **Saline areas** [Khewra (KW), Khabeki lake(KL) and Kallar khar (KK), **Roadside** [Choa sidan shah (CS), Buchal (BU), Munara (MN) and Faisalabad (FD). **Stem morphological attributes:** Shoot fresh weight (SFW), Shoot dry weight (SDW), Shoot fresh to dry weight ratio (Sfdw), Shoot Length (SL). **Shoot ionic contents:** Shoot sodium (S-Na), Shoot potassium (S-K), Shoot calcium (S-Ca), Shoot phosphorus (S-P) and Shoot magnesium (S-Mg). **Stem epidermal cell area (SDA), Stem epidermal thickness (SDT), Stem Cortical cell area (SCA), Stem protoxylem cell area (SPA), Stem metaxylem cell area (SMA), Stem vessel number (SVN), Stem vascular bundle area (SVA), Stem phloem thickness (SPhT), Stem cortical thickness (S.CRT), Stem sclerenchyma thickness (S ScT), Stem area (SA), Stem radius (SR), Stem collenchyma thickness (S ChT), Pith cell area (PCA), Xylem to phloem ratio (XPR).**

Supplementary Files

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